EFFECT OF ANTISTRESS DRUGS (ALPRAZOLAM, BUSPIRONE AND FLUOXETINE) ON STRESS INDUCED CHANGES OF BRAIN AND OTHER ORGANOHISTOPATHOLOGY IN MALE ALBINO RATS.

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CERTIFICATE

This is to certify that thesis entitled ***"Effect of antistress drugs (Alprazolam, Buspirone and Fluoxetine) on stress induced changes of brain and other organohistopathology in male albino rats"*** is a bonafide work of Mrs. Kori Rohini Sharanappa and was carried out under our supervision and guidance in the department of Anatomy, ***BLDE (Deemed to be University) Shri B.M.Patil Medical College, Hospital and Research centre,*** Vijayapura, Karnataka, India.

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I declare that the thesis entitled **"Effect of antistress drugs (Alprazolam, Buspirone and Fluoxetine) on stress induced changes of brain and other organohistopathology in male albino rats"** has been prepared by me under the guidance of Dr. S D Desai, Department of Anatomy and Dr. K K Das Department of Physiology, BLDE (Deemed to be University) Shri B M Patil Medical College Hospital and Research Centre, Vijayapura, Karnataka, India. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

μg	Microgram	HCL	Hydrocloric Acid
μm	Micrometer	HPA	HyperthalamicPitutaryAdrinal
ACTH	Adrenocorticotrophin Hormone	HPA	Hyperthalamic-Pitutary-Adrinal
ANOVA	One Way Analysis of Variance	HPG	Hypothelamus -Pitutary – Gonadal
ATP	Adenosine Triphosphate	Hrs	Hours
BW	Body Weight	IAEC	Institutional Animal Ethecal Committee
CCF	Congestive Cardiac Failure	IBS	Irritable Bowel Syndrome
Cm	Centimeter	IUPAC	International Union of Pure and Applied Chemistry
CNS	Central Nervous System	IP	Intraperitonial
CRH	Corticotrophin Releasing Hormone	Kg	Kilogram
CRF	Corticotrophin Releasing Factor	MCH	Mean Corpuscular Hemoglobin
CPCSEA	Committee for the Purpose of Control and Supervision on Experiments on Animals	MCHC	Mean Corpuscular Hemoglobin Concentration
DNA	Deoxy Ribonucleic acid	MCV	Mean Corpuscular Volume
$C_{10}H_8N_2$	2.2'-bipyridal	Mg	Milligram
$C_{24}H_{30}$	Xylene	min	Minute

$C_{29}H_{50}O_2$	α-tocopherol	ml	Milliliter
$C_6H_6N_4O_4$	2,4-dinitrophenyl hydrazine	NaCl	Sodium Chloride
$C_6H_8O_6$	Ascorbic Acid	NO	Nitric Oxide
CH_3COOH	Glacial Acetic Acid	OCD	Obserssive Compulsive Disorder
DXM	Dexomethorphan	OD	Optical Density
ELISA	Enzyme Linked Immunosorbent Assay	°C	Degree Celsius
$FeCl_3$	Ferric Chloride	PCV	Packed Cell Volume
FDA	Food And Drug Administration	PVN	Paraventricular Nucleus
FSH	Follicular Stimulating Hormone	PCP	Phencyclidine
GABA	Gamma Amino Butyric Acid	PMS	Premenistrual Syndrome
GAD	Generalised Anxiety Disorder	RBC	Red Blood Cells
GAPDH	Glyceraldehydes	ROS	Reactive Oxgen Species
GAS	General Adaptation Syndrome	SE	Standard Error
GC	Glucocorticoids	SNRIs	SerotonineNorephinephrin Reuptake Inhibitors
GH	Growth Hormone	SSRIs	Selective Serotonine Reuptake inhibitors
GnRH	Gonadotrophin Releasing Hormone	SUL	Sulpiride Drug
GR	Glucocorticoid Receptor	TCA	Trichloroacetic acid
H_2SO_4	Sulphuric Acid	TMB	Tetramethylbenzidine
HB	Hemoglobin	TWBC	Total White Blood Cells

ABSTRACT

Introduction: Stress has an impact to make changes in a range of anatomical, physiological, biological responses consist of psycho-physiological responses. Many studies showed that serum cortisol levels and antioxidant vitamins C and E are a reliable indicator of stress responses in animals. Based on the current available information on exposure to restrained stress standpoint, it was noticed that majority of the studies were centered on their modifications of the immune function rather than the configuration and available reports concerning persuade of antidepressants and reversible assessment after withdrawal on assessment of antistress effects in restrained rats is lacking. Hence, present experimental trials have been designed to examine the consequence of restrained stress and efforts have been directed for studying those novel commonly used antistress drugs viz., alprazolam (benzodiazepine anti-anxiety agent), fluoxetine (a selective serotonin reuptake inhibitor) and buspirone (a non-benzodiazepine anxiolytic drug) to compare for their protective effects in restrained stress 21 days in continuance with stress. While, in contrast, the withdrawal of stress also designed meant for residual 21 days might perhaps counteract or reversible of such restrained stress provoked damage.

Materials and Methods: Acclimatized Wistar albino rats weighing about 175 to 225g were obtained for the study and were randomly divided into six grouping of six animals each. Untreated control rats (Group I) reserved uninterrupted in the metabolic cage all through the experimental duration intended for 42 days; Stress induced rats (Group II) were strained daily for 6hrs in wire mesh restrainer intended for 42 days; Group III rats were stressed intended for 21 days by means of observance in mesh restrainer and then

retaining animals in normal cages for residual 21 days designed for stress withdrawal and rest of other drug treated groups i.e., groups IV,V and VI rats were stressed intended for 21 days and then treated with alprazolam (5mg/kg body weight, intraperitoneally, BW, IP), buspirone (12mg/kg BW, IP) and fluoxetine drug (20mg/kg BW, IP), respectively for residual 21 days in continuation with stress. All animals were observed daily for mortality and morbidity, physical examinations and clinical observations like general appearance of the animal's behavior; individual food consumption record of the animals. At the end of the final day subsequent to an overnight fast, all the animals were sacrificed and gravimetrical parameters, relative organ weights, haematological parameters, blood serum cortisol, antioxidant vitamins levels were assessed. Finally, whole brain and relative organs of control and experimental rats were dissected out, blotted free of mucus, weighed to the adjacent milligram and subjected to histopathological evaluations.

Results: Results revealed that restrained stress (Group II) being a psychological stress significant ($P \leq 0.05$) change in the food consumption, BWs, gravimetrical parameters, relative organ weights, haematological parameters and serum levels of cortisol and antioxidant vitamins C and E and drugs treated rats. On the other hand, stress withdrawal rats (Group III) and administration of drugs alprazolam (Group III), buspirone (Group III) and fluoxetine (Group III) for residual 21 days in continuance with strain illustrated a remarkable ($P \leq 0.05$) improvements by means of neutralizing or recovering of altered parameters while compared to only group II restrained stress induced rats.

Histopathological evaluations of stress induced brain sections of cerebral cortex exhibit neuronal cells with indistinct in profile with shrunken intensely stained nuclei, bounded

in vacuolated parts, mild focal vacuolar degeneration and there were no features of necrosis, infarcts, inflammation or glial proliferation. Liver sections demonstrated distorted architecture including congestion blood vessels, infiltration, vasodilatation, hydropic, fatty changes and hypertrophy. Kidney sections exhibited mild thickening of tubular basement membrane, focal cloudy swelling of tubular cells and glomerular shrinkage. Testis sections showed atrophic tubules and spermatogenesis did not proceed ahead of pachytene spermatocytes with exhibited signs of degeneration. The Sertoli cells showed vacuolization and intercellular spacing that become wider with occurrence of Leydig cells with shrunken nuclei. However, these histopathological changes were remarkably reversed in stress withdrawal rats (Group III) and neutralized to normal level in stress induced alprazolam (Group IV), buspirone (Group V) and fluoxetine (Group V) drugs treated animals designed meant for remaining 21 days with continuation with stress.

Conclusion: Results presented here led us to that, exposure to restrained stress resulted alter the levels of gravimetry, haematological, biochemical parameters and marked histopathological alterations due to peripheral oxidative stress in male albino rats. While, withdrawal of stress or antistress drugs designed meant for residual 21 days in continuance through stress might possibly neutralize restrained stress provoked injure to escorting antioxidant equilibrium and modify hypothalamic-pituitary adrenal (HPA) axis

Key words: Stress, Antistress drugs, Withdrawal, Gravimetry, Haematology, Cortisol, Antioxidant vitamins, Histopathology and Rats.

CHAPTER – 1

GENERAL INTRODUCTION

1.1. Stress

Stress is a broad, vague often poorly unstated conception and be able to expressed because the amount of each response of the body, which perturb the regular physiological balance as well as consequences in a condition of frightened homeostasis. It is globally accepted observable fact reinforced by development of tradition and insisting civilization. Contemporary existence is beset with annoys, time limits, frustration, anxieties and each individual nowadays faces distressing circumstances in day to day continuation. Stress stands for response of body to inducement that tend to alter its usual physiological balance or homeostasis and it has been described as non precise reaction of the body to any demand imposed on it.[1] For best possible endurance of the human being it is essential that physical roles are theme to homeostatic organize. Hence, there is a incessant venture to preserve these tasks within a sure variety changeable to maintain through a method referred to as allostasis.[2] It has been reported that 43% of every adults suffer from hostile health effects because of strain and condition stay untreated more than 50% endure from life span exciting disorders as per remarks from American Institute of stress.

Individuals respond to stress in their own ways. While stress goes far beyond what one really feels, causes expected alterations in immune function, hormone levels, enzymes and gastrointestinal function. Indeed, prolonged stress, whether consequences of mental/emotional disturb or due to physical factors like malnutrition, chemical exposures, excessive exercise, sleep deprivation or environmental sources resulting in expected systemic effects. All individuals have diverse abilities to confront and accommodate when faced with anxiety. Stress that persists with no aid be able to escort towards a situation called a negative stress response, distress. Distress can escort to bodily signs including headaches, disturb stomach, raised blood pressure, chest pain and emotional signs such as,

sense of aloneness, irritability and disturbance. Behavioural symptoms involving eating too much or less, neglecting responsibilities and cognitive signs including recollection problems, incapability to concentrate , poor decision , nervousness etc. This condition of build up stress can augment the threat of both acute and chronic psychosomatic sickness and deteriorate the immune system. Reports show that diseases such as hypertension, asthma, diabetes, heart ailments and even cancer are caused by stress augmentation.[3]

In a demanding situation the brain organizes the body for defensive action called fight or flight reaction by releasing stress hormones, namely, cortisone and adrenaline.[4,5] Probable disorders from internal or else external causes, which communally observed by the person because stress make active two coordination's that providing regulate the perturbed tasks referring as sympatho-adrenomedullar system and hypothalamo-pituitary-adrenocortical (HPA) axis. Commencement of earlier quickly consequences in enhancement of adrenaline and nor adrenaline release, which through commence of the vagal nerve circuitously raises the exploit of noradrenergic neurons in nucleus tractus solitarius and locus ceruleus.[3,6] Stress activates the HPA axis, resultant in the discharge of corticotropin releasing hormone (CRH) from the hypothalamic paraventricular nucleus (PVN). Then CRH causes anterior pituitary to discharge adrenocorticotropic hormone (ACTH) which consecutively motivates the adrenal cortex to discharge corticosterone.[4] Within the brain, corticosterone performs at those sites where corticosteroid receptors are expressed such as in limbic areas of hippocampal subfields, lateral septum and central amygdala, etc.[7]

In addition to these hormones, the enhancement of neuropeptides release also escorted by stress exposure in the brain such as vasopressin and corticotrophin releasing hormone (CRH).[8] Together, catecholamines, neuropeptides and corticosterone modify the electrical

possessions, form and propagation ability of cells in the brain, consequently giving rise in the direction of the central and behavioural stress. Stress hormones too influence other features of brain task, for instance bio-availability of neurotransmitters[9] and metabolic processes[10] both adding to the vigorous variety of cellular consequences of strain.

In the areas of stress causes lying on hormones, neurotransmitters, chemical messengers, vital enzyme organizations as well as metabolic performances are still mysterious hence investigate on these regions could assist in the direction of describe how anxiety be able to offer to despair, unease and its assorted consequences on gastrointestinal tract and various other organs. Consideration of over perceptions is extremely significant in evolving pathophysiology of the illness cause by anxiety and to expand the beneficial mediators intended for executive of stress and anxiety induced diseases. The root cause of many diseases like diabetes mellitus, hypertension, allergies, CCF, Alzheimer etc has been studied with respective to stress. However, due to lack of diagnostic tests to recognize aetiology of stress and dealing is based only on symptoms and there is no safely reputable treatment advice for this situation and the obtainable therapies in modern medicine are restricted.[11]

Consecutively to lessen the incidence of side consequences linked with other former antidepressants, newer groups of antidepressant, which perform further selectively on one or more monoamine transporters exclusive of binding to other receptors, were afterwards industrialized. These take account of the selective serotonin-reuptake inhibitors (SSRIs), the selective noradrenaline-reuptake inhibitors (NRIs), the combined serotonin, noradrenaline-reuptake inhibitors (SNRIs), benzodiazepines and azaspirodecanedione anxiolytics in addition to other drugs with assorted mechanisms. These categories of medicines that are

employed to indulgence the signs of anxiety disorders amongst most usually recommended types of anxiety prescriptions are antidepressants, anxiolytics and beta blockers. Antidepressants and anxiolytic prescriptions work for the most part through influencing the stability of convinced elements or neurotransmitters in the brain. Beta blockers and other types of drugs are employed to deal with physical signs that might escort an anxiety molest. For example: i) fluoxetine drug, belongs Selective serotonin reuptake inhibitors (SSRIs) family, is often employed to indulgence main depressive disorder, post traumatic stress disorder, panic disorder and premenstrual dysphoric disorder. This drug eases despair through enhancing serotonin levels in the brain and hinders the serotonin reabsorption in the brain. However, the fundamental means of its therapeutic effectiveness linger uncertain; ii) alprazolam drug, belongs benzodiazepines family, is mainly employed to indulgence generalized anxiety disorder and panic disorders. The precise means of act of this drug is not known excluding it come into view to exploit through influencing messengers in the brain; iii) further, buspirone a azaspirodecanedione anxiolytic agent, is consented for the short or long standing dealing of anxiety disorders. Its compound structure and method of exploit are exclusively not associated to those of the benzodiazepines and its competence is not similar to that of components of the benzodiazepine family. Contemporary explores shown that its major neuropharmacologic consequences are arbitrated through the 5-HT_{1A} receptors.

For the treatment of stress, the allopathic medicines are employed including benzodiazepines, antidepressants, Barbiturates etc., generally for curing or controlling the neurological or psychiatric implications of stress. To correct altogether neuronal, endocrinal melabolic disorders caused by stress non-specifically, as there are no drugs available; the modern allopathic medicines used are only for indicative treat and cannot be used for prevention. Further, allopathic medicines also bring development of addiction or medicine

dependence along with serious side effects on prolonged use.[12] Besides, these drugs are not confirmed as awfully effectual against chronic stress induced unfavourable effects on endocrinal system, immunity, behaviour and cognition. As there are no consistent and safe pharmacotherapeutic agents available for treatment of chronic stress, these drugs consequently are generally used for symptomatic management of acute stress.

Stress is a convoluted physiological mechanism that represents a range of integrative physiological and behavioural routes that take place when there is an actual or apparent risk to homeostasis. These processes are adaptive and generally believed that insufficient or excessive or prolonged activation of stress systems can alter normal physiological and behavioural task which leads in a series of unfavourable consequences such as depression, cardiovascular diseases, impaired immune function with increased susceptibility to disease, reproductive function, diabetes, dementia and reduced life anticipation.[13]A perceptive of the neuroendocrine mechanisms that cause usual physiological states of stress receptiveness might offer information that could be employed to formulate physiological treatments for people at threat of sickness due to chronic stress and disorders of the stress systems. This is theoretically striking because the most effectual mechanisms to suppress stress reaction will certainly be those that the body itself uses. Hence to know the nature of stress response and mechanisms by which stress disorder increase, it is essential to make out how and why stress response extended. It has been suggested that capability of living individual to adapt to the huge changes in their surroundings,[14] the mechanisms of action of presently obtainable antidepressant dealings, all offered antidepressants perform by means of the monoamine neurotransmitters, serotonin or noradrenaline based on serendipitous along with discussed the exploration of prospective drugs which are requirement to conquer stress and for overall prevention or treat stress and stress related diseases.[15]

1.2. Historical Development of concept of Stress

Conception of homeostasis goes back to the historic Greeks; the theorist Empedocles believed in the direction of each subject was a melodic combination of components and merits. Hippocrates extended this early on appearance of homeostasis to livelihood and believed that health the same as condition of harmonious equilibrium and illness the same as situation of disharmony. He explained worrying forces of nature as reasons of illness and the curing might intrinsic to the organism the same as curing influencing the environment. Then this psychogenic stress put forwarded that managing with exciting stressors was an approach to recover the fine quality of living.[16] A theory was introduced during early 19th century and signifying that since life forms happen to extra self-governing of their environs by developing additional composite means of steadying their interior situations to counteracting the alters in their exterior environs. This consequence of amendment mechanism theory was then expanded and demonstrated that numerous trials that the sympathoadrenal organization be accountable for harmonizing the fight or flight reaction essential to convene exterior confronts.[17] Further, it was supposed that an individual organism's vulnerability to critical stress varies under different general situations and in the course of the usual and pathologic alterations of continuation in common living series.[17]

The concept of the General Adaptation Syndrome (GAS) was presented by Selye[18] and thought modified as of the sympathetic nervous system to the adrenal glands. He suggested that hypothalamic discharging or hindering factors control the anterior pituitary task. A factor as of the hypothalamus was controlling ACTH release of pituitary and named this factor corticotropin-releasing factor (CRH) and then this factor was isolated and structure characterized as 41 amino acid.[19] After identifying its chemical structure, then phrase factor was modified to hormone.The concept of stress biology theory that integrated stress system

is consist of neuroanatomical and functional configurations that meaning to make the behavioural, physiological and biochemical change focussed to maintaining homeostasis.[16] Then outcomes in neurobiology representing anatomical and efficient associations among the hypothalamus and many sympathetic nuclei in the hindbrain supported this theory.[20,21]The adrenocortical and sympathetic divisions of the stress system (in the periphery) comprise supplementary integrative deeds including harmonizing and tolerant communications of glucocorticoids and catecholamines in the ruling and protection of metabolic and cardiovascular homeostasis.[22,23] The word stress explains a situation of threatened homeostasis or threatened balance. The threatening or distressing forces are described the same as stressors, whereas the offsetting forces state to counteract the consequences of the stressors and restore homeostasis are defined as adaptive response.[24]

1.2.1. Types of stressors

Stressors comprise a broad range of surroundings stimuli that provoke important homeostatic variations in the crowd that might be a consequence as of usual alters in the surroundings or non-natural alters caused by organization restraint or caused via a variety of trial stimulus. Stress perhaps convinced through unkind of the stressor, the period all through which it is concerned i.e., acute vs. constant otherwise whether the animal be able to flee the stressor if it is affected repetitively i.e., avoidable vs. unavoidable.

a) Environmental: Usual exposures towards acute of in climatic situations or else unnaturally in animal accommodation or holding amenities are amongst the commonest stressors. Bodily restraint linked with parting, handling or transfer of animals is an additional major stressor. Surroundings alterations which create odd or novel noises, prospects; odours or flavours can as well stimulate stress. Drugs or compounds employed in

organization or conduct of animals be capable of take steps as stressors, whereas lethal commodities discharged by communicable agents, atmosphere contaminants or deficient aeration comprise a related consequence. Trimming, harbouring as well as castration carried out through schedule executive too reason for stress.

b) Behavioural: Congestion, hierarchical endeavour, extrication, introduces to unusual surroundings or else separation can comprise a foremost behavioural impact taking place in animals and incite strain. Alterations otherwise limits in cut down can too perform as stressors solitary or in combination with stressors for instance physical restraint.

c) Psychological: Confined to wild animals otherwise introducing of tamed animals to restraint, shift acute may provoke adaptative anxiety in the crowd. As perspective of Selye's GAS, the reaction is identifiable primarily as unease, which might advance to fear or else fright. Unsuccessful animals to come into view that flight-fight reaction consequences in the phrase of aggravation or temper and such stimulus continues for a longer period results in aggravation or vulnerability. Since stress effects mostly as of individual's insight of the risk causes via the incentive before its nature as such.

1.3. Stress defense Mechanisms

The HPA axis along with sympathetic system be the key controllers of an animal's homeostatic purposes.[25] Hence, organism's reaction to strain is gathered of organization between behavioural, endocrinal, and autonomic components to counteract the disrupting results of the stressors on homeostasis.[26,27]

1.3. 1. Nervous defense mechanism

The initiations of sympathetic limb of autonomic nervous system consequences the discharge of norepinephrine in certain brain areas of locus ceruleus, frontal cortex amygdala and other limbic structures. From the hypothalamic vegetative centre, the nervous impulses move down through the autonomic nerves to the peripheral organs. Adrenal medulla is stimulated by the splanchnic nerve to release adrenergic hormones (adrenaline and noradrenalin) in to the blood and other adrenergic nerves persuade their target organs directly through fibres.

Norepinephrine discharged causes vasoconstriction and augmented cardiac output which elevates blood pressure. Epinephrine discharged stimulates the pancreas to release glucagon and a result in turn leads to insulin decline and glucose uptake in the skeletal muscle and peripheral tissues. Catecholamines consist of neurotransmitters for example dopamine, epinephrine and norepinephrine which are released during the body's stress response. Epinephrine is the strongest of catecholamine secreted by stress.[28] The immediate effects of catecholamines during stress includes increasing glucose in bloodstream, increase cardiac output, increasing glycogenolysis in muscle, retaining sodium and constricting the blood vessels in the skin etc.

1.3.2. Hormonal defence mechanism: Hypothalamic Pituitary Adrenal axis (HPA axis)

The main endocrine reaction to strain is considered by the shift in anterior lobe hormone production which consist of diminished discharge of somatotrophins and gonadotrophins (FSH, LH, and prolactin) and thyrotrophin which are not important for maintenance of life during circumstances of emergency. The HPA axis (Figures. 1.1 & 1.2) activates when adaptive capability of individual is plagued by stressors. During stress

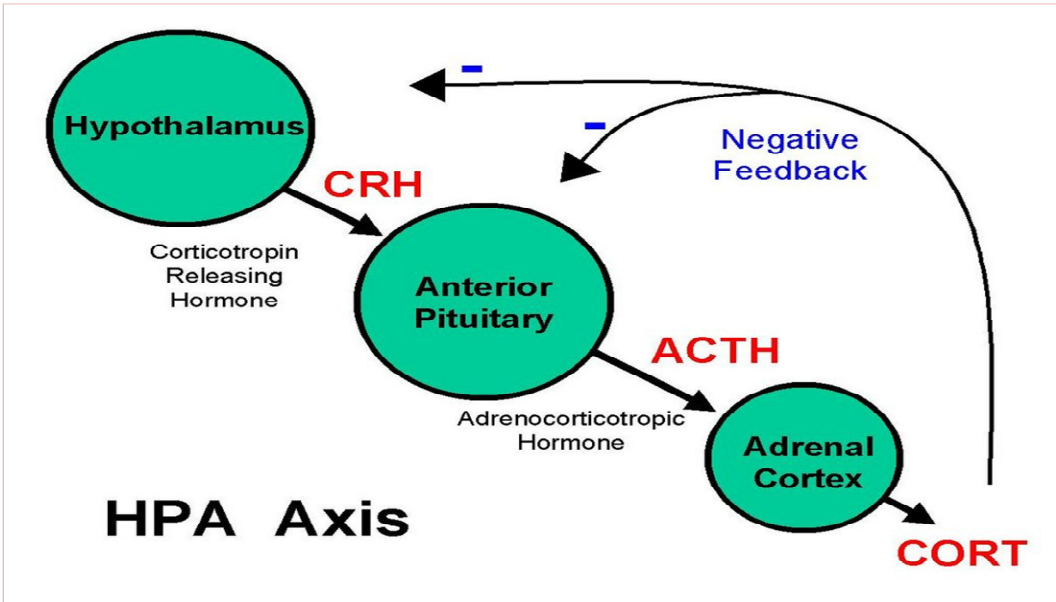

Figure.1.1 Schematic of the HPA axis (CRH, Corticotrophin-releasing hormone; ACTH, Adrenocorticotropic hormone)

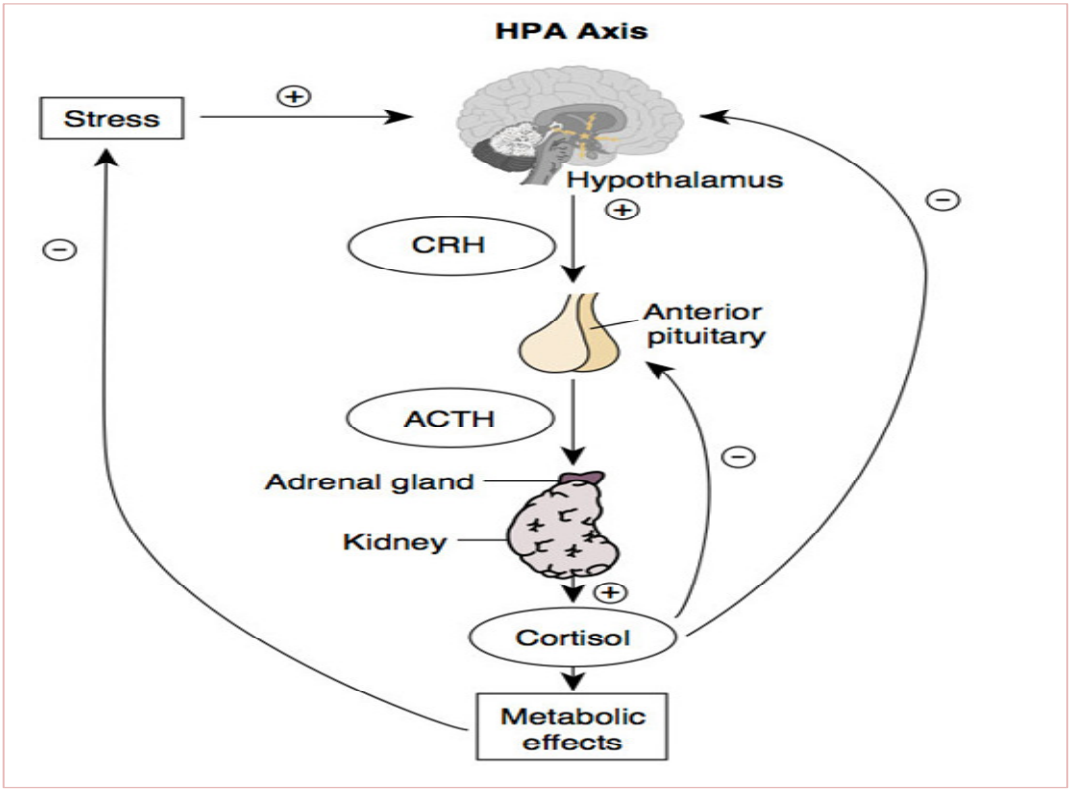

Figure.1.2. Main constituents of the stress reaction arbitrated via the hypothalamic-pituitary adrenal (HPA) axis [29]

respond from central nervous system (CNS) activates the hypothalamus to discharge CRH or corticotrophin releasing factor (CRF) which motivates both posterior and anterior pituitary glands causing elevate in the secretion of ACTH.

A) Corticotrophin releasing hormone (CRH)

CRH act as neurotransmitter and neuromodultor in the brain and is expressed in amygdala, prefrontal cortex, Cingulate cortex. Centrally introduced CRH induces behaviours similar to natural stress (anxiogenic). Since it is released in brain areas, there it coordinates behavioural and emotional response to stressors. CRH is located in neuronal cell bodies of PVN.[20,21] CRH is discharged taken with the hypophyseal portal system and then it is carried to the anterior pituitary where it incites pituitary corticotrophs to together produce and secrete ACTH. A divergent set of PVN-CRH neurons pass on protrusions to the hindbrain where they motivate the stimulating action of their object neurons in arousal as well as sympathetic centres.[30] Also the additional CRH neurons have been recognized in the brainstem, midbrain, striatum, hippocampus, cerebral cortex, spinal cord, sympathetic ganglia and adrenal gland.[31,32] The huge allocation of CRH and its receptors in CNS affords extensive behavioural consequences of this peptide.

Rat and several primates exhibited that the CRH receptor is deeply intense in brain, anterior pituitary, adrenal medulla and sympathetic.[30,33] CRH discharge be able to influenced by incentive for instance impression, hurt and alteration in blood pressure.[34] Numerous neurotransmitter systems control PVN CRH discharge together nor-epinephrine and epinephrine stimulate CRH release. Various products of the immune system for example some cytokines or inflammatory mediators elucidate to motivate the discharge of hypothalamic CRH in vitro and in vivo.[35-37] The allocation of CRH inside and beyond the

hypothalamus offers an anatomical perspective meant for the examination that CRH be able to concurrently stimulate as well as harmonize metabolic, circulatory plus behavioural reactions throughout acclimatize condition.[38-40]

B) Adrenocorticotropic hormone (ACTH)

By the influence of CRH, ACTH released, from the pituitary gland, targeting on the adrenal cortex to secrete cortisol and aldosterone. ACTH networks with adrenal cortex cell membrane receptors and through its second messenger, cyclic AMP, stimulates steroidogenesis process escorting to cortisol discharge. The gratis flowing cortisol performs in a negative response mode to manage the ACTH discharge as of the pituitary gland. Stress is so as to overrule the negative response of cortisol on ACTH discharge. Stress motivates discharge of neurogenic amines which consecutively motivates CRH discharge. The levels of ACTH can rise able to tenfold in period of strain, following in elevated cortisol stages. Hypoglycaemia can as well enhance CRH discharge, eventually escorting to elevate in cortisol. This consequence is arbitrated through glucose receptors in the hypothalamus which motivate the discharge of CRH.

C) Glucocorticoids (GC)

Glucocorticoids are the main anxiety hormones in order to role to conserving homeostasis. They are produced and discharged through the adrenal cortex subsequent stress provoked commencement of the HPA axis and influence almost each organ and tissue in the body. Studies have been shown that a surplus of biological procedures comprising immune task, skeletal development, reproduction, behaviour and cell propagation are regulated by glucocorticoids.[41,42] In concert, physiological and pharmacological exploits of glucocorticoids are arbitrated through the glucocorticoid receptor (GR) and existing

hypothesis so as to a solitary receptor protein is accountable meant for the various deeds of glucocorticoids.[43] The glucocorticoid receptors are present all through the brain together with the CRH neurons of hypothalamus.[44] Glucocorticoids performances on the CNS are arbitrated via two separate receptor systems, GRs type I [45] and type II [45] and these receptors type I participate a function in changing the response to surroundings and emotional incentive with consequential alters in behaviour and HPA axis action and Type II, it is expected so as to the receptors take part within the behavioural, neuroendocrine and autonomic responses to strain. Appending of glucocorticoids activates a compositional modification in GR resulting in the separation of the heterocomplex, nuclear localization indicators revelation and importin arbitrated nuclear access.[47] GR can as well control the expression of gene via connecting by means of other transcription aspects. Studies have been revealed that GR heterogeneity could also take part in significant task deciding the glucocorticoid signaling profile,[48] though divergences in ligand bioavailability, GR expression levels, and cofactor availability contribute to the tissue-specific effects of glucocorticoids.

1.3.3. Effect of stress on various physiological functions of the body

I) Effect on Psyche

It is anticipated that an important factor in the pathophysiology of various psychiatric diseases, for instance main despair, anorexia nervosa as well as fear unease effecting in CRH otherwise central catecholamine hypersecretion. Specially, it is being assumed the irregularity during affirmative regulation or deficiencies in counteract directive of the adrenocortical central constituents as well as adrenergic system are accountable meant for these anarchies.[26,27] Acute stress provokes hormonal and behavioural alterations intimately resemble the indication depression composite.[49] Hypercortisolism of melancholia is a

reliable characteristic of the typical type of major depression.[50] It is a controlled state of anxiety, resultant in a thoughtful sense of insignificance and desperation concerning the prospect are linked among other indications of hyperarousal or general stress response commencement which comprise increased vigilance and reticence of vegetative tasks.[26,27] The pituitary corticotroph cells in main despair are inhibited by elevated flowing glucocorticoids. Hypercortisolism in despair imitates the hypothalamus resultant in the hypersecretion of endogenous CRH. [26,27,51,52]

It has been shown that concurrently persuade changes in behavioural, cardiovascular and neuroendocrine role feature of those examined for the period of strain.[53,54] Hypothetical evidences exhibited to stress-provoked alters during emotional, neuroendocrine, sleep and heart tempo outlines might imitate a central muscarinic cholinergic constituent. Data from the in vivo and in vitro imply to muscarinic cholinergic agonist arecoline motivates the HPA axis with this consequences are arbitrated mostly through the discharge of endogenous CRH.[55] Besides, it also shown the efficient action of ACh and the discharge of hypothalamic CRH are enhanced in affecting disorders.[53,56,57]

II) Effect on Growth

In individual, linear growth and ultimate mature stature depend on several aspects. These comprise inherited charter,[54] nutrition,[53] systemic illness,[54,56] hormones[58] and psychosocial atmosphere.[59] Physical stature, intellectual and behavioural advance consequently influenced by stressful psychosocial atmosphere.[60,61] Psychosocial dwarfism alias mistreatment dwarfism be an individual state happening due to normal care withdrawal which might perform as an affecting stressor[59] and such condition is categorized with three main reversible destructions which are postponed physical maturation, retardation of

intellectual age, postponed social maturation.[58,59,62] It is a generally speculated that in response to chronic stress, glucocorticoids and opioids secreted inhibit pituitary growth hormone discharge on the pituitary point and decline objective tissue receptivity to growth hormone, somatomedin-C or other growth aspects. Also it has been reviewed that the central administration of CRH declines GH discharge which proposed likely part of endogenous CRH in the variation of GH discharge all through strain.[61,63]

III) Effect on Immunity

The main effectors of the stress reaction put forth several and composite effects on the immune aptitude during stress. Immunologic function can be influenced by the HPA axis during diversity of means comprising, CRH-arbitrated activities on the somatostatin discharge by following reticence of growth hormone.[65] Generally, review of on immunologic studied, consequences of HPA axis are interceded via glucocorticoids.[63,64] Glucocorticoids usually exert immunosuppressive and antiinflammatory consequences[63,64] comprising the reticence of leukocyte passage, intrusion with cell interceded immunity with increase in suppressor T-cell role.[23,55,62,64,65] In various cases, glucocorticoids increase definite elements of immune reaction, comprising the role of precise distinguished copy of lymphocytes.[66]

It has been shown that CRH neuron of HPA axis contribute in a negative response circle, making pituitary adrenal commencement with linked glucocorticoid arbitrated immunosuppression in reply to inflammatory peripheral arbitrators reactions.[62,64] From the observations, it indicated to patients by means of main despair illustrate immunosuppression due to hypercortisolism.[55,67] Norepinephrine is consideration to have a diversity of effects on the immunologic response.[66] The HPA axis sequentially restrains the immune or

inflammatory reaction mainly by means of enhancing in glucocorticoids discharge. This glucocorticoid- arbitrated immunosuppression might protect intense inflammatory or immune responses through severe strain.

IV) Effect on Reproductive system

The condition of frightened homeostasis made by means of bodily or disturbing strain is extensively documented the same as a reflective disruptive feature in reproductive function. Reports have been shown that females in anxiety show postponed puberty, need of behavioural approachability, ovulation malfunction or embryo implantation, impulsive termination or increased newborn death.[42,66-68] Reports on males may perhaps demonstrated the testosterone discharge repression, spermatogenesis and libido.[42,61,68,69] It has been reviewed that suppression of reproduction is come into view to be reasoned via a number of hormones discharged all through strain for example CRH, ACTH, beta-endorphin and glucocorticoids on HPA axis function.[61,69-73]

The mechanisms of stress consequences on reproductive role are not completely revealed, however the probable sites are associated during stress, for examples, include: 1) decline in circulating levels of LH and sex steroid which might be due to centrally arbitrated reticence of GnRH discharge through hormones discharged resultant in diminished sexual behaviours in males and need of behavioural approachability as well as absolute ovarian idleness in females[73-76]; 2) at the gonadal point, testicular Leydig cell function directly inhibited via glucocorticoids because of glucocorticoid provoked decrease in testicular LH receptors.[72,77]

V) Effect on sleep

It is supposed that sleep is a neural condition during which combine of assertive recollections are happening.[78] Stress due to sleep deficiency enhances the homeostatic vigour to sleep among resultant alters within the proinflammatory cytokines as well as glycogen levels. Also lessened sleep is linked through enhanced risk for obesity, which signifies elevating probability of cardiovascular disease in addition to diabetes. Among diabetes, there is also lesser cognitive task with raised despair[79] and enhanced threat meant for Alzheimer's disease.[80,81] Depressive infirmity is approximately commonly linked by means of distressed sleep.[78,82] Therefore, there are associations not merely flanked by the manifold, networking intermediaries so as to concerned in allostasis and allostatic heap, but also overlaps i.e., co morbidities for instance; diabetes, hypertension, cardiovascular illness and despair to linked through severe strain and by means of deregulation of the systems that usually endorse allostasis or adaptation.

VI).Effect on Memory

Stress effects on memory comprise interference with an individual's capability to set memory and the aptitude to recover in order.[83,84] and might cause acute and chronic alterations in certain brain areas which might cause lasting obliteration.[85] Excess discharge of stress hormones often obliterate long-term delayed recall memory; in particular, the hippocampus, prefrontal cortex and the amygdala are pretentious.[85,86] Glucocorticoids, which are responsible for negatively affecting long-term and delayed recall memory, create probable damage the deeds of anxiety in brain reminiscence course.[87] Cortisol is recognized biomarker for strain.[88] In natural conditions, the hippocampus controls cortisol production through negative feedback since it has several receptors that are receptive to anxiety hormones. On the other hand, an overload of cortisol can damage the capability of the

hippocampus to both set and recollect recollections.[84] It has been shown that the activation of the anterior pituitary gland's release of ACTH, during stress, which in turn triggers the different region of adrenal gland towards dispose of cortisol into the bloodstream. Cortisol binds to cells of brain's hippocampus and this binding in-fact interrupt the memory forming course. Finally, if stress prolongs, the synaptic areas worsen, making the impairment permanent.[87] In recent years a significant advances made in understanding the stress consequences on reminiscence. For example, it has turn out to be gradually apparent so as to stress influences not only hippocampus-dependent memory however also striatum-dependent memory besides the communications between manifold memory systems.[88]

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CHAPTER – 2

REVIEW OF LITERATURE

2.1. Anti stress drugs

Antidepressants are regularly used for the dealing of major depressive disorder, which is one of the most important worldwide healthiness confronts; however, in the scientific literature, there remains significant disputes regarding both their efficiency as a group and the potential divergences in efficiency and acceptability between individual drugs.[1] Among the advertising of novel antidepressants and mounting numbers of tests published each year, a rationalized methodical review and system meta-analysis was necessary to synthesize the proof in this imperative clinical area. The antidepressants are a group of medicines employed to indulgence the signs of despair disorders by précising chemical inequities of neurotransmitters in the brain. Chemical inequities might also be on expenses for alters in mood and actions. According to etiologic of depression, the chemical inequities description may put forth a significant effect on treatment looking for activities plus the structures that are fashioned and preserved for such dealing.[2,3]

Antidepressants are helpful to treat several conditions of moderate or severe and can be employed alone or jointly with substitute medicines. It has been suggested that the main antidepressant medicine efforts to fine during early phase of treatment. Though, if it does not reduce the signs, it causes unfavourable consequences on the individuality.[4] For the resolution of antidepressant drug sulpiride in pharmaceutical formulation and plasma, several methods have been developed.[5-9] Realistic use of antidepressants that includes all possible profits and difficulties consists in aiming their claim to the most severe and unrelenting cases of despair, limiting their employ to the shortest probable period and minimizing their use in anxiety disorders unless presence of main depressive disorder or other treatments have been ineffectual.[10,11] The acute mechanisms of action of antidepressant drug's information guide to the common assurance to each effectual antidepressant

medicines perform through boosting the activity of serotonergic or noradrenergic system of brain. Nevertheless these medicines ought to be specified for as a minimum quite a few weeks for their antidepressant performances to become obvious.

Although quite a few decades of research with many hopeful escorts, the drugs influence in the brain that lie beneath their therapeutic exploits linger uncertain. The mainstream of antidepressant medicine finding attempts during earlier times of decades have focused on discovering additional selective serotonin or noradrenaline receptor agonists or antagonists, which might cause performances similar to those of the already accessible medicines. Such endeavours are still in progress with some promising leads.

2.2. Review of literature of some drugs reported with antistress effect

An inventory of anxiety medicines consist of several types of medicines including antidepressants, antipsychotics, beta blockers and benzodiazepines. Food and Drug Administration (FDA) endorsed the antianxiety medicines comprising all drugs for anxiety disorders treatment plus those frequently advised off-label.[12]

Four main categories of medications are employed in the anxiety disorders treatment:

2.2.1 Selective Serotonin Reuptake Inhibitors

Selective serotonin reuptake inhibitors (SSRIs) are largely employed to look after a numeral of other mental health situations among obsessive compulsive disorder, panic disorder severe phobias and generalised anxiety disorder. To treat other state of affairs for instance early ejaculation, premenstrual syndrome, fibromyalgia and irritable bowel syndrome, infrequently SSRIs can be employed. It is known that the SSRIs activate by raising the levels of serotonin, a neurotransmitter, in the brain. Following, transporting a

message, generally nerve cells reabsorbed (term as reuptake) serotonin. SSRIs role is inhibiting or blocking reuptake which indicates additional serotonin is obtainable to bypass more messages among nerve cells. For instance, some of the more generally recommended SSRIs include: Prozac, Sarafem, Rapiflux, Selfemra (fluoxetine); Pexeva, Paxil, Brisdelle, Paxil CR (paroxetine); Luvox, Luvox CR (fluvoxamine); Lexapro (escitalopram); Celexa (citalopram); and Zoloft (sertraline).

2.2.2 Serotonin-Norepinephrine Reuptake Inhibitors

Serotonin and norepinephrine reuptake inhibitors (SNRIs) are a category of medicines that are helpful in treating despair. Occasionally SNRIs are too employed to indulgence other situations for instance nervousness disorders and extended term aches. SNRIs reduce despair through forcing neurotransmitters employed to correspond among brain cells. Similar to majority antidepressants the role of SNRIs is eventually implementing alterations in brain chemistry and communiqué in brain nerve cell synapses to assist reduce despair. SNRIs ease symptoms by blocking the reabsorption of the neurotransmitters serotonin (ser-o-TOE-nin) and norepinephrine (nor-ep-ih-NEF-rin) by brain's nerve cells. The FDA has agreed these SNRIs to indulgence despair, for instance, some of the generally recommended SNRIs consist of Desvenlafaxine (Pristiq, Khedezla); Duloxetine (Cymbalta) Levomilnacipran (Fetzima); and Venlafaxine (Effexor XR) .

2.2.3 Benzodiazepines

Benzodiazepines (benzos) are psychoactive, this category of medicines are the most recommended medicines worldwide.[13] As the name urged, the core chemical structure of benzodiazepines is a mixture of benzene and diazepine rings. The benzodiazepines designated intended for curing of general nervousness disorders, panic disorders

(agoraphobia) as well as skeletal muscle relaxation.[14,15] Though, the precise mode of benzodiazepines deed is not known, they appear to endeavour through influencing neurotransmitters in the brain. Based on the literature, benzodiazepines are claimed greatly because of their efficient in endorsing relaxation and decreasing muscular strain, other physical indications of nervousness and a variety of psychological and neurological disorders due to its consequences on the neurons that activate stress and anxiety responses.

Gamma-amino butyric acid (GABA), one of these neurotransmitters, that suppresses the activity of nerves and it is believed that the main pharmacological effects of benzodiazepines are the consequences from their binding and modulating of the $GABA_A$ receptor.[16,17] These benzodiazepines might act by increasing the effects of GABA in the brain. GABA decreases the action of nerves in the brain and enhancing the effect of GABA results decrease in brain activity. for instance, some of the generally recommended benzodiazepines include: Alprazolam (Xanax); Chlordiazepoxide (Librium); Clonazepam (Klonopin); Diazepam (Valium); Lorazepam(Ativan); Midazolam (Versed); Oxazepam (Serax); Clorazepate (Tranxene); Estazolam (Prosom); Temazepam (Restoril); Flurazepam (Dalmane); Clorazepate (Tranxene); and Triazolam (Halcion).

2.2.4 Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) be a group of antidepressant medicines and these generally known to be by increasing the levels of two neurotransmitters, norepinephrine and serotonin and block the action of another neurotransmitter, acetylcholine. These cyclic antidepressants relieve depression through forcing chemical messengers employed to correspond among brain cells. These antidepressants block the reuptake of the messenger's serotonin and norepinephrine by raising the levels of these two messengers in the brain. In

addition to relieving depression, it is believed that these antidepressants also cause sedation and somewhat block effects of histamine. Some of the more commonly prescribed TCAs include: Clomipramine (Anafranil); Amoxapine (Ascendin); Amitriptyline (Elavil); Desipramine (Norpramin); Nortriptyline (Pamelor); Doxepin (Sinequan); Trimipramine (Surmontil); Imipramine (Tofranil); and Protriptyline (Vivactil).

2.2.5 Ketamine

Ketamine is a class III designed drug, generally known as Ketanest or Ketaset and is accepted to employ in hospitals and other medical settings as an anaesthetic. Other drugs in this class comprise hallucinogen, phencyclidine (PCP), dextromethorphan (DXM) and nitrous oxide. Details indicated a possible employ of ketamine as a therapeutic implement for the management of despair when administered in lesser doses.[18] Ketamine increases descending inhibiting serotoninergic pathways and can exert antidepressive effects. It can be explained that the effect of ketamine as painkiller by the prevention of central sensitization in dorsal horn neurons plus by the inhibition on the synthesis of nitric oxide. Studies have been elucidated that ketamine is interacts with known receptors of N-methyl-D-aspartate, opioid, monoaminergic and muscarinic, however, distinct other general anaesthetic agents, it does not interact with GABA receptors.[19]

2.3. Targets for the actions of antistress drugs:

It has been reviewed that the main site of exploit of drugs by means of antistress activity appears to be the hypothalamic-pituitary adrenal (HPA) and their secondary sites of effecting appear to be on metabolism, liver, immune components and cardio vascular systems. Stress, is a defensive reaction to exterior factors, induces the configuration of endogenous messenger stuffs for example prostaglandins, cytokines, catecholamines, nitric

oxide and platelet activating factor which consecutively trigger other factors that might also compensate anxiety, on the other hand persuade or facilitate illness. According to stress perception, the turn on mechanism triggers the sympathoadrenal coordination and more than the extended as well make triggers the HPA collectively with different controllers of cell and organ rationale. Neutralizing this is the turn off coordination which defends cells and organ coordination and consequently whole organism from destructing in excess of response to stress. Switch-off system comprises antioxidant enzymes that down regulate a range of features of the immune response, corticosteroids and anti-inflammatory intermediaries. Further antistress activity of medicines can be represented as representatives that minimize the host-defence system reactivity to a range of stressors by assisting to reinstate regular homeostasis.[20]

The information from the literature and it has been revealed that the mechanism of the antistress activity of various emphasized drugs is related with their consequences. The intermediaries of stress reaction particularly on the cortisol formation, NO and stress activated protein kinase pathways.[21] The appearance of molecular chaperons Hsp70 and Hsp16 proteins engaged in stress influenced cytoresistance and in acclimatization to reiterated exposure to preliminary concerner.[22,23] NO formation is able to effectively reduce the creation of cellular energy in anxiety by the mechanisms of inhibition of mitochondrial respiration linked with constitutive NO synthase (NOS) isoforms or through the irreversible inhibition of P450, linked with inducible NOS (i-NOS)[24] or another type of glycolysis reticence via alteration of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) group, which is a key enzyme engaged in the formation of ATP.[25] In this perspective, medicines by means of antistress results protect the anxiety tempted enhance in NO and the connected

decline in formation of ATP therefore consequential in increased performance and endurance.[26]

2.4. Review of literature of current used drugs

2.4.1. Alprazolam

Alprazolam (Figure 2.1), be industrialized, amongst earlier trade names as Xanax and Xanor. This drug be the largely utilized member of the benzodiazepines family,[27] assumed to be the most toxic drug [28] and has a long residence time in the body.[29] Alprazolam structure is consisting of four distinct benzene, chlorobenzene, diazepine and triazole ring structures. It is one of the most generally approved squat performing benzodiazepines and validated by the modest probability of its accretion and through the comforting consequences of numerous doses.[30] Several studies have been emphasized that the nature consequences of this drug might be due to unusual connections with receptor of benzodiazepine [31] or in the direction of the participation of other mechanisms similar to an interaction with adrenergic,[32,33] adenosinergic[34] and serotonergic systems[35] or an antagonistic action of the platelet activating factor.[36]

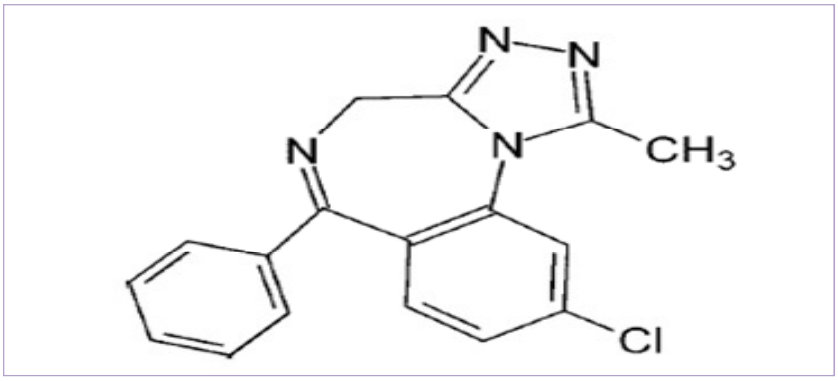

Figure 2.1. Chemical Structures of Alprazolam [87]: (8-chloro-1-methyl-6-phenyl-4H-[1, 2, 4] triazolo-[4,3-a][1,4] benzodiazepine, Molecular Formula: $C_{17}H_{13}C_lN_4$, Molecular Weight: 308.76

Alprazolam is a drug of choice for the management of anxiety disorder and panic disorders. The most general side effect of alprazolam is sedation which is directly related to drug's dosage.[37] Studies on rodents, it has been indicated that the alprazolam found suppressed aggression and muricide by olfactory bulbectomized rats,[38] habituation effects on aggressive mice and monkeys[39] and also reduce shock-induced aggression in mice.[40] Mice exposed prenatally to alprazolam results increased individual rather than group activity and male aggression.[41] Reports revealed, alprazolam, similar to other benzodiazepines, it has antianxiety, anticonvulsant and muscle relaxant properties,[42] conversely, in dissimilarity to other benzodiazepines, it is made known in placebo controlled trials to have antidepressant properties.[43-45]

The effectiveness of alprazolam defends against the unfavourable results from diverse types of stressors [46,47] and is broadly prescribed for the treatment.[48] In addition, alprazolam relieves a few of stress consequences on the immune system [46,49] and defends aligned with oxidative injure.[50,51] Besides to the benzodiazepine objectives GABA, spectroscopic observations have anticipated its binding to a variety of key bio molecules comprising haemoglobin, [52] albumin [53,54] and even DNA.[55]

2.4.2. Fluoxetine

Fluoxetine, (Figure 2.2), is an antidepressant medicine employed for the treatment of unipolar mental despair. Amongst others, fluoxetine, a first molecule of a new generation of antidepressants, is besides branded by other names Prozac and Sarafem and be generally recommended SSRI antidepressant drug.[56] To advance in understanding of its genuine impact in the psychiatric vicinity, several controlled experimental studies and meta-analyses were carried out on this antidepressant drug.[57] This antidepressant has been shown

efficiently to treat stress disorders[58] and defends from the damaging effects of different types of stressors.[59,60] Reports shown on stress consequences indicates that fluoxetine attenuates effects of stress on haematological parameters,[61] obsessive compulsive disorders [62] and prevent from oxidative damage.[63]

Fluoxetine has come out as the dealing of selection for despair owing to its safer report, less side consequences and progressed acceptability contrast by means of the leading tricyclic antidepressants.[64] For example, it has been shown to have similar efficiency to tricyclic antidepressants, though with fewer cardiovascular and anticholinergic side effects.[65,66] Observation from studies, fluoxetine being a SSRI does not considerably inhibit norepinephrine and dopamine reuptake at remedial doses, though at higher dose levels in rats, have been revealed to persuade a considerable enhance in synaptic norepinephrine and dopamine.[67] Thus, it is hypothesized that these results might be mediated by 5HT2C receptors which are inhibited by higher concentrations of fluoxetine.[68] Nevertheless, the core methods of its beneficial effectiveness remain uncertain.

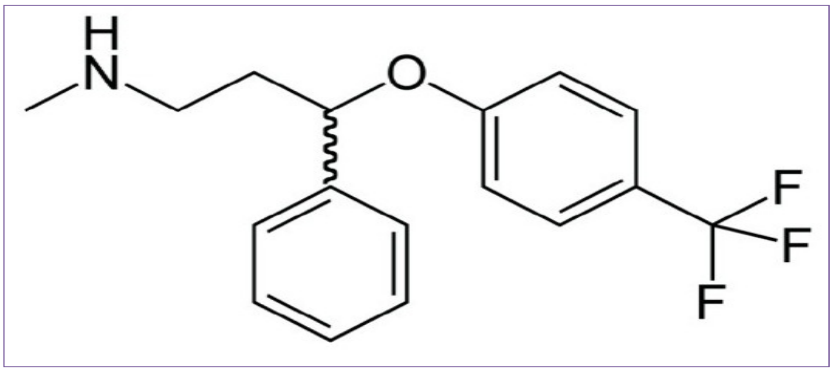

Figure 2.2. Chemical Structures of fluoxetine [88]: (dl)-N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy]-propan-1-amine, Molecular Formula: $C_{17}H_{18}F_3NO$, Molecular Weight:302

2.4.3. Buspirone

Buspirone (Buspar, Figure 2.3), is an anxiolytic drug mainly or augment antidepressants employed to treat prevalent anxiety disorder.[69] Unlike predominantly anxiolytics, its compound structure and method of exploit are exclusively not associated to those of the benzodiazepines and its competence is not similar to that of components of the benzodiazepine family.[70-71] Reports revealed that buspirone perform like as antidepressant activities [72-74] and commonly proposed that remedial effects of buspirone are not because of its instant action on the 5-HT system, but quite adaptive modification that happen on following prolonged treatment.[75]

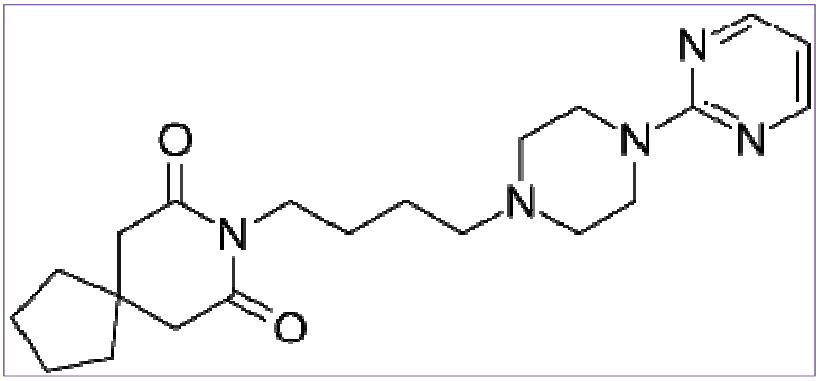

Figure 2.3. Chemical Structures of Buspirone [89]: [8-(4-(4-(pyrimidin-2-yl) piperazin-1-yl) butyl)-8-azaspiro[4.5]decane-7,9 dione}, Molecular Formula:$C_{21}H_{31}N_5O_2$,M.W Weight:385.512 g/mol

This anxiolytic drug acts on the CNS, particularly, on certain regions of mammalian brain and it has been confirmed that the anxiolytic exploit of buspirone is associated to its influence on the synaptic transmission of signals moderated by serotonin receptors localized in the brain neuronal membranes.[76] Some studies on animal models have proposed that

buspirone acts postsynaptically on the 5-HT_{1A} receptor [77,78] and in some studies disputing that buspirone be able to act presynaptically.[79,80] Nevertheless, the primary mechanisms of its remedial efficiency remain uncertain, but might execute by motivating serotonin type 1A receptors on nerves in consequence altering the chemical messages in order to nerves receive.

Disparate benzodiazepines, buspirone doesn't interact with the GABAA receptor complex.[81] In accordance with animal studies it is established that, in precise brain areas, buspirone dose reliant declines serotonin levels as enhancing the levels of dopamine and norepinephrine. Consideration to the major consequences of buspirone are arbitrated through its dealings by means of serotonin 5-HT_{1A} receptor with high affinity[81] or might be arbitrated through oxytocin discharge secondary to 5-HT_{1A} receptor agonism.[82,83] In animal models, it has been observed that a main metabolite of buspirone, 1-(2-pyrimidinyl) piperazine (1-PP) is recognized to act as a effective α2-adrenergic receptor antagonist which might be accountable for the enhanced noradrenergic and dopaminergic activity.[84,85] Besides, buspirone moreover has very weak and perhaps clinically insignificant affinity for the α1-adrenergic receptor, though, the noteworthy and selective intrinsic effectiveness on the α1-adrenergic receptor expressed is species dependent manner.[86]

2.5. Scope of Investigation

Stress has an impact to make changes in a range of anatomical, physiological, biological responses consist of psycho-physiological responses. During stressful activities, our bodies discharge chemicals (adrenaline and cortisol) into the bloodstream which activate the 'fight-or-flight' response. In reaction to stressors, a sequence of behavioural, neurochemical, and immunological alterations happen that should to provide in an adaptive

capability.[90,91] Stress generate life frightening dealings in premature progress and can consequence in extended expression outcomes and claimed to be involve d in the etiopathogenesis of a range of illness form and consequential disorder might differ depending upon nature, potency and the length of a particular stressor and the strain or sex discrimination of the themes.[92] It has been broadly studied on the stress effects on different organs of the body in both humans and animals and observed that relentless anxiety long-lasting weeks or months be able to damage cell communiqué within the brain's certain region.[93]

In rodents, an accepted neurobiological implement to persuade anxiety is the disclosure to movement restraint, which even though comprising a corporal interfering, cause psychological anxiety while applied in a persistent mode in addition to escorts to the appearance of despair resembling behaviours for instance anhedonia, intellectual vulnerability and nervousness. In same manner, the movement restraint can be accomplished with restraint in small wire mesh cages or with immobilization in flexible artificial rodent immobilization plastic bags.[94,95] From the description, together methods are believed as corresponding with the exemption of the intensity factor, which is hypothetical to be elevated in the case of complete immobilization.[96] Restraint stress as well as immobilization procedures are one of the most generally used protocols to persuade stress associated behavioural, biochemical and physiological alterations in laboratory animals.[97] Immobilization representations create an unavoidable physical and mental stress with a low tempo of adaptation. Following restraint or immobilization stress, animals show signs of higher levels of anxiety in the tests.[98]

To evaluate the antistress activity of compounds which belongs to synthetic origin or natural origin, different animal models have been using and investigate allied with focusing on recognition, enumeration and categorization of the damaged tissue meant for assessment of diverse beneficial modalities and realizing the methods of anxiety reaction.[99] Stress is able to be revealed because some inducement that makes an inequity in the homeostasis procedures.[100] The blood is one of the main homeostatic systems of the body preserving regular feasibility, integrity and adaptive responses. The efficient state of the blood systems changes animatedly according to the nature, effectiveness and period of exposure to exterior factors. Evidences from several studies on humans and animals, it is revealed that a significant changes in physiological and biochemical courses during the modelling of emotional stress, for instance, the nervous and blood systems are the quickest to respond to emotional stimuli. Stress and emotional responses influence the immune system is by means of total blood combine, comprising the hematopoietic system, leukocyte outline otherwise biochemical stress indicators.

It has been shown that the antioxidant defense system is also damaged by stress.[101] Oxidative damage is a documented consequence of strain that has been involved in the mood pathogenesis and nervousness disorders.[102,103] Vitamin C is well recognized an antioxidant and needed of by every mammalian cells meant for appropriate performing to manage a range of biochemical reactions.[104] Another a type of lipid soluble antioxidant, i.e. Vitamin E, is most effectual sequence splitting an antioxidant in the cell membrane where it defends membrane fatty acids from lipid peroxidation.[105] In addition to this, other studies elucidated that the levels of serum cortisol be a dependable marker of anxiety reactions in animals.[106,107]

On reviewing the available information on exposure to restrained stress standpoint, it was observed that there was diminutive update concerning about persuade of antidepressants viz., alprazolam (benzodiazepine anti-anxiety agent), fluoxetine (a selective serotonin reuptake inhibitor) and buspirone (a non-benzodiazepine anxiolytic drug) on assessment of antistress effects in restrained rats. In general, for the most part, studies were centred on their amendment of the immune role quite than the structure. Hence, it was considered to study the antistress consequences of these generally employed drugs particularly on food consumption, gravimetry, organ weight, histopathology, haematological parameters, serum cortisol as well as levels of antioxidant vitamin C and E in restrained stress albino rats at their maximum therapeutic doses.

Further, extensive research has been done on the consequences of stress over the various hormones levels in the blood but information about reversible assessment of restrained stress after withdrawal in rats is lacking. Therefore, the present study was intended to evaluate the consequence of restrained stress and its withdrawal probable amendment on gravimetry, organ weight, histopathology, haematological parameters and serum cortisol level in restrained stress rats. Thus our entire efforts have been directed for studying these novel commonly used antistress drugs to compare for their protective effects in restrained stress for 21 days, while, in contrast, the withdrawal of stress designed meant for remaining 21 days might perhaps neutralize restrained stress provoked injure so as to escort to oxidant antioxidant equilibrium and modify HPA axis.

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CHAPTER – 3

HYPOTHESIS AIMS AND OBJECTIVE

HYPOTHESIS

1. It may be expected that withdrawal from the stress has a beneficial recovery of pathophysiological changes due to stress.

2. Drugs to be specific; alprazolam, buspirone and fluoxetine counterbalance the stress induced alteration of pathophysiology.

3. A relation between antistress elements like serum Vitamin C and Vitamin E concentration may be used as predictor for clinical evaluation in associated with stress patients

4. Antistress drugs of alprazolam, buspirone and fluoxetine have potential beneficial consequences on stress.

AIMS AND OBJECTIVES

1. The present study is undertaken to evaluate the effect of stress on various biochemical, histopathological and haematological changes in male albino rats.

2. The present study also made on assessment of stress withdrawal consequences on biochemical, histopathological and haematological changes in male albino rats.

3. Additionally, study also designed to examine the consequences of restrained stress and efforts have been focused directed for studying antistress drugs; alprazolam, fluoxetine and buspirone to compare for their protective effects in restrained stress 21 days in continuance with stress.

OBJECTIVES OF THE STUDY

A) Considered to study the effect of restrained stress and its withdrawal or protective effects of antistress drugs for the possible alteration on remaining 21 days in continuation with stress particularly on

i. Clinical observations

ii. Food consumption

iii. Gravimetry

iv. Organosomatic index

v. Histopathology changes -whole brain, liver, kidney and testis

vi. Haematological parameters including PCV, Hb, RBC, MCV, MCHC, MCH, TWBC, Platelets, Neutrophils, Lymphocytes, Eosinophils and Monocytes.

vii. Biochemical parameters Serum cortisol and levels of antioxidant vitamin C and E

B) Purpose of the present study was address the issues to support to the existing information pertaining the stress and antistress drugs may conceivably a vital means connecting the defensive pharmacological consequences over restrained stress induced haematological, biochemical and histopathological adverse change.

CHAPTER – 4

MATERIALS AND METHODS

4.1. Animals and Ethics

Colony strain healthy mature Wistar strain male albino rats weighing 175-225g were used in our experiments. Rats were fed by laboratory store diet (Hindustan lever, Mumbai, India) and water *ad libitum*. They get acquainted meant for a week to the laboratory environments at 22-24°C, relative humidity (55%) and a 12 h light: dark (circadian) cycle. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura and the experiments were performed as per norms of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA).

4.2. Drugs, Chemicals and Reagents

Alprazolam (ALPRAX tab) from Torrent Pharmaceuticals Ltd., Ahmadabad, INDIA, Fluoxetine (fludac) from Cadila Pharmaceuticals Ltd., Ahmadabad, INDIA and Buspirone (Buspin) from Intas Pharmaceutical Ltd., Ahmadabad, INDIA were obtained as commercially available pharmaceuticals. Cortisol ELISA Kit (Ref; EIA-1887) procured from the DRG International Inc., USA. All other chemicals and solvents utilized in the tests were of analytic quality and acquired from local commercial sources.

4.2.1. Preparation of Alprazolam solution

The preparation of alprazolam solution was prepared the same as method explained by Lau and Hetherington[1] and Anwar et al[2] by means of commercially obtainable alprazolam (ALPRAX tab, 1mg). Briefly, five tablets contain 1mg alprazolam was dissolved in 50 ml of 1.2 N HCl, diluted with saline solution (0.9% NaCl) and selected dose level of alprazolam was employed for further stress experimental investigations.

4.2.2. Preparation of fluoxetine-hydrochloride solution

The preparation of fluoxetine solution was prepared following the technique of Brandes et al.[3] by means of commercially obtainable fluoxetine (fludac tab, 20mg). Briefly, capsules were opened and the powder enclosing 20 mg of active pills as the soluble HC1 salt and 205 mg of insoluble cellulose type filler per capsule was included to sterilize distilled water (2ml/capsule). The resultant suspension was transferred into a 50ml conical polypropylene tube and centrifuged at 2800xg meant for 20 mins at room temperature. The supernatant, containing 10 mg/ml fluoxetine-HCl was decanted off and stored at 4°C until used. Then, prior to use, the selected dose level of fluoxetine was employed for further stress experimental investigations.

4.2.3. Preparation of buspirone-hydrochloride solution

Using commercially available buspirone (Buspin, 10mg), the preparation of buspirone solution was prepared following the modified methods described by Brandes et al.[3] and Abdel-Ghani et al.[4] Briefly, tablets of brand buspirone hydrochloride (Buspin) label assert were opened and the powder, containing 10 mg of active pills, powdered, mixed well and subsequently dissolved in sterilize distilled water (2ml/capsule). An opaque solution was quivered well, filtered through a filter paper to get an obvious solution and was decanted into a 50ml conical polypropylene tube and centrifuged at 2800xg meant for 20 mins at room temperature. The supernatant, containing 10 mg/ml buspirone-HCl was decanted off and stored at 4°C until used. Then, prior to use, the selected dose level of buspirone was employed for further stress experimental investigations.

4.3. Experimental study

4.3.1 Stress Procedure

Acclimatized animals (n=36) divided at random into six groups of six animals each were reserved in each metabolic wire mesh restrainers cage (60cm x 30cm x 20cm) was wooden base fabricated by stainless steel turned to the base (Figure 4.1). A pad lock and clasp helped to safe the rat in the restrainer.

- Group I. Untreated control rats reserved uninterrupted in the metabolic cage all through the experimental duration intended for 42 days
- Group II. stress induced rats were strained daily for 6hrs in wire mesh restrainer intended for 42 days.[5]
- Group III. Rats were stressed intended for 21 days by means of observance in mesh restrainer and then retaining animals in normal cages for residual 21 days designed for stress withdrawal.
- Drug treated groups, to be precise, rats were stressed intended for 21 days and then treated with alprazolam (Group IV, 5mg/kg body weight, intraperitonealy, BW, IP), [6-8] buspirone (Group V, 12mg/kg BW, IP) [9-11] and fluoxetine drug (Group VI, 20mg/kg BW, IP), [12-14] respectively for residual 21 days in continuation with stress.

Proceeding to use, each drug solutions were arranged instantly and injected in a quantity of 2 ml/kg BW and drug's administration were done every day at 9:30 am through all duration of stress application. Drug doses and the testing time intervals were selected based on those reported in literature and conducted in our laboratory (Figure.4.2).

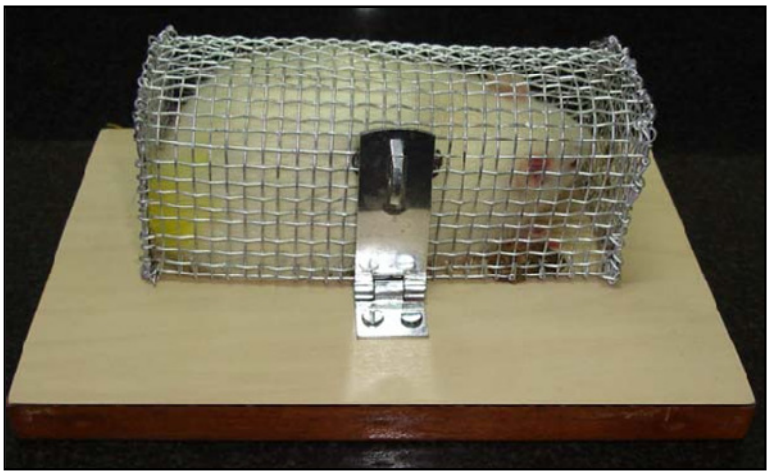

Figure 4.1. An image of rat in metabolic wire mesh restrainer cage

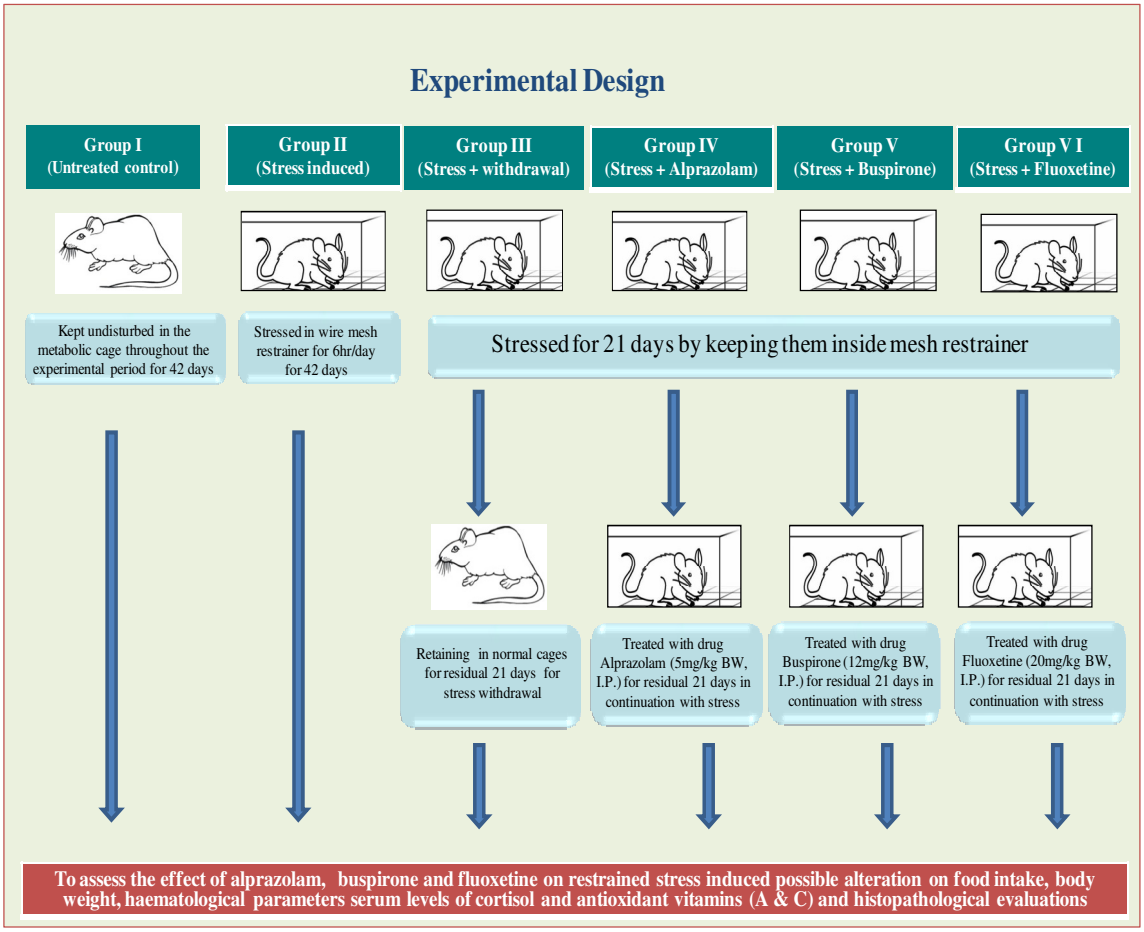

Figure 4.2. Schematic illustration of experimental design on following initiation antistress drugs and recuperation by withdrawal possible in continuation with restrained stress rats.

4.3.2. Clinical observations:

All animals were monitored every day for mortality, morbidity and physical assessments were specified weekly. The clinical remarks that are recorded include assessing the animal's general appearance and determining if behaviour is affected.

4.3.3. Food consumption:

The individual food consumption documentation of the animals was maintained all through the whole experimental procedure. The food left over and spilled rat feeds by the rats were accumulated daily and weighed. Discrepancy among food supplied and residual along with spill out food by the animal in 24 hours was obtained the same as food consumption.

4.3.4. Gravimetry

The BW of all the rats was documented on the day 1 of stress, alternate 10^{th} day and the day of sacrifice (i.e., 42^{nd} day). Percent BW gain was determined in experimental groups by means of a ratio of final BW to the initial BW. The ratio of organ weight to rat BW prior to sacrifice (i.e., final BW) is considered as organ somatic index. At the end of the final day subsequent to an overnight fast, all animals were sacrificed by cervical and relative organs such as whole brain, liver, kidney, adrenal gland along with testis were dissected out, blotted free of mucus and weighed to the adjacent milligram.

4.3.5. Haematological parameters

From the retro orbital plexus, blood was collected in centrifuge tubes, maintained at room temperature meant for about 2 hrs, followed by centrifuged at 1500×g for 15 min to collect serum. Using SYS MAX-35 automated cell counter machine, serum was then used for analyzing the haematological parameters[16] including packed cell volume (PCV),

Haemoglobin (Hb), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), total white blood cell (TWBC), Platelets, Neutrophils, Lymphocytes, Eosinophils and Monocytes.

4.3.6. Biochemical parameters

Collected blood serum was employed meant for the evaluation of vitamin C level by Roe and Koether method,[17] vitamin E level by modified Baker and frank method[18] along with level of cortisol by the Enzyme-linked Immunosorbent Assay (ELISA) kit (DRG, USA) method.[19]

4.3.6.1. Estimation of Vitamin C

Principle

In presence of strong acid solution the ascorbic acid is oxidized to diketogluconic acid. This diketogluconic acid reacts with 2,4 dinitrophenyl hydrazine to form dinitrophenylhydrazone, which dissolves in strong sulphuric acid solution to turn out red coloured complex which be able to be determined spectrophotometrically at 500nm.

Reagents

i) 2,4-dinitrophenyl hydrazine ($C_6H_6N_4O_4$) in 9N sulphuric acid (H_2SO_4); ii) Activated Charcoal; iii) Trichloroacetic acid (TCA); iv) Concentrated H_2SO_4 (36 N); and v) Ascorbic acid ($C_6H_8O_6$) standard: 50 mmoles//l in double distilled water including 0.1 ml glacial acetic acid (CH_3COOH).

Procedure

Taken 0.4 ml of trichloroacetic acid is added to the 4.6 ml of serum sample to the tube and then to this100 mg of acid cleaned charcoal was added, mixed and filtered. In a separate tube, 1.0 ml of filtrate and 0.4 ml of dinitrophenyl hydrazin were added and incubated at 37ºC meant for 3hrs. Following incubation, tubes were cooled on ice bath, subsequently added 1.6 ml of sulphuric acid and tubes were kept at room temperature meant for 30 mins and read optical density (OD) at 520 nm. A standard curve was prepared by means of taking ascorbic acid in the range of 10-50 mmoles/l.

4.3.6.2. Estimation of Vitamin E

Principle

Vitamins E estimation method was based on previous Baker20 and Martinel21 methods by make use of 2.2'-bipyridal ($C_{10}H_8N_2$), ferric chloride ($FeCl_3$) and xylene($C_{24}H_{30}$). The composite of Ferrous ions produced in this result by 2-2'-bipyridal and it was determined by employing simple enzyme linked immunoabsorbent assay (ELISA) microplate (non-antibody coated) at 492nm.

Reagents:

i) Stock standard solution of α-tocopherol ($C_{29}H_{50}O_2$): 270 mg of α-tocopherol acetate diluted in 100ml ethanol and mixed systematically; ii) 2.2ˈ-Bipyridyl (0.12% w/v): 120 mg 2.2ˈ-Bipyridyl is dissolved and volume is made upto 100 ml with n-propanol and is kept in a brown bottle; iii) Ferric chloride (0.12% w/v): 120 mg $FeCl_3.6H_2O$ is dissolved in 100 ml ethanol and is also kept in a brown bottle. All these solutions are stable at room temperature. Working standard of α-tocopherol: 1 ml of stock standard solution was taken and the volume was made up to 100ml with ethanol to attain concentration of 27µg/ml.

Procedure:

To the centrifuge tubes, 750µl of ethanol (aldehyde free) and 750µl serum were added and marked as sample and for another tube, marked as a blank, 750µl of distilled water and 750µl of ethanol (aldehyde free) were added. All tubes were enclosed firmly and shaken forcefully for 30 seconds. By adding 750ml of xylene, again all tubes were enclosed firmly and shaken forcefully for an additional 30 seconds and centrifuged meant for 10 minutes at 3000 rpm. Then xylene layer was transferred in small sized test tubes. 50µl of 2.2'-bipyridyle solution was added, subsequent by 100µl of $FeCl_3$ solution to each test tube and waited for 2 minutes. The absorbance was measured at 492 nm. The serum α-tocopherol concentration of sample was achieved by means of standard curve and prepared by taking α-tocopherol in the range of 10-50 µmoles/l.

4.3.6.3. Estimation of Serum Cortisol

Principle

The DRG® Cortisol ELISA Kit is a solid phase enzyme-linked Immunosorbent assay (ELISA) which is based on the principle of competitive binding. In this, the microtiter wells are coated with a monoclonal antibody directed to an antigenic site on the Cortisol molecule. Sample's endogenous Cortisol competes with a Cortisol-horseradish peroxidase conjugate used for binding to the coated antibody. Following incubation the unbound conjugate is washed off. The concentration of Cortisol in the sample is inversely proportional to the amount of bound peroxidase conjugate. Subsequent adding of substrate solution, the intensity of colour produced is inversely proportional to the concentration of Cortisol in the sample.

Contents of the Kit:

Microtiter wells, anti-Cortisol antibody (monoclonal), standard (Standard 0-6) vials, Preservative Proclin, Enzyme Conjugate, Substrate Solution, Tetramethylbenzidine (TMB), Stop Solution and Washing Solution.

Assay Procedure

20µl of each standard, control and samples distributed into suitable microtiter wells, added 200µl enzyme conjugate into each one well and mix methodically meant for 10 seconds. Then incubated for 60 minutes at room temperature, quickly shake out the contents of the wells. Washing process followed by accurately rinsing the wells three instances with diluted wash solution (i.e., 400µl per each well) and stroked the wells sharply on porous paper to take out left over droplets. After that 100µl of substrate solution added to each well, incubated meant for another 15 minutes at room temperature. The enzymatic reaction was blocked by addition of 100µl of stop solution to each well and within 10 minutes read the OD at 450±10 nm with a microtiter plate reader.

4.3.7. Histopathological evaluations

The whole brain, liver, kidney, adrenal gland and testis of control and the experimental rats were dissected out and subjected to histopathological evaluations.[22] The microscopic study on routine stain (Haematoxylin and Eosin stain) was done and sections (5 µm thickness) were examined under compound microscope for histopathological changes in the architecture of tissues and their microphotographs were captured. If any changes in the cytoarchitecture was noticed.

Statistical analysis

Statistics were suggested as mean ± standard deviation of the mean. Statistical contrasts were carried out by means of one-way image result for Analysis of variance (ANOVA) employing the Graph Pad Prism software mode and pursued by post-hoc t-test. Values demonstrated are mean ± SEM (n=6). $P \leq 0.01$ is deliberated to designate a significant differentiation among experimental and controls.

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CHAPTER – 4

RESULTS

5.1. Clinical observations:

All the rats in groups control and other rest groups remained active and healthy with normal behaviour during the entire period of experimentation.

5.2. Food consumption:

Stress induced rats (group II), were strained daily for 6hrs intended for 42 days, exhibited a significant decrease in the final food consumption ($P \leq 0.05$) as compared to control (Group I), stress withdrawal group (group III) and drug treated groups that is group IV (Alprazolam), group V (Buspirone) and group VI (Fluoxetine) respectively, meant for residual 21 days in continuance with stress, revealed no changes in food intake. However, drug treated groups had some following days of variation in food consumption at different instances in the study, but this variation was insignificant to the on the whole food intake rates (Figure 5.1).

5.3. Gravimetry

The body weight (BW) of each rat was documented scheduled conduct day first and the day of sacrifice (i.e. 42^{nd} day). Percent BW gain was determined in experimental groups with a ratio of final BW to the initial BW. Stress induced rats observed to be tired with significant reduce in final BW. However, administration of drug alprazolam, buspirone and fluoxetine in rats meant for residual 21 days in continuance with stress or withdrawal of stress for residual 21 days demonstrated noteworthy progress in BW gain (%) when compared to stressed (Table 1; Figure 5.2a &b).

Group & Treatment	Initial BW(g)	Final BW(g)	Weight gain (% g)
I Control	212.5 ± 5.44	217.5 ± 4.23	2.36 ± 0.65
II Stress	211.7 ± 4.60	208.0 ± 4.55	1.89 ± 0.24
III Stress+ Withdrawal	210.8 ± 5.83	216.3 ± 4.18	4.10 ± 0.41*
IV Alprazolam (5mg/kg BW)	211.5 ± 2.45	215.8 ± 2.23	2.32 ± 0.23
V Buspirone (12mg/kg BW)	211.2 ± 2.39	215.5 ± 2.73	2.59 ± 0.77
VI Fluoxetine (20mg/kg BW)	211.2 ± 3.52	214.2 ± 1.08	2.96 ± 0.84*

Table 1. Effects of Antistress drugs on body weight in control and experimental rats (Values are expressed in SEM of 6 animals)

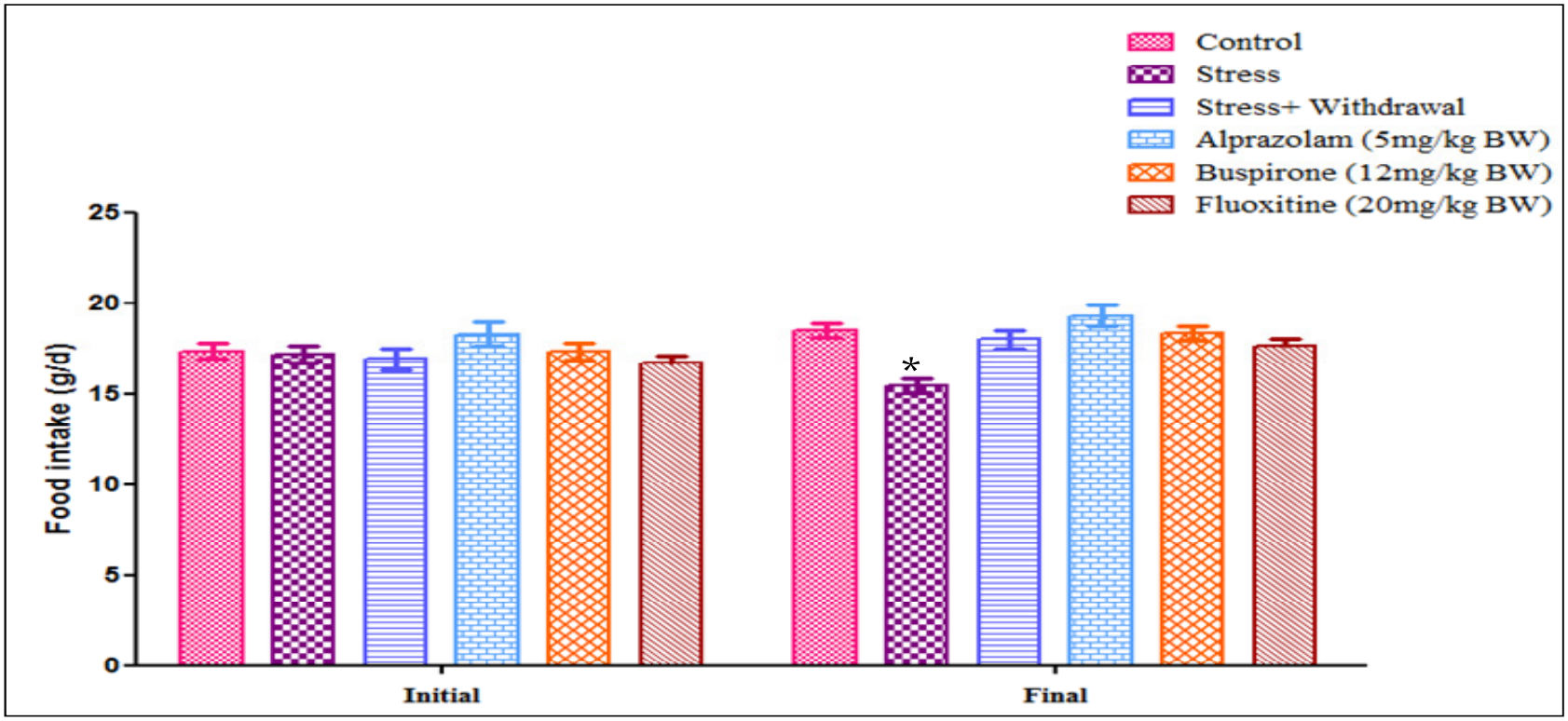

Figure 5.1. Effects of Antistress drugs on food intake in control and experimental rats. Values are expressed as mean ± SEM (n=6) and * indicates significant (P ≤ 0.05) compared to control

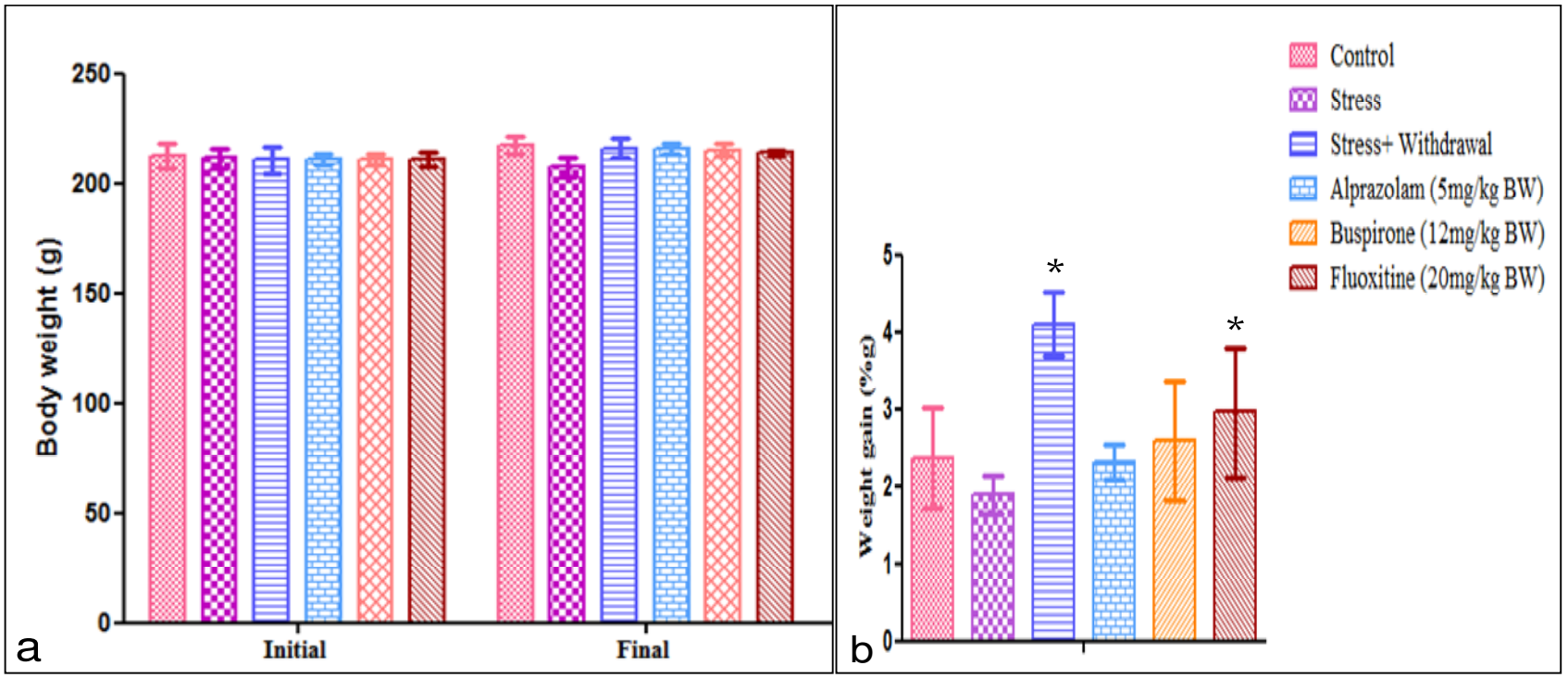

Figure 5.2a &b. Effects of Antistress drugs on body weights in control and experimental rats. Values are expressed as mean ± SEM (n=6) and * indicates significant ($P \leq 0.05$) compared to control.

5.4. Organosomatic index:

The virtual organ weights of control with experimental animals were revealed in Figure 5.3. Organ somatic index was determined by the proportion of organ weight to BW of rat prior to sacrifice (final BW). Gross examination of internal organs of control and treated groups III, IV, V and VI disclosed no obvious deformities. Organs like brain, liver, kidney, adrenal gland as well as testis of rats did not show any major alter in their weights, however, some alterations in the weight of few organs which were insignificant level when compare to the controls. However, the relative weight of organs weight of brain, liver, kidney, adrenal gland and testis exhibit a significant ($P \leq 0.05$) decrease in stress induced (group II) rats compare to all rest groups.

5.5. Haematological parameters

Haematological parameters of control as well as experimental rats were revealed in Figure 5.4. Stress induced rats explained statistically noteworthy ($P \leq 0.05$) decrease in Hb, total WBC and Platelets, whereas statistically major ($P \leq 0.05$) enhance in RBC, MCV, MCHC, neutrophils, lymphocytes, eosinophils along with monocytes when compared to untreated control rats. No considerable alters observed in the levels of MCH and PCV amongst all rest groups. Withdrawal of stress along with drug treated rats' demonstrated a notable ($P \leq 0.05$) improvement in lymphocytes count when compared to the stress group. However, there was an increase and decrease level of some haematological parameters in withdrawal of stress and drug treated groups; the difference was insignificant against the control.

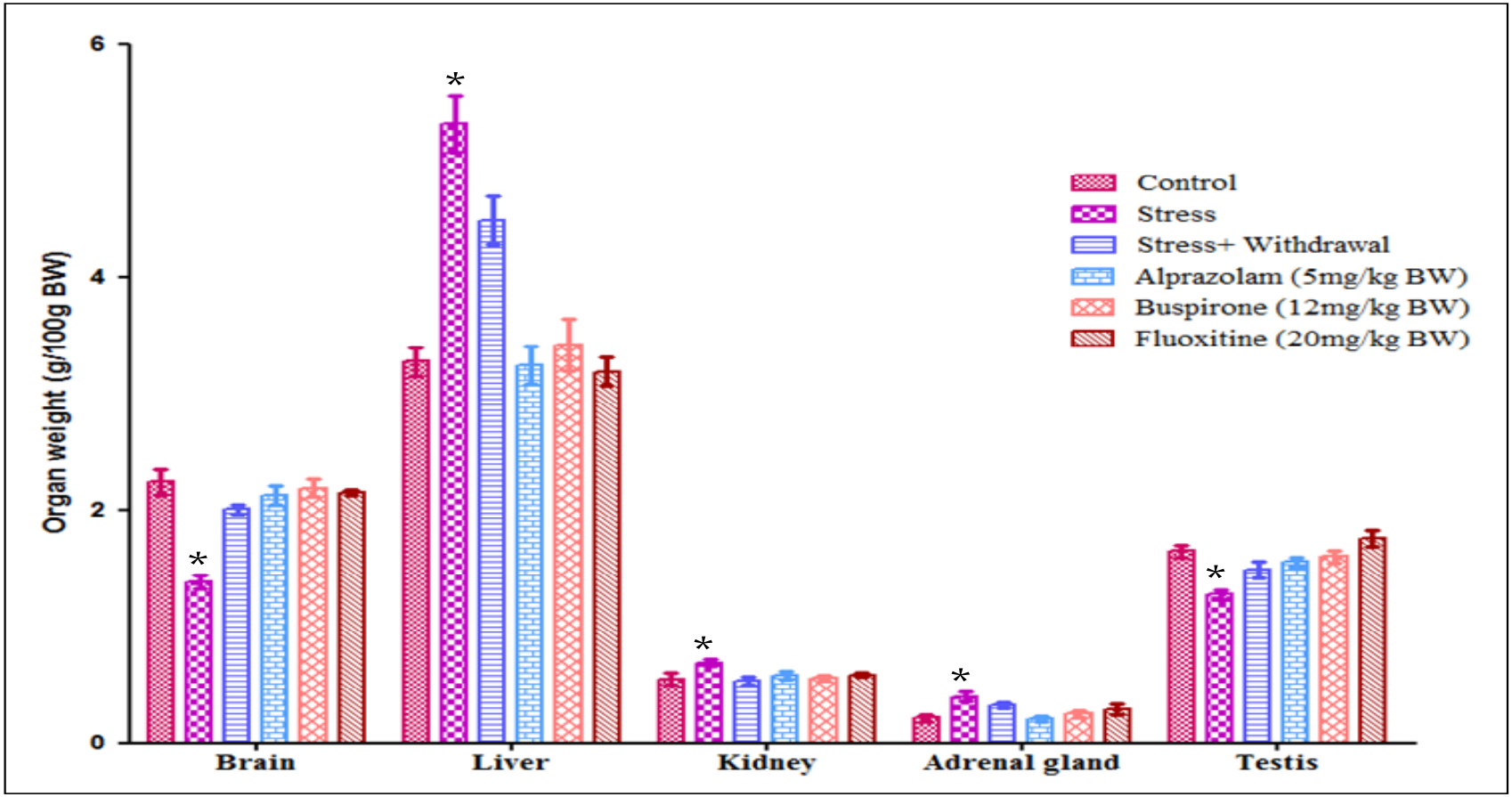

Figure 5.3. Effects of Antistress drugs on organ weights in control and experimental rats. Values are expressed as mean ± SEM (n=6) and * indicates significant ($P \leq 0.05$) compared to control

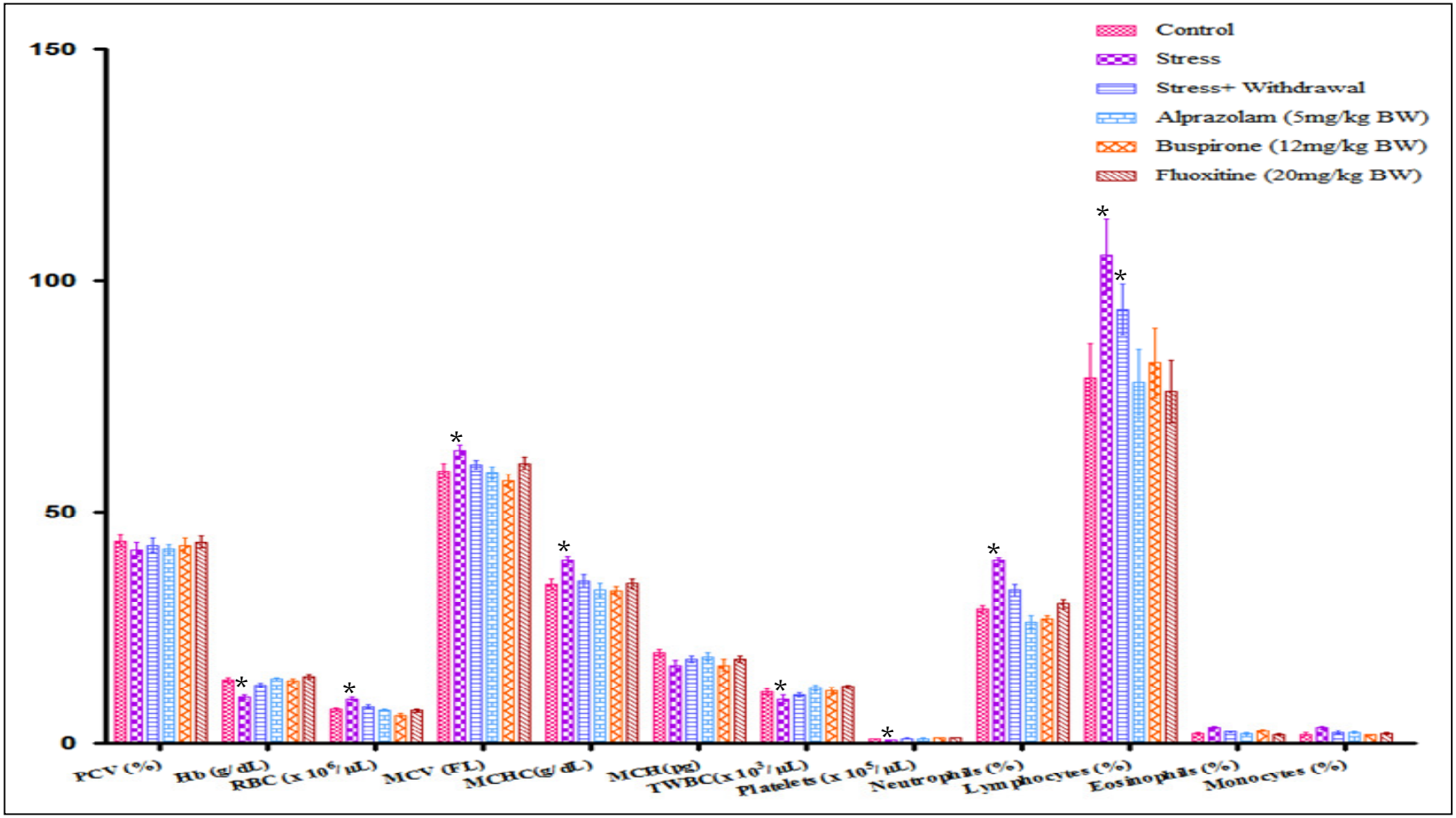

Figure 5.4. Effects of Antistress drugs on haematological parameters in control and experimental rats. Values are expressed as mean ± SEM (n=6) and * indicates significant ($P \leq 0.05$) compared to control

5.6. Biochemical parameters

5.6.1. Serum cortisol:

Our results of stress induced rats were strained daily for 6hrs intended for 42 days exhibited considerably ($P \leq 0.05$) augmented in serum cortisol level moreover partially reversed this change by reducing the cortisol level in withdrawal of stress. However, drug treated groups of alprazolam, buspirone and fluoxetine meant for residual 21 days in continuation with stress revealed greatly noteworthy diminish ($P \leq 0.05$) in serum cortisol level while contrast to merely stress induced rats (Figure 5.5A).

5.6.2. Antioxidant Vitamins:

The levels of serum vitamin C and vitamin E were highly significant decrease ($P \leq 0.05$) in stress induced rats than the control. Whereas withdrawal of stress and alprazolam, buspirone and fluoxetine drugs treated rats explained significant development in levels of both vitamin C and E while contrast to merely stress induced rats (Figure 5.5B & C).

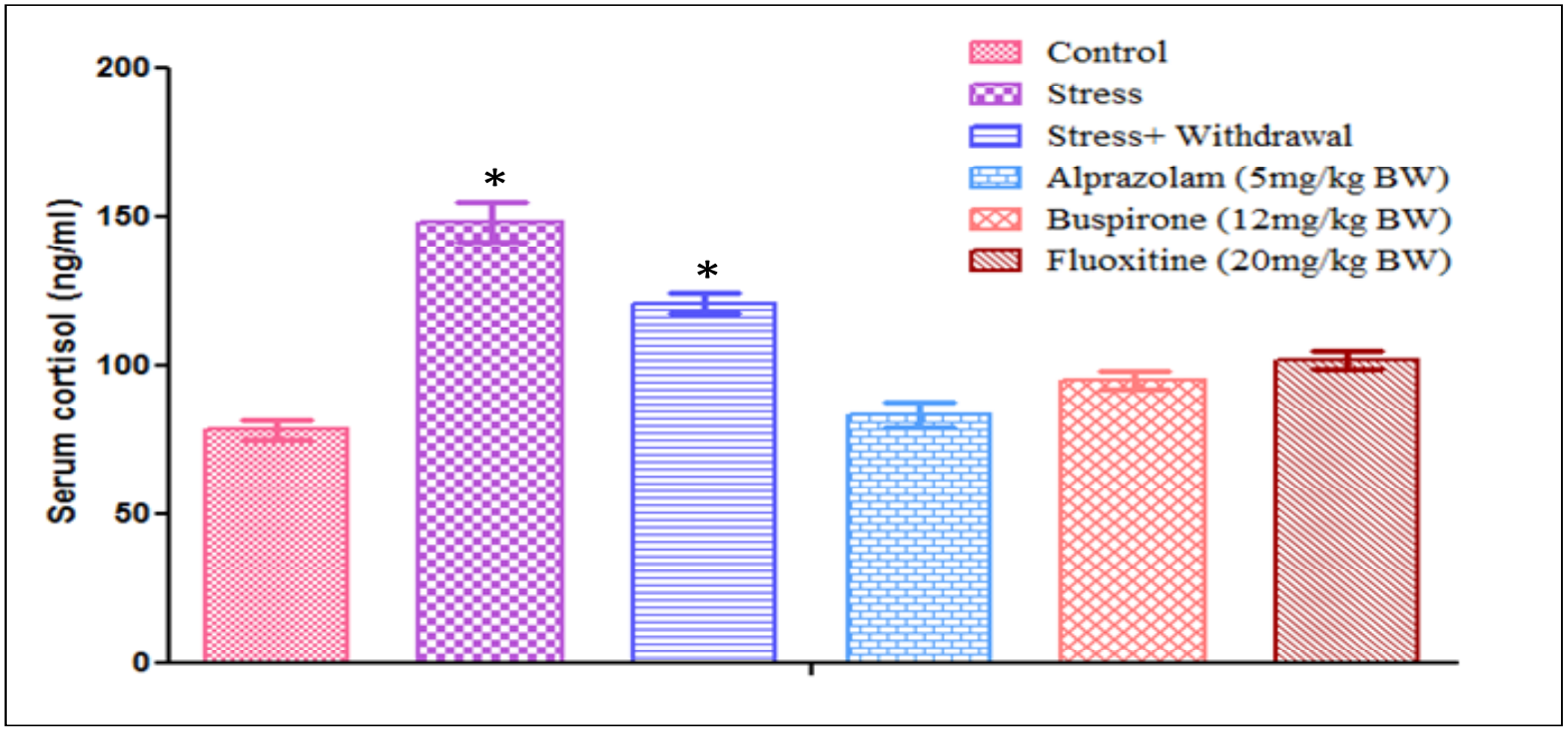

Figure 5.5A. Effects of Antistress drugs on serum cortisol concentration in control and experimental rats. Values are expressed as mean ± SEM (n=6) and * indicates significant (P ≤ 0.05) compared to control

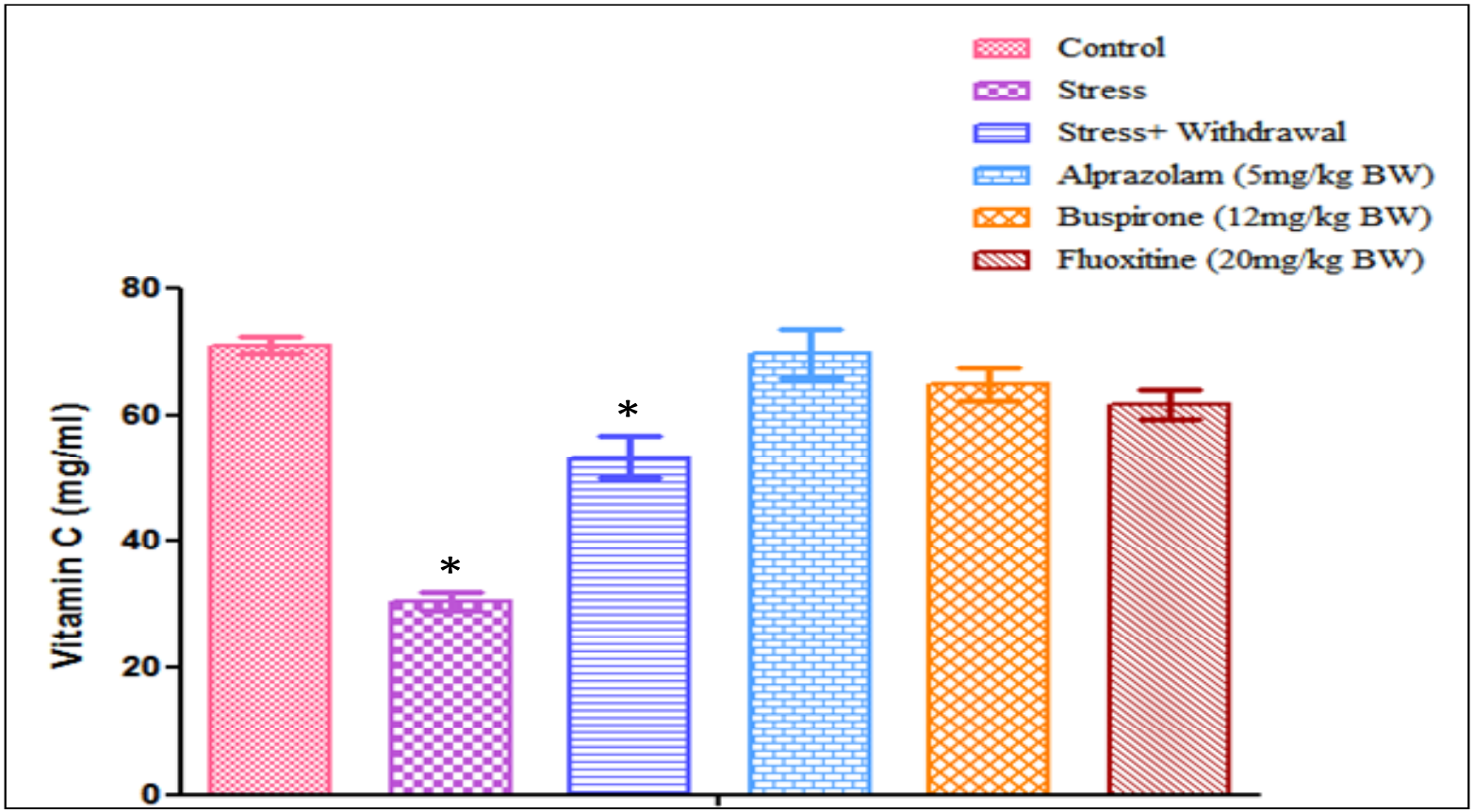

Figure 5.5B. Effects of Antistress drugs on Vitamin C concentration in control and experimental rats. Values are expressed as mean ± SEM (n=6) and * indicates significant ($P \leq 0.05$) compared to control

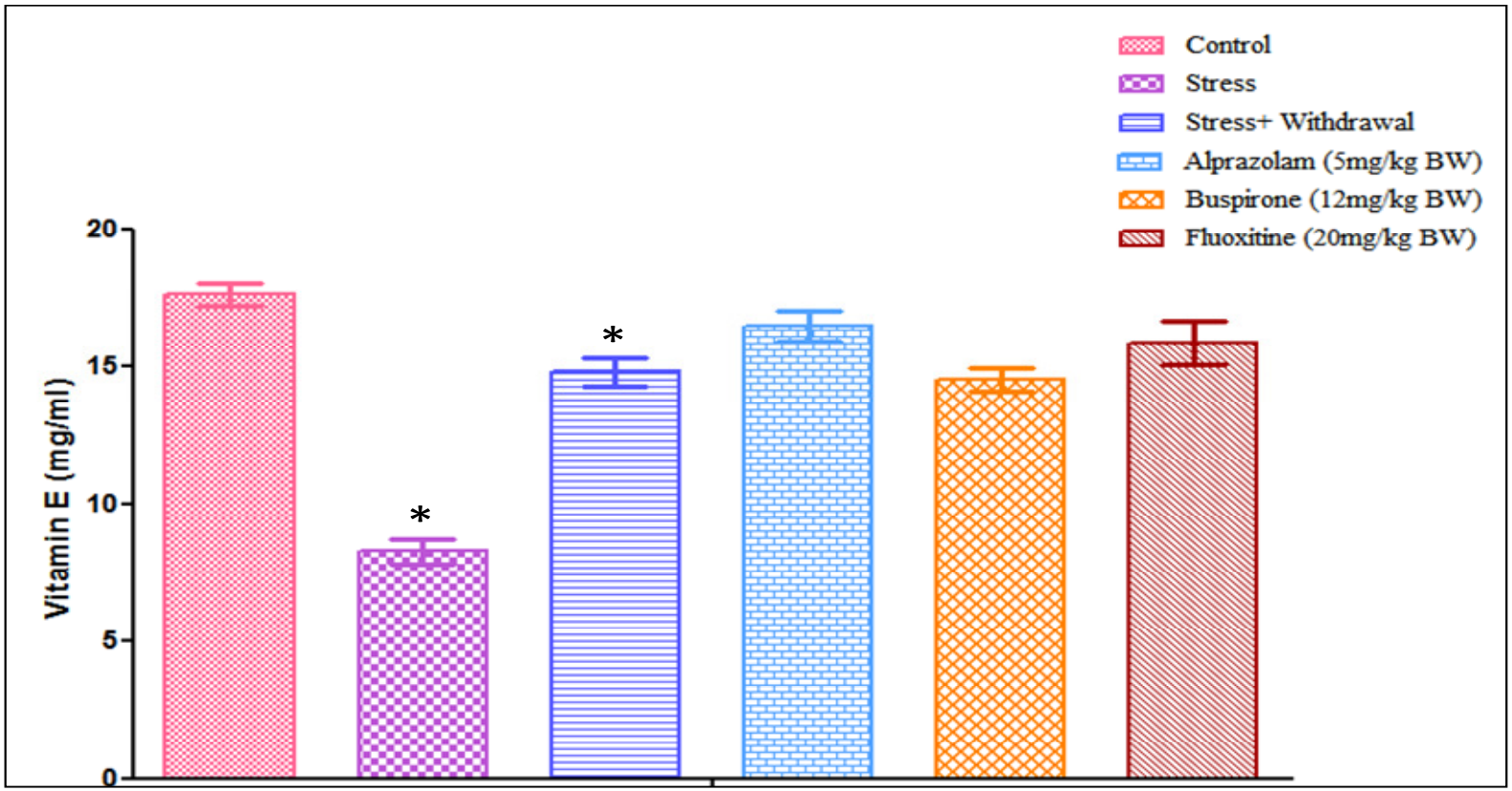

Figure 5.5C. Effects of Antistress drugs on Vitamin E concentration in control and experimental rats. Values are expressed as mean ± SEM (n=6) and * indicates significant ($P \leq 0.05$) compared to control

5.7. Histopathological evaluations

5.7.1. Brain (cerebral cortex)

Histological assessment of the brain section of the controls (Group I) illustrated usual cerebral cortex consisting of neuronal cells possessing round central placed nuclei in moderate amphophilic cytoplasm and entrenched in fibrillar network (Figure 5.6 A&B). Pyramidal cells, granular cells and perineural neuroglia illustrated the key cellular elements of cortical area and were dispersed in an eosinophilic milieu produced of neuronal and glial cell processes. Pyramidal cells demonstrated vesicular nuclei, basophilic cytoplasm and long apical dendrites. Granular cells emerged encircled in form, huge rounded open face vesicular nuclei with prominent nucleoli. Glial cells explained lesser in size by little intensely stained nuclei.

Microscopic impression of stress induced brain sections (Group II) exhibited cerebral cortex with neuronal cells, mild focal vacuolar degeneration and there were no features of necrosis, infarcts, inflammation or glial proliferation. On the whole, neuronal cells were of normal appearance however, few of them appeared deformed in form and emaciated with intensely stained nuclei and enclosed by vacuolated pallid areas most likely apoptotic cells. Neuropil illustrated vascular dilatation (Figure 5.6 C&D).

These histopathological changes were remarkably reversed in stress withdrawal rats (Group III, Figure 5.6 E&F) and recovered to normal level in stress induced alprazolam (Group IV, Figure 5.6 G&H), buspirone (Group V, Figure 5.6 I&J) and fluoxetine (Group V, Figure 5.6 K&L) respectively, drugs treated animals. Microscopic impression illustrated cerebral cortex consisting of neuronal cells possessing round central placed nuclei in moderate amphophilic cytoplasm; embedded in fibrillar network. Stress induced alprazolam

and buspirone drug treated rats exhibited some features of focal vasodilatation, comparatively increased focal vacuolar degeneration and mild glial proliferation. Where as in stress induced fluoxetine treated rats exhibited no features of glial proliferation. No features on necrosis, infarcts or inflammation in stress withdrawal rats and rest of other three drug treated animals were comparable to that of stress induced rats.

5.7.2. Liver

Histological assessment of liver sections of the controls (Group I) designated the regular liver lobular architecture and cell structure (Figure 5.7 A&B). Sections exhibited a typical hepatic parenchymal tissue made of many hexagonal to pyramidal lobules. Each lobule consists of a central vein from which the hepatic plates radiate outwards towards the portal areas and the central veins are lined by endothelial cells enclosed by a ring of collagen fibres. The sinusoids were lined by both endothelial cells and Kupffer cells both of which have unremarkable flattened nuclei and vague cytoplasmic margins. The hepatocytes were polygonal in shape with clear borders. The nucleus was single, round and had a fine chromatin pattern with 1 to 2 obviously defined amphophilic prominent nucleoli. The cytoplasm was eosinophilic and finely granular. Zones of periportal, mid-zone and centrilobular appear normal.

Microscopic impression of stress induced liver sections (Group II) show mild deformed lobular architecture of liver parenchyma and hepatocytes were appeared to be little swollen having vague cell borders with difference in cellular size and shape. The nuclei were round, regular and contain 1to2 nucleoli. The cytoplasm was microvesicular. There were foci of fatty alterations, swelling deterioration and hepatocytes necrosis in centrilobular regions. The portal area appeared mildly distended with mild diffusion of varied

inflammatory cells. The sinusoidal spaces were mildly extended. Central vein showed features of dilatation and congestion (Figure 5.7 C&D).

These histopathological changes were remarkably reversed in stress withdrawal rats (Group III, Figure 5.7 E&F) and recovered to normal level in stress induced alprazolam (Group IV, Figure 5.7 G&H), buspirone (Group V, Figure 5.7 I&J) and fluoxetine (Group V, Figure 5.7 K &L) respectively, drugs treated animals. Microscopic impression is there were mild or lesser vacuolar degeneration, hepatic necrosis, hepatocellular degeneration and less inflammatory cell infiltration. Areas of periportal, mid-zone and centrilobular appeared normal. Fine preserved hepatocytes with polygonal in shape were observed on the whole areas and the recovery from degeneration of hepatic cells in stress withdrawal rats and all drug treated animals were compared to that of stress induced rats.

5.7.3. Kidney

Histological assessment of kidney sections of controls explained typical structure of glomeruli and renal tubules (Group I). Renal parenchymal tissue which was made of glomeruli and tubules partitioned with diminutive quantity of interstitial connective tissue enclosing peritubular capillaries. Each glomerulus was spherical set of inter-linked capillaries in a Bowman's space line up with compressed parietal cells. The outer portions of the glomeruli capillaries were enveloped with a coating of visceral epithelial cells and representing normal architecture of interstitium and vessels (Figure 5.8 A&B).

Microscopic impression of stress induced kidney sections (Group II) exhibited mild congestion of the renal blood vessels and renal tubules illustrated mild degenerative changes with presence of eosinophilic debris in their lumina. No clear significant changes noticed, however, there were some features of mild thickening of tubular basement membrane, focal

cloudy swelling of tubular cells and slight glomeruli shrunken with normal appearance of interstitium and vessels (Figure 5.8 C&D).

These histopathological changes were remarkably reversed in stress withdrawal rats (Group III, Figure 5.8 E&F) and recovered to normal level in stress induced alprazolam (Group IV, Figure 5.8 G&H), buspirone (Group V, Figure 5.8 I&J) and fluoxetine (Group V, Figure 5.8 K&L) respectively, drugs treated animals. Microscopic impression is there were still few residual lesions with minimal focal glomerulopathy and was characterized by swelling and vacuolation of podocytes and by hypertrophy and increase of parietal epithelial cells and condensing of capsular basement membranes in stress withdrawal rats and other drug treated animals were comparable to that of stress induced rats.

5.7.4 Testis

Histological assessment of the testis sections of controls described usual testicular parenchymal tissue whose architecture appeared normal and is divided into lobules containing many seminiferous tubules. Each seminiferous tubules displayed varied phases in seminiferous rudiments encompassing of normal appearance of germ cells, Sertoli cells along with interstitial cells. Headed for the lumen, the primary and secondary spermatocytes, early spermatids as well as late spermatids were connected among Sertoli cells and arrangement of mature spermatozoa with formation of residual bodies might be observed (Figure 5.9 A&B).

Microscopic impression of stress induced testis sections (Group II) exhibited deterioratic tubules and detained spermatogenesis in majority of the tubules. Tunica propria was deteriorated with disrupted basement membrane. Spermatogenesis detained either at the primary spermatocytes or else the spermatogonial stages. Some of tubules exhibited

cytolysis of the whole spermatogenic rudiments. The Sertoli cells also illustrated the vacuolization with cell debris. The spermatogenesis not advanced ahead of pachytene spermatocytes and the minority of these displayed indications of disintegration. The intercellular spacing becomes wider, Leydig cells were decreased in number or the interstitium holds mainly fibroblasts. There was occurrence of fibroblasts like rudiments above the Leydig cells with emaciated nuclei (Figure 5.9 C&D).

These histopathological changes were remarkably reversed in stress withdrawal rats (Group III, Figure 5.9 E&F) and recovered to normal level in stress induced alprazolam (Group IV, Figure 5.9 G&H), buspirone (Group V, Figure 5.9 I&J) and fluoxetine (Group V, Figure 5.9 K&L) respectively, drugs treated animals. Microscopic impression is there was partial or complete restoration of spermatogenesis, besides Leydig cells with rounded nuclei with outermost basement membrane. However, this restoration of restoration of spermatogenesis process was preceded merely up to primary or secondary spermatocytes stage of few tubules and there was retention of intraepithelial vacuoles of differing size are situated or commenced from basal lamina to the lumen of the tubules were observed in the interstitium of stress withdrawal rats and other drug treated animals were comparable to that of stress induced rats.

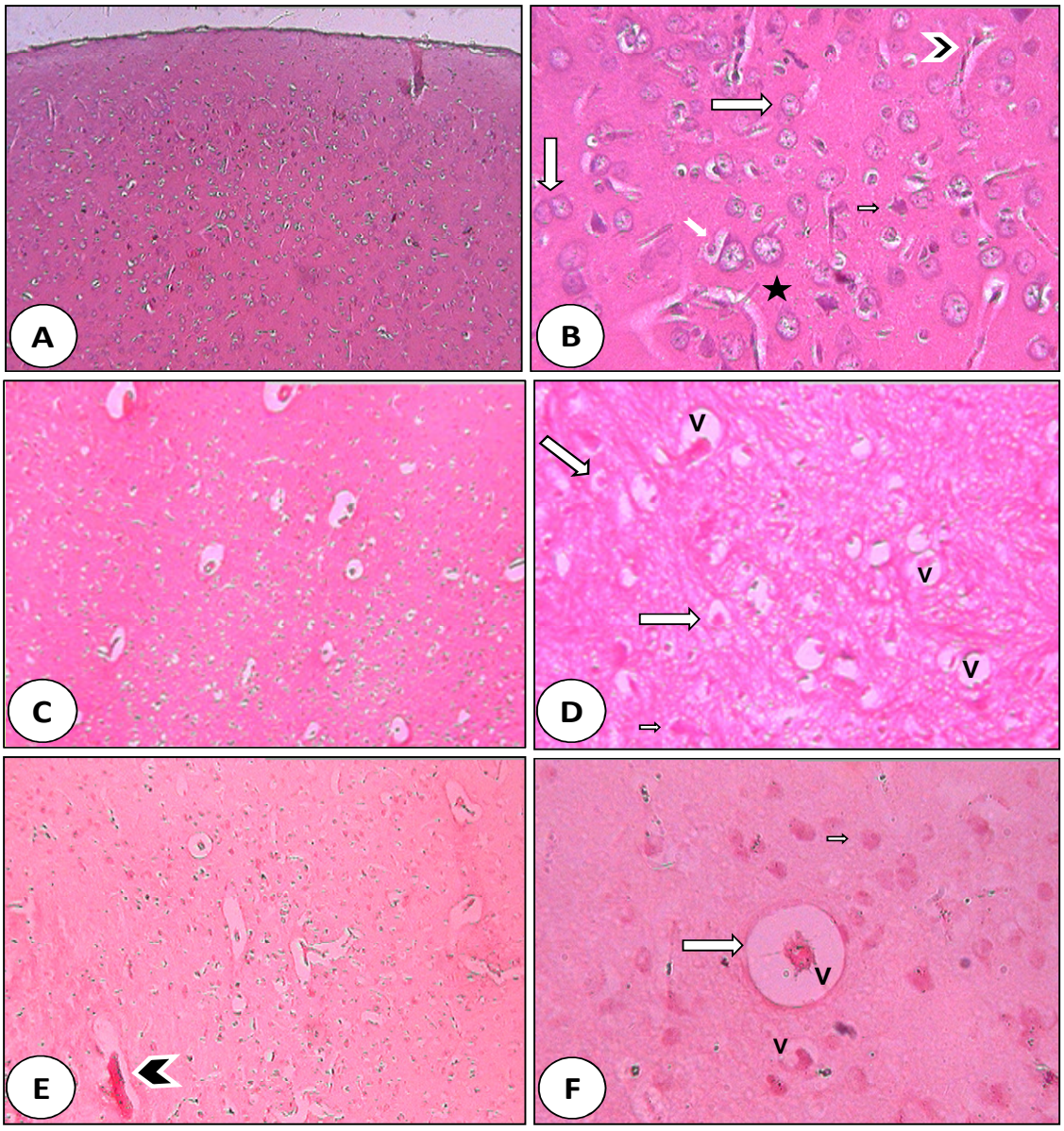

Figure 5.6 A-F: A photomicrograph of histopathological changes occurred in the brain section of cerebral cortex after restrain induced stressed and recuperation by withdrawal possible alteration in restrained stress rats. Figure A-B: Brain section in cerebral cortex of a control rat showing Pyramidal cells (notched white arrow) with bulky rounded vesicular nuclei, basophilic cytoplasm, and processes. Note: the neuroglia cells with small dense nuclei (small arrow head) and granular cells with open face vesicular nuclei and prominent nucleoli (long arrow head) in the eosinophilic neuropil that forms the background for the cells (*). Blood capillaries (arrow head) are seen among neurons. Figure C-D: Brain section in cerebral cortex of stress induced rat showing that most neurons were distorted in shape and shrunken with deeply stained nuclei and surrounded by vacuolated pale areas most probably apoptotic cells. Neuropil showed vascular dilatation. Few cells appeared normal or appear distorted and shrunken with deeply stained nuclei (arrow). Note: the dilated blood vessels (V). Figure E-F: Brain section in cerebral cortex of stress withdrawal rats showing that multiple neurons were of normal appearance however, few distorted cells and dilated vessels (V) were still present. H & E stain; Original magnification: figures (A, C, E × 10x) and (B, D, F × 45x) respectively.

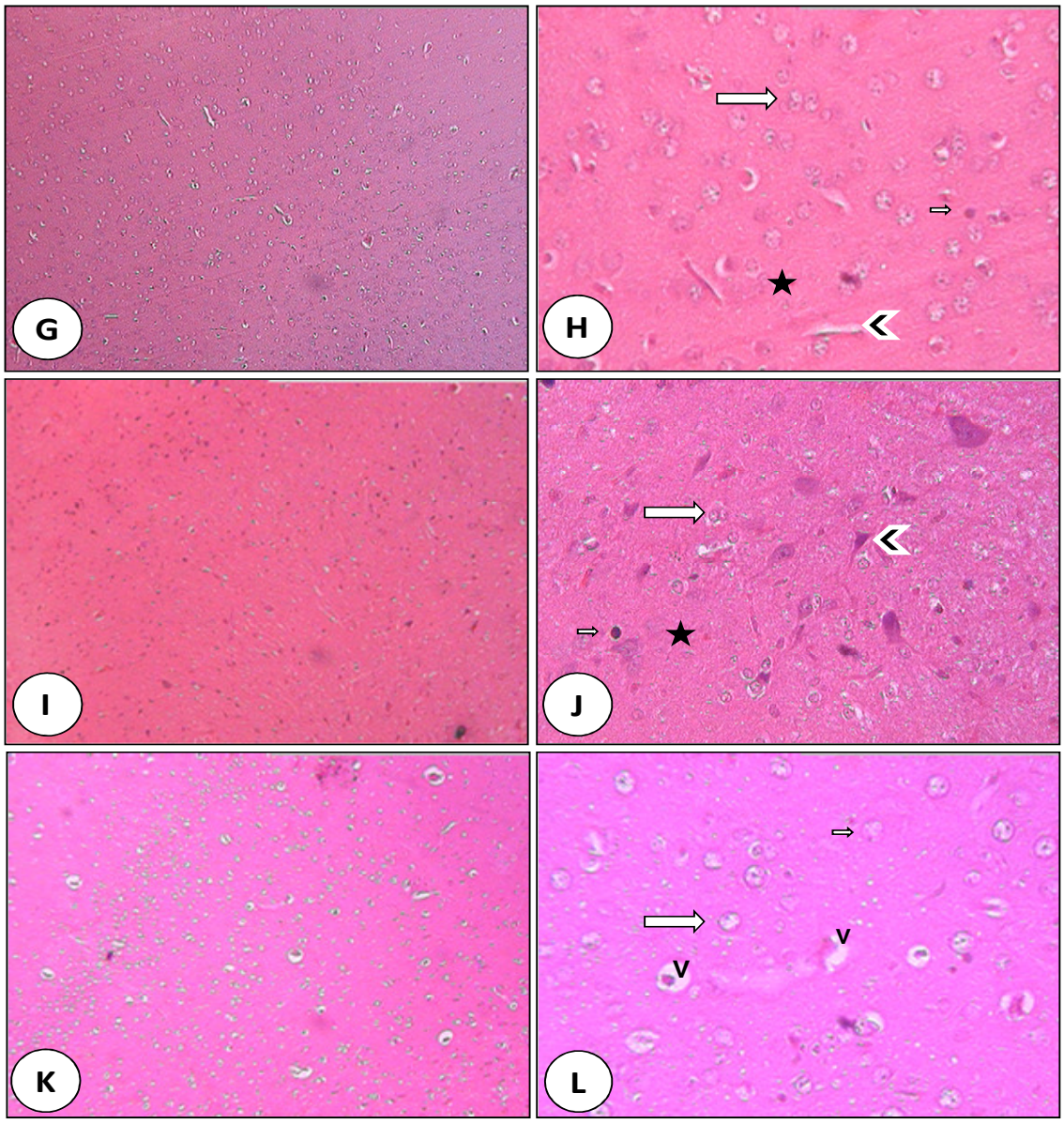

Figure 5.6 G-L: A photomicrograph of histopathological changes occurred in the brain section of cerebral cortex after initiation antistress drugs to compare for their protective effects in restrained stress rats. Brain sections in cerebral cortex of stress induced alprazolam (Figure G-H, 5mg/kg BW), buspirone (Figure I-J, 12mg/kg BW) and fluoxetine (Figure K-L, 20mg/kg BW) rats showing normal features of Pyramidal cells with large rounded vesicular nuclei and basophilic cytoplasm. Note: the neuroglia cells with small dense nuclei (small arrow head) and granular cells with open face vesicular nuclei and prominent nucleoli (long arrow head) in the eosinophilic neuropil that forms the background for the cells (*). Blood capillaries (arrow head) are seen among neurons. In stress induced fluoxetine (Figure K-L) section most neurons were of normal appearance (long arrow head) however, few distorted cells and dilated vessels (V) were still retained. H & E stain; Original magnification: figures (G, I, K × 10x) and (H, J, L × 45x) respectively.

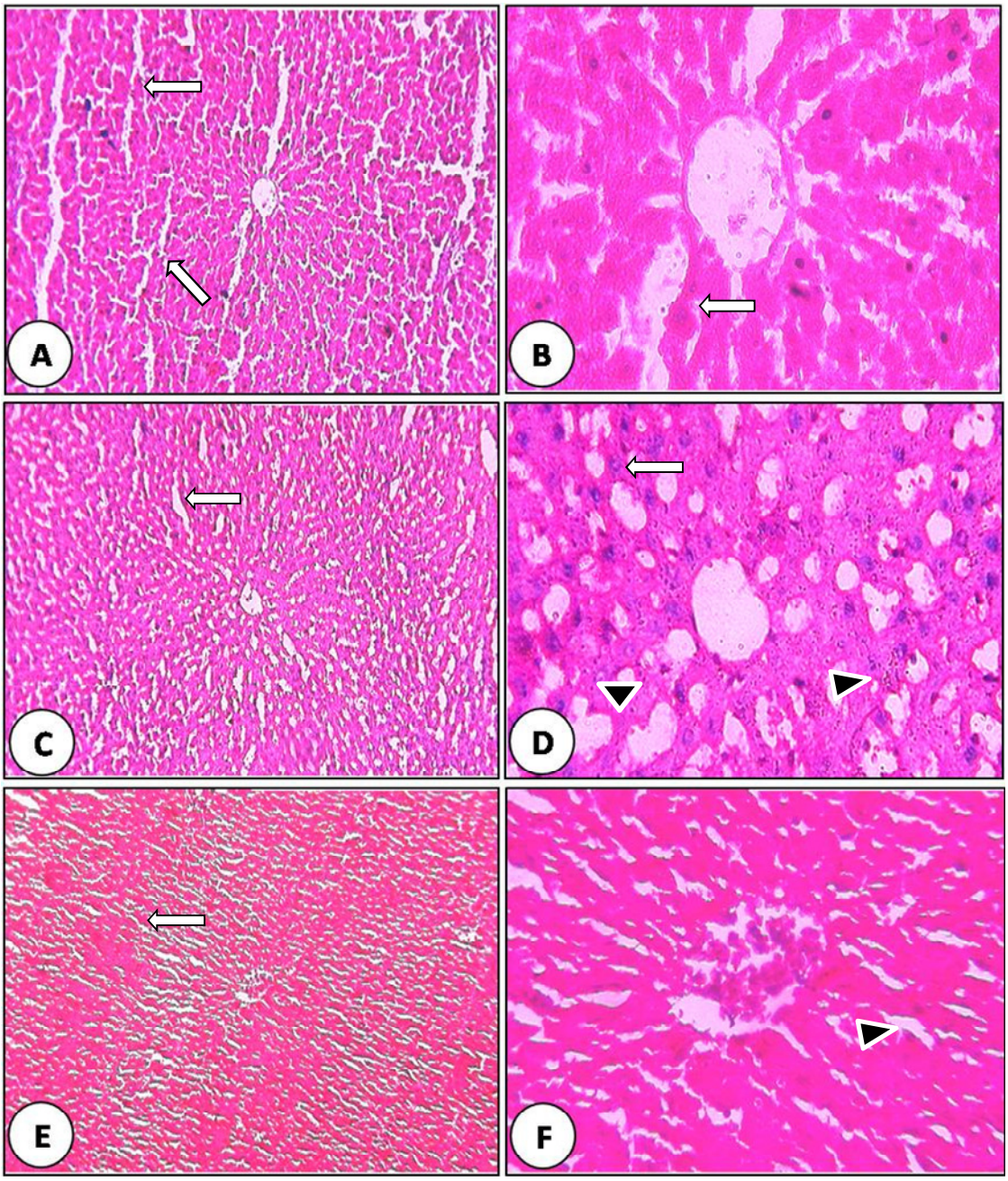

Figure 5.7A-F: A photomicrograph of histopathological changes occurred in the section of liver of restrain induced stressed and recuperation by withdrawal possible alteration in restrained stress rats. Figure A-B: Liver section of control rat showing normal liver having histological structures of normal hepatic lobules and central vein; Figure C-D: Liver section of stress induced rat showing hepatocytes with hepatocellular vacuolization with extensive area of necrosis (arrow head) profound inflammation and congestion of hepatic sinusoids (small arrows); and Figure E-F: Liver section of stress withdrawal rats showing recovering appearance of normal hepatocytes; showing reduced inflammation, degenerative changes, steatosis, mild vacuolization with necrosis (arrow head); reduction of centrilobular necrosis, however degeneration in some hepatocytes at some places were clearly visible mild improvement in sinusoidal dilatation (arrows) was observed. H & E stain; Original magnification: figures (A, C, E × 10x) and (B, D, F × 45x) respectively.

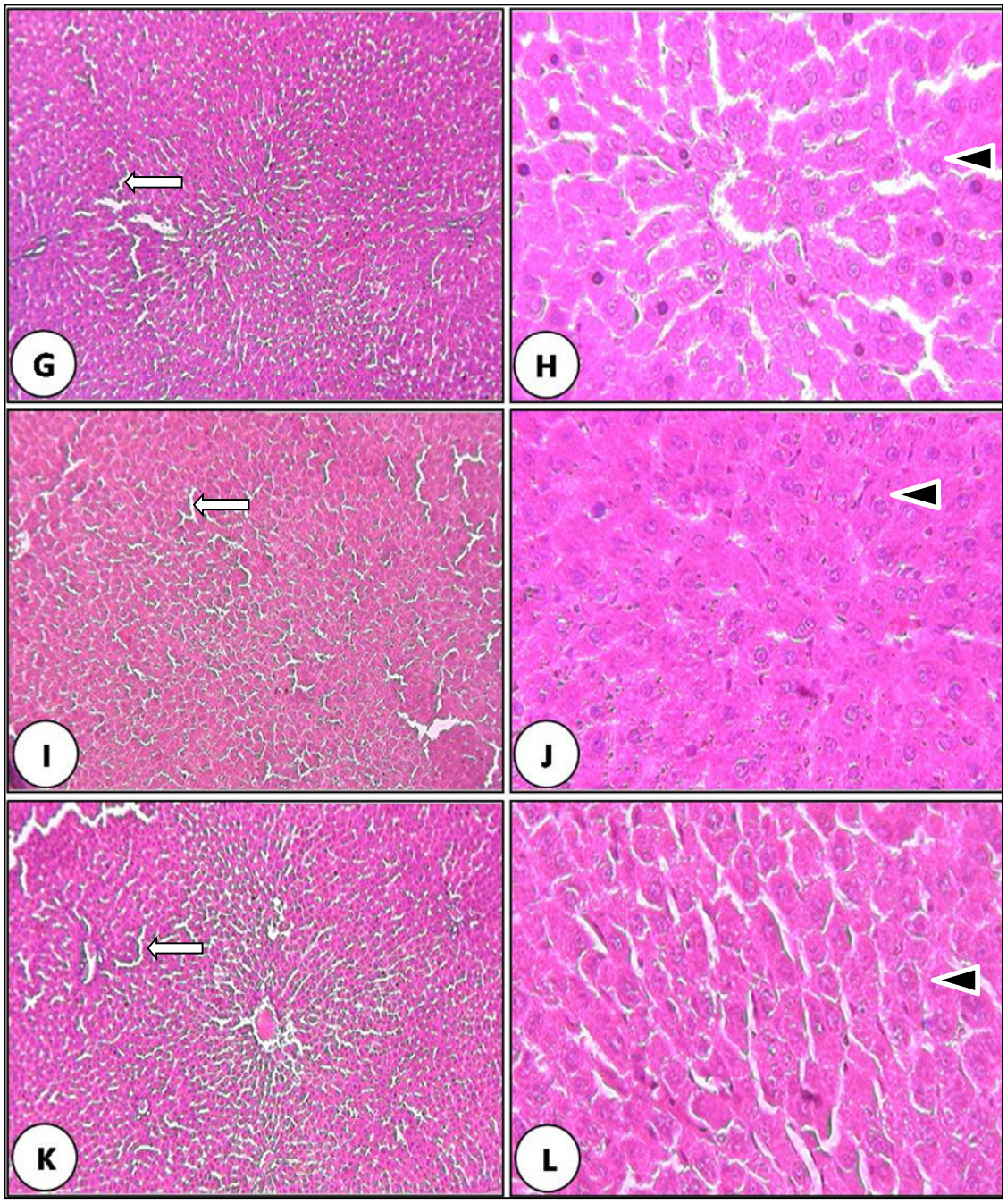

Figure 5.7 G-L: A photomicrograph of histopathological changes occurred in the liver section of after initiation antistress drugs to compare for their protective effects in restrained stress rats. Liver section of stress induced alprazolam (Figure G-H, 5mg/kg BW), buspirone (Figure I-J, 12mg/kg BW) and fluoxetine (Figure K-L, 20mg/kg BW) rats showing normal features of hepatocytes with vesiculated nuclei; showing reduced inflammation, degenerative changes, steatosis, mild vacuolization with necrosis (arrow head); reduction of centrilobular necrosis, however degeneration in some hepatocytes at some places were clearly visible prominent improvement in sinusoidal dilatation (arrows) was observed. H & E stain; Original magnification: figures (G, I, K × 10x) and (H, J, L × 45x) respectively.

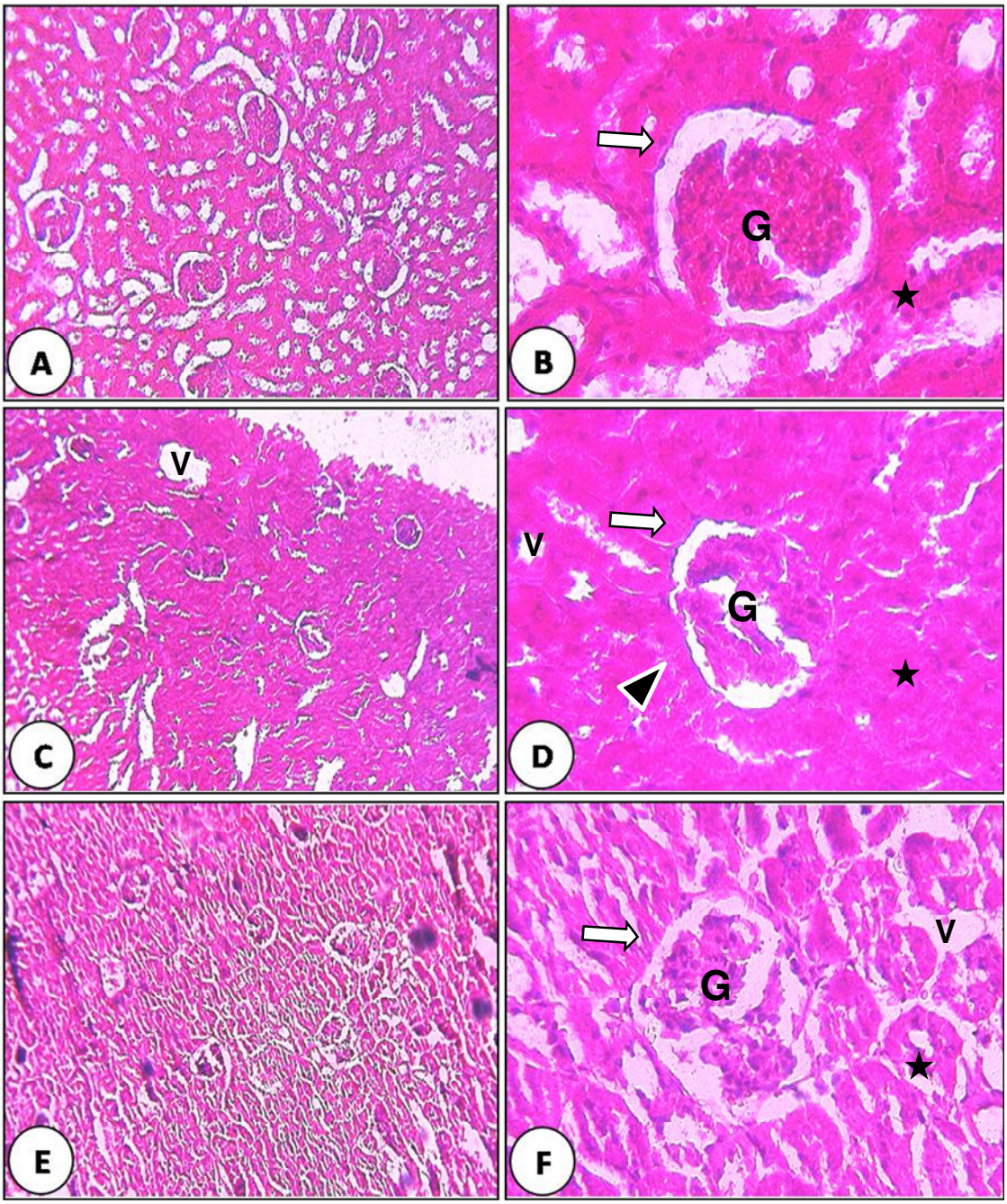

Figure 5.8A-F: A photomicrograph of histopathological changes occurred in the section of kidney of restrain induced stressed and recuperation by withdrawal possible alteration in restrained stress rats. Figure A-B: Kidney section of control rat showing normal showing normal structure of glomerulus's (G) and renal tubules (Long arrow); Figure C-D: Kidney section of stress induced rat showing tubular dilatation, vacuolar (V) and cloudy in epithelial cells lining (arrow head). Interstitial inflammatory cells (*), hemorrhage, cellular debris and glomerulus's hyper cellularity and apoptotic nuclei in renal tubules epithelial cells; and Figure E-F: Kidney section of stress withdrawal rats showing recovering or some improvement in tubular structure but the interstitial fibrosis still presents. H & E stain; Original magnification: figures (A, C, E × 10x) and (B, D, F × 45x) respectively.

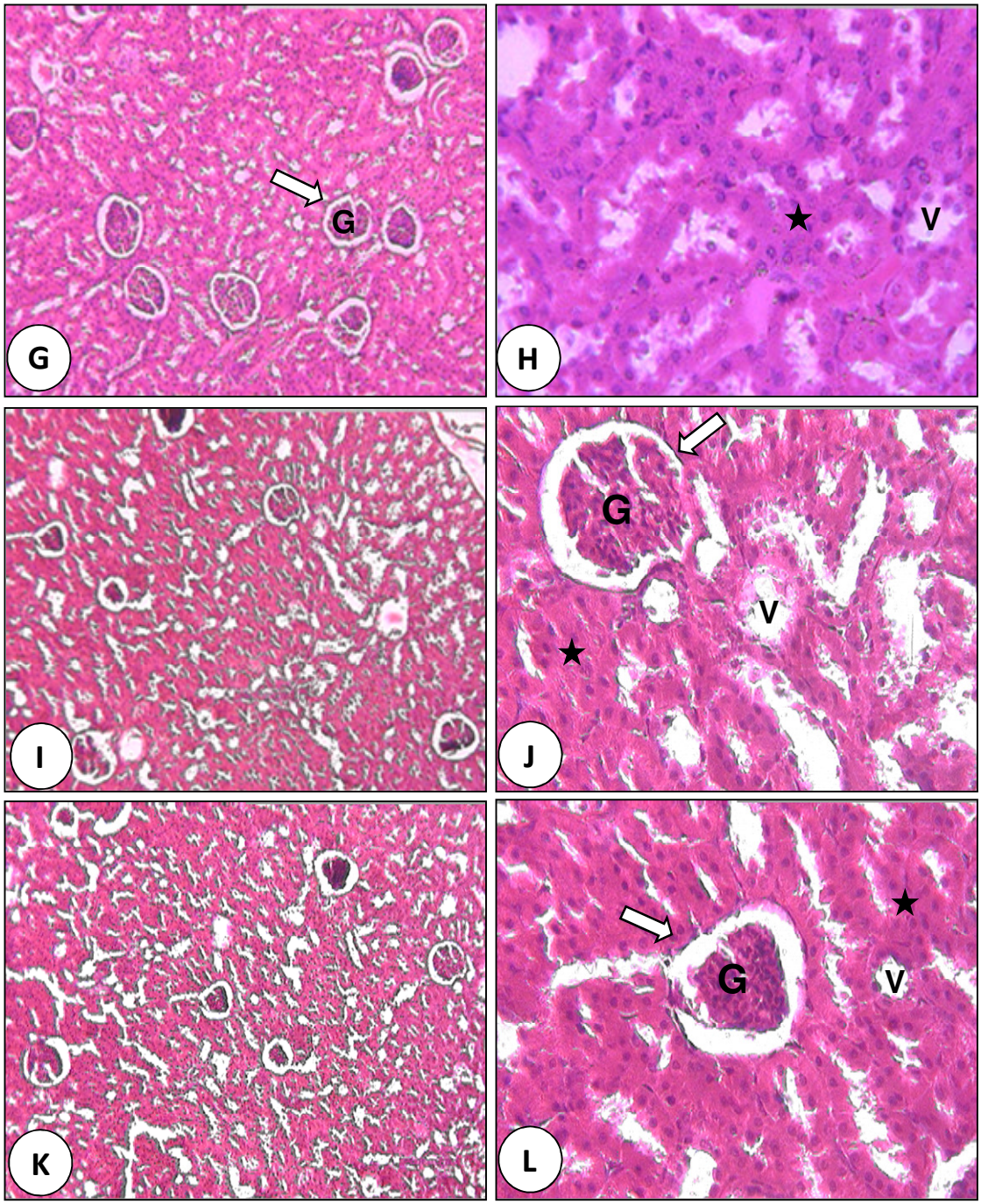

Figure 5.8 G-L: A photomicrograph of histopathological changes occurred in the kidney section of after initiation antistress drugs to compare for their protective effects in restrained stress rats. Kidney section of stress induced alprazolam (Figure G-H, 5mg/kg BW), buspirone (Figure I-J, 12mg/kg BW) and fluoxetine (Figure K-L, 20mg/kg BW) rats showing some improvement in tubular structure (Long arrow) and normal features of glomerulus's (G) ; but the still tubular dilatation, vacuolar (V) and cloudy in epithelial cells lining (arrow head) and interstitial fibrosis (*) still present in the buspirone and fluoxetine treated rats. H & E stain; Original magnification: figures (G, I, K × 10x) and (H, J, L × 45x) respectively.

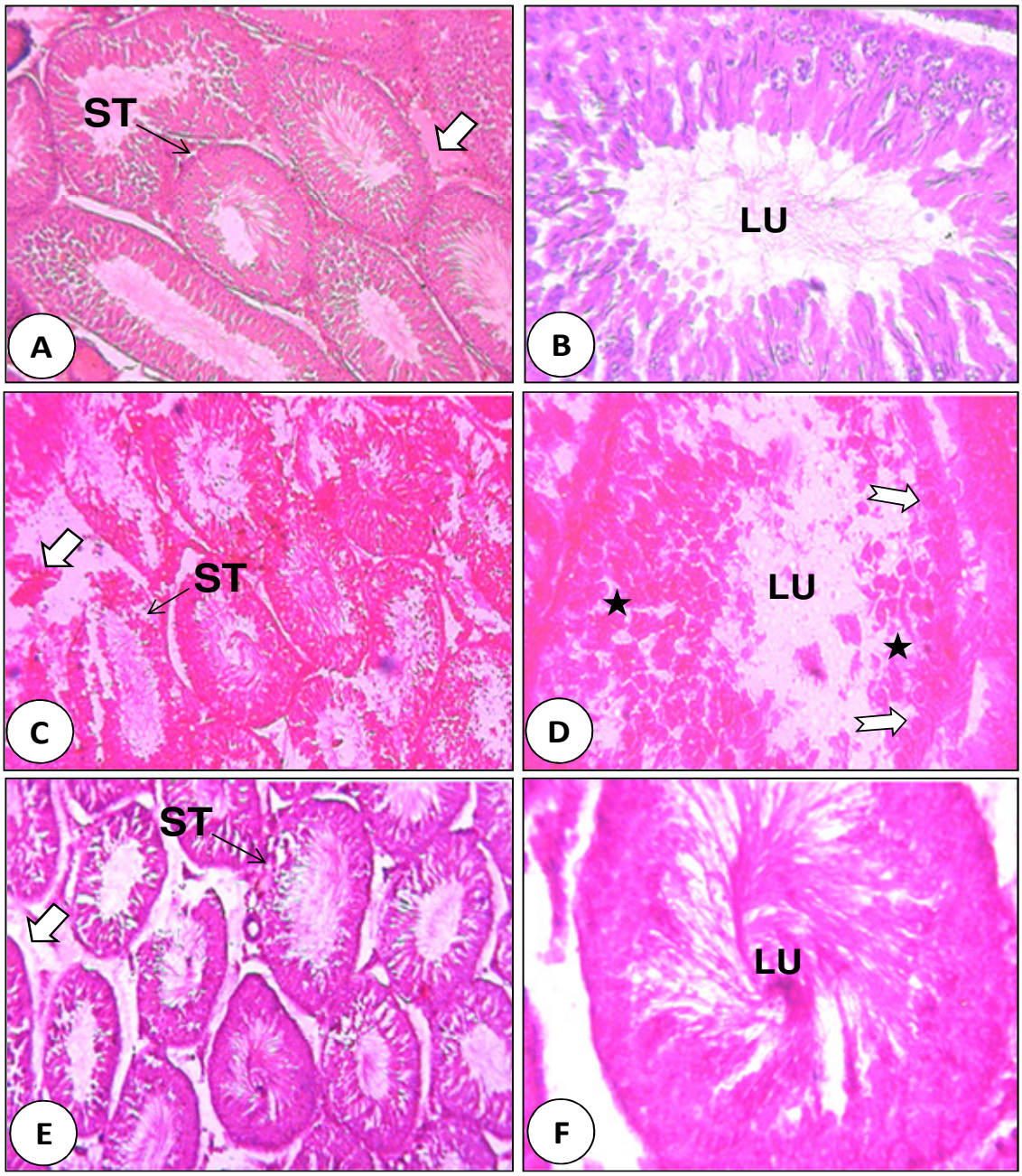

Figure 5.9 A-F: A photomicrograph of histopathological changes occurred in the section of testis of restrain induced stressed and recuperation by withdrawal possible alteration in restrained stress rats. Figure A-B: Section of the seminiferous tubules (ST) of control rat exhibiting normal spermatogenesis (LU) with normal features consisting of spermatogonia, spermatocytes, spermatids, elongated spermatids and interstitial elements (arrow); Figure C-D: Testis section of stress induced rat showing the disruption of seminiferous epithelia and the evident of spermatogenesis detained at the primary spermatocytes stage (snatched arrow). Interstitial spaces increased and atrophy of Leydig cells which are sparsely distributed (arrow). The germ cells show overall decrease in cytoplasmic ground substance followed by vacuolation (*) at the basal lamina and towards the lumen (LU). Testis section of stress withdrawal rats showing recovering or prominent improvement in ST features but the interstitial fibrosis still presents. H & E stain; Original magnification: figures (A, C, E × 10x) and (B, D, F × 45x) respectively.

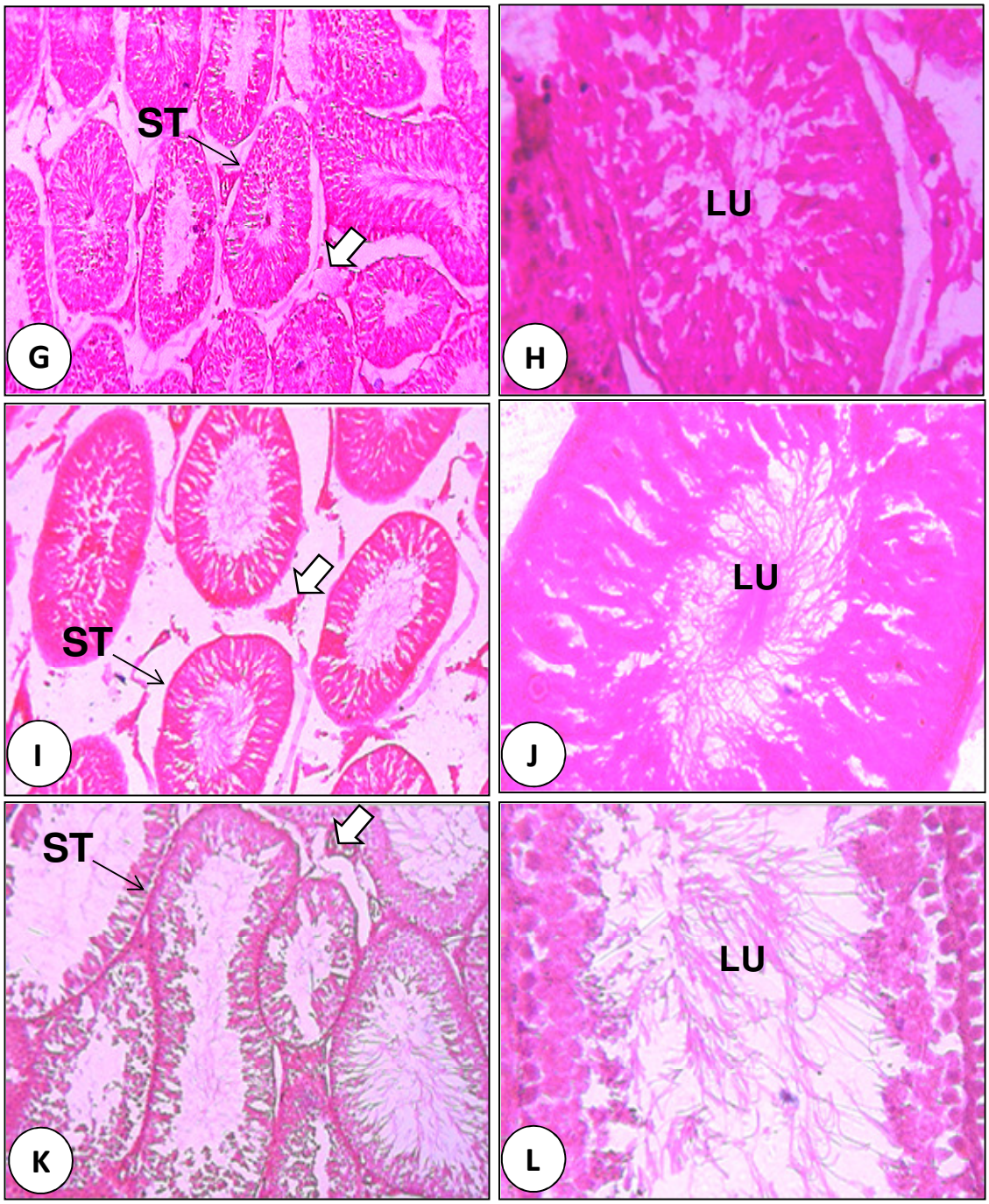

Figure 5.9G-L: A photomicrograph of histopathological changes occurred in the testis section of after initiation antistress drugs to compare for their protective effects in restrained stress rats. Testis section of stress induced alprazolam (Figure G-H, 5mg/kg BW), buspirone (Figure I-J, 12mg/kg BW) and fluoxetine (Figure K-L, 20mg/kg BW) rats showing remarkable improvement in the structural and cellular morphology of the seminiferous tubules (ST) and interstitial space (arrow). Note: There is still retention of intraepithelial vacuoles (*) of varying size are situated or started one cell layer away from the basal lamina to the lumen (LU) of the tubules H & E stain; Original magnification: figures (G, I, K × 10x) and (H, J, L × 45x) respectively.

CHAPTER – 6

DISCUSSION

The hypothalamic-pituitary-adrenal (HPA) axis is a key endocrine adaptor against stressors and contributes an imperative part in stress pathophysiology associated psychiatric sickness for instance depression and anxiety disorders.[1] A number of issues influenced by the effect of stress comprising individual compassion.[2] Test animal representations must reproduce the pathophysiological aspects of individual illness. Amongst, the generally employed representations is restraint stress which is a tailored appearance of immobilization/restraint stress, thought to be a unkind sort of stress in rodents as well as relative result in humans besides. A sequence of behavioural, neurochemical and immunological alterations take place in response to stressors in order to must serve in an adaptive ability.[3,4] Restraint stress procedure is a authenticated examination stressor involving together bodily and emotional consequences on the related time.[5,6] Brain regions of frontal cortex along with hippocampus are responsive to strain provoked injure.[7,8]

Stress is noticed by the brain and consequences in amendment responses in other organ structures by means of neural and neuroendocrine pathways and these stress reactions are specific for various regularly assessed limitations and organ systems so as to receptive to strain. The features of stress reaction any of these systems might be influenced by strain which differs depending on the species for instance, the nature of strain, animal's physiologic condition and intrinsic animal's unpredictability of facts.[9] Studies have been shown that the exposure to continual restraint strain results changes in cognitive functions for example learning, reminiscence and impaired concert in rats[10,11] and some behavioural parameters as anxiety like disorders.[12] In contrast, some studies were verified that reproductive functions are also suppressed under diverse stress conditions.[13]

In our study, the observation indicates that restrained stress for 6 hours per day for 42 days unfavourably affects food consumption of the rats exhibited tired along with their body weights (BWs) reduced which is specified with the mean proportion of BW increase when compared to their mean preliminary BW gain. The reduction in BW could be by reason of lessened in food intake in the rats under the influence of stress and this observation is in accordance with other reported studies.[14-16] Food consumption is one of the uneven receptive to strain and it is mainly fascinating in stress research not just owing to the force of food on development and healthiness other than since it be able to deliberated by means of minimum frustration in animals. Foreword to stressors by average to severe meant for a extended phase results the reduce food consumption and later BW decline in rat.[17,18] Evidences shown the decline in BW might be because of the straight consequence of strain on the food consumption activities,[19] metabolic processes are arbitrated by glucocorticoids,[20] enhance within metabolic demands and increased adrenal steroid discharge.[21] Stress can also enhanced the protein catabolism in addition to obstructed the food received all through the stress phase thus the reason of reduction in BW.[22,23]

In present study, a significant reduction of BW may be by reason of straight consequence of stress on the food consumption activities of rats, low food consumption or apparently related with stress provoked enhance in metabolic demands, decreased absorption and enhanced adrenal steroid discharge in the rats under the influence of stress. Further, we observed weight loss and statistically significant decrease between initial and final weights in the stress group. Profuse studies on chronic exposure otherwise stressors with medium to severe intended for a long period to restraint stress have revealed a decrease in food utilization and subsequently diminution in BW of rodents,[17,18,24-26] furthermore the declining in BW possibly because of direct consequences of strain on the food consumption

performance,[27] hormonal imbalance and protein metabolism. We believe that the cause of weight loss is reduced feed and water consumption or tiredness of body reserves as consequences of increased metabolic activity.[28] Corticotrophin releasing hormone (CRH) persuades feeding behaviour, arbitrate in behavioural plus physiological response to stress. Studies confirmed that the CRH provoked anorexia all through stress by either triggering of serotonin pathways otherwise reticence of neuropeptide Y discharge.[29-31] Neuropeptide Y is a effective inspirer of food consumption since restraint stress persuades weight attain proceed repression of because of despair as well as anorexia.[31] However as per hypothetical evidences point of view on the stress reaction might fluctuate concurrence to its nature, period, austerity strains and sex of trial animals, the methods connecting these restraint persuaded alters in BW and food consumption remain to be elucidated.

Organ weight be capable of for the most part receptive marker of a result of trial ones because noteworthy diversities in relative organ weights among treated and untreated animals might happen in the absence of whichever morphological alters.[32] In our study, restrained stress produces a greater a psychological stress[33] consequences of an increase in the weights of liver, kidneys with adrenal glands furthermore decrease in the weights of brain and testis. Studies have been shown that the decrease in brain weight has been attributed to decline in the number of neurons due to ongoing cell loss or due to decrease in the size of pyramidal cells or might be due to a decrease in the number of synaptic connections.[33,34] In addition to this, it also results from either decline of neuronal pathways or from myelination of the already existing ones.[22,36,37] Increased in liver weight all through stress might be because of enhanced stress hormones discharge which are identified to enhance the actions of metabolic and mRNA levels in hepatic cells.[38] Liver is a vital organ meant for metabolism as well as detoxification and it contain substantial quantities of

polyunsaturated fatty acids which are prone to injure through free radicals. Surplus quantities of proteins are necessary intended for restore of deterioration reasoned by stress and as a consequence of this during stress the metabolic changes are higher. This might be the reason for significantly increase in the liver weight of stress induced study.

Kidney has an imperative function in the multifaceted inter-organ communiqué in order to take place among the growth of inflammation and fibrosis with fatness. Further, it has been shown that increases in cardiac output during exercise, linked with renal vasoconstriction and up regulation of endothelial NOS, might increase blood flow velocity and shear stress in kidney circulation.[39] Alteration within the homeostatic mechanism, for example, enhanced cardiac production with blood pressure all through stress may possibly have added to the enhanced kidney weight following stress.[40-42]

Stress influenced adrenal hypertrophy is a fine ascertained occurrence and burly stimulation of the adrenal glands all through long-standing stress conditions is known reason for adrenal hyperplasia and hypertrophy.[27,43] Stress provoked adrenal hypertrophy is a fine predictable incidence and enhance in the adrenal gland weight is measured as one of the individual preliminary responses to diverse strains. Studies have been shown that the hyperactivity of adrenals in strained animals is by reason of the stress provoked adrenomedullary reaction escorting to enhanced producing corticotropic hormone so as to leads to elevate in adrenals weight.[44,45] In present study, an elevation in adrenal gland weight in restrained stress induced rats point to because of the invariable motivation of adrenal cortex via adrenocorticotropic hormone (ACTH) hypersecretion.[46]

Observation of major differ in the testes weight be a sign of bioavailability reduction otherwise androgen production in restraint stress rats. Reports shown change in the weight

of testes reflects reduced production of androgen. Using animal models study has been shown that stress can influence circulating testosterone levels.[47] During stress, glucocorticoids discharge influence blood testosterone, directly[48] or indirectly.[49,50] In present study, decrease in the weight of testis might be due to deterioration of germinal epithelium by direct action on the spermatogenic compartment or an indirect consequence through inhibition of testosterone production.

In this study, the restrained stress being a psychological stress might cause noteworthy decreased in brain and other relative organ weights which consecutively leads to better normal levels in the groups of withdrawal of stress and antistress drugs designed for residual 21 days in continuance through stress while compared to controls. In present study we be able to conclude that the improvement in the BWs and the relative organ weights of alprazolam, member of the benzodiazepines family, treated rats specifies a favourable result in this regard probably by modification of metabolism or nature consequences of this drug might be due to unusual connections with receptor of benzodiazepine[51] or in the route of the participation of other mechanisms.[52] Anxiolytic drug buspirone could act on the central nervous system (CNS), in particular, on certain regions of brain which consecutively a noteworthy reversal of the stress induced changes in relative organ weights.[53,54] Experimental studies shown that fluoxetine, a selective serotonin re-uptake inhibitor (SSRI), has feature of reverse the behavioural deficits[55] and sub acute toxicity[56] in rats which are considered to be a valid and useful experimental model of depression. In present study, fluoxetine causes a remarkable reversal of the stress induced alterations of relative organ weights via its indirect central and peripheral effects through the modification concentration of free Serotonin (5–HT) which escort to composite secondary responses.[57,58]

Stress stimuli, amongst other confront appearing as of the exterior and interior surroundings modify flowing plasma composition and it is consideration to damage immune function through emotional or behavioural signs. Stress induced alterations in the haematological parameters and CNS is defended from these variations through barriers, amongst which the blood brain barrier participate in a key task in preserving homeostasis.[59] The blood brain barrier provides the brain by means of oxygen, glucose and other nutrients needed meant for neural functions. It adds to the most favourable ionic and neural microenvironment transmitter composition meant for synaptic signaling and defends the CNS aligned with neurotoxic stuffs.[59,60] The decline in haemoglobin content, RBC count, total WBC count and MCV might be because of non-regenerative anemia occurring from stress provoked disorder of hematopoietic stem cells resultant in reduced erythrocyte, leukocyte and platelet count. Similar to this study, other experimental studies shown by significant reduction in haematological parameters when exposed to immobilization stress.[61,62] Nevertheless, conflicting results regarding the consequence of continual stress on eosinophil counts.[63-65]

Following an acute stress event, eosinophils levels were considerably lesser in destructive animals contrast to faintly destructive ones.[66] Eosinophils level is a hormone dependent limit, as a whole with neutrophils and lymphocyte ratios; it may perhaps assist discriminate linking leukocyte reactions to strain. Together emotional[67] and physiological stress, for instance long-standing cold[68] or immobilization[64] have been confirmed to decrease the level of eosinophil. Diminished in the level of haematological parameters in the stressed induced and together treated with antistress drugs or withdrawal groups designed for residual 21 days in continuance through stress have effectively improved the

haematological disturbances and demonstrated a defensive role as antioxidant, antilipidperoxidation and immunostimulation effects of antistress drugs.

It has been shown that alprazolam acts as an immunoenhancer, emerged to improve immune receptiveness in non-strained and strained animals and these effects are arbitrated via the alteration in the immune system organs' structure.[69,70] Studies revealed that alprazolam was established to moderate various of consequences of strain in rodents immune systems[71,72] and capability of alprazolam to reduce the stress provoked enhance of ACTH levels indicates an major role in the immunoprotective consequences.[73] However, the means of act of benzodiazepines lying on the immune system surplus need to be described. Central pharmacological results associated to the central form benzodiazepine receptors so as to assist inhibitory GABA neurotransmission within the CNS may control the discharge of neuroendocrine hormones entailed in the immune reaction to stress.[71,74]

The mechanism of action of buspirone, a 5-HT_{1A}, agonist anxiolytic, on the immune system might be also through or circuitous. The immune reaction regulation might consequence as of an arbitrator entailed in conveying the drug's cause. Study shown buspirone exploits its deed at the receptor location apart from GABA-benzodiazepinechloride ionophore complex[75] and dose related effects of buspirone moderately reversed the oppressive stress that results in T-cell populations.[76,77] From the information on fluoxetine, it has been indicated to cytoprotective consequence causing restraint of calcium ions more production,[78] proficient in decreasing the immune and inflammatory components[79,80] to support the reactive oxygen species (ROS) production.[81] Further, it is proposed that the inhibitory consequence is arbitrated in some way by the protein kinase-A.[79] Taking into description the available evidences of ROS production key

pathways in the line of depression have been illustrated, in present study, the potentially positive antistress result of the fluoxetine might be arbitrated by initiation of immune as well as inflammatory reaction systems.[81]

Results revealed that prologue to restraint stress persuaded peripheral oxidative stress, which is described with an enhancing ROS generation in peripheral blood lymphocytes, granulocytes as well as monocytes furthermore the observed unfavourable consequences were partly rescinded by antistress drugs or stress withdrawal group indicating that it is able to improving oxidative damage influenced through restrained stress on the tangential immune system. Present results are in concurrence among other studies explaining that the ROS generation by immune cells possibly persuaded by psychological stress. However, the accessible outcomes in direction to the convince of psychological strain on ROS generation are conflicting,[23,82] whereas others have revealed reduced generation ROS.[83,84] Further, initiation of three drugs separately evidently indicates as potential antistress drug as part of blood cortisol concentration concern.[85,86]

Cortisol provides like a key regulator meant for neurohumoral reactions and it is believed that the enhance in the level of blood serum cortisol as marker for organism's response to stress.[87-89] Stressors generally persuade sensory neuronal pathways proposed to diencephalon centers in the brain and extend a reaction in the appearance of behavioural, autonomic, endocrine otherwise oxidative stress.[90,91] Several diverse animal models of stress have been accounted that immobilization stress enhances the levels of cortisol and declines levels of testosterone which consecutively interrupts the testicular function.[92] In stress conditions, glucocorticoids discharge enhances and inhibits the reproduction. Therefore it is consideration that stress inhibits reproduction by means of the glucocorticoids.[89] It has been

revealed that inhibition of reproduction is as a result of the hindered gonadotropin discharge in long-standing strain. [93] This examination method is awfully imperative intended in favour of endurance with stress.[91] Corticosteroids revealed to endorse the immune reaction all through severe stress and to hinder the immune reaction for the duration of continual stress.[94] Conversely it was found that restraint stress tempts corticosterone discharge [95] Studies illustrated so as to HPA axis is triggered through novel or irregular conditions.[91,96] In present study a significant increase in levels of serum cortisol is due to the increasing stress response via HPA axis in restrained stress rats.

Results of a remarkable neutralization or reversed in the serum cortisol levels after administration of antistress drugs groups or withdrawal group designed for residual 21 days in continuance through stress may possibly by reason of diminution of strain reaction through HPA axis. Results were supported by other's experiment on alprazolam since it was established to efficient in improving activities changes in mice owing to restraint and oxidative stress.[97] The serotonergic coordination of brain participate a fundamental task in autonomic, neuroendocrine and behavioural amalgamation of strain reaction and alprazolam possibly persuade it.[98] The consequence of alprazolam on corticosteroids production and it was concluded that it has an oppressive effect on cortisol production.[99]

Buspirone is established to enhance plasma ACTH and corticosterone-cortisol quantities and from the reports it has been indicated that the defensive effects of buspirone on T-cell immune reactions were escorted with a proportional decrease in ACTH levels in stressed mice and suggested that buspirone provoked decrease in ACTH levels and ought to partly assist to elucidate the immunoprotective results of this drug.[76] In addition to this, other experimental studies demonstrated that strain provoked raise in corticosterone

discharge which was reduced by the dose linked effects buspirone, which enhanced the discharge level of corticosterone in stressed rats.[75,77]

The involvement of the antioxidant vitamins C and E in defending cellular organelles from oxidative damage is well established.[100,101] Exploit be able to enhance free radical generation with 2 to 4 fold up [102] and generate alters in redox eminence which might provide oxidative stress on muscles and other tissues escorting to modification of lipids, proteins, and genetic material.[103]Also, during stress, decline in vitamin C and E obviously specify both over utilization of vitamin C and E or deficiency of biosynthesis of vitamin E and over deprivation of vitamin C during metabolism.[104] The consequence of restrain stress persuaded lowering vitamin C and E represent so as to certainly provoked oxidative stress. Administration of antistress drugs groups or withdrawal group designed for residual 21 days in continuance through stress confirmed the improvement of together vitamin C and E are the key oxidative stress indicators.[105] Improvement in levels of vitamin C and E indicates after supplementation of drugs are active possibly the supplement itself is having good concentration of vitamin C which make possible and interactive metabolic pathway for vitamin C synthesis.[106,107] In the present study, stress has reduced the actions of vitamin C and E might interact directly with free radicals, in consequence preventing oxidative damage.[108-110]

A pathogenic aspect of stress also contributes to the sequence of neuroinflammation furthermore shown to be influence many brain actions and endorse long-standing alters within manifold neural systems.[111] Brain is the object meant for diverse stressors owing to its lofty compassion to strain persuaded disintegrative situations. In contrast to other related organs, brain have comparatively enzymatic low levels and nonenzymatic antioxidants

providing it additional susceptible to oxidative stress.[112] Moreover, brain tissue includes huge quantities of polyunsaturated fatty acids, which are predominantly susceptible to free radical assails [113-115] and also showed distinct differences in cellular with area allocation of antioxidant biochemical fortifications.[116] Therefore, neural cells otherwise brain areas are probable to diversely react to changes in metabolic rates linked through ROS generation.[117]

In this study, histological observations of stress induced brain section of cerebral cortex exhibited mild focal vacuolar degeneration. Stresses incentives, amongst other confront rising from the exterior and interior surroundings, modify flowing plasma composition. These effects are in order with others experiments; bulging of astrocytes is frequently related by means of disturbance in the cerebral cortex [60,118-119] and suggested that these structural alterations might represent morphological signs of brain blood barrier dysfunction. Though, the impact of strain on the cellular and molecular elements of the brain blood barrier is still unexplored. Our results could assist to describe the stress persuaded brain pathological alters contributing a function in the pathogenesis of various neurological and psychiatric illnesses.

Stress factor be able to cause liver damage due to improperly associated or misalignments in HPA axis. In present study, the frequent vacuolisation or degeneration of hepatic cells were observed with rudimentary cytoplasm and presence of clustered nuclei cause improper function of liver. Therefore, liver atrophy in restrained persuaded stress rats could be by reason of modification in the cell membrane permeability.[120-121] Further, restraint stress induces formation of autophagic vacuoles surrounded by several regions,[122] which is related with damage of DNA oxidative, [123] lipid peroxidation,[124] protein oxidation[125] and eventually all these alterations may be causes hepatocytes injury and

depresses liver function.[126] Kidney tissue is very receptive and the stability of cellular life depends on the balance in the execution of composite biological responses. Endogenous or exogenous features that could disrupt this balance lead to cellular damage.

Kidney tissue is awfully receptive and the constancy of cellular life reliant on the equilibrium in the implementation of multifaceted biological feedbacks. Endogenous or else exogenous aspects so as to may perhaps disrupt this equilibrium escort to cellular injure. The present study indicated that restrain stress induced marked histopathological alterations in the kidney such as congestion of the renal blood vessels and renal tubules. It is justified that since the renal tubules are mainly responsive to stress influences, partially because they have high oxygen utilization and susceptible enzyme systems and such notable alterations were distinct in the in the proximal convoluted tubules.[127,128] The occurrence of necrosis might be associated to the reduction of ATP, which after all escorts to the loss of the cells.[129] The mechanism of which is inadequately understood, but it seems to involve a vascular alteration or tubular lesions which is a direct restrained stress consequence on the cell function.[130] Further, kidney exhibited a feature pattern of changes in the glomerus in addition to gentle condense of glomular basement membrane in stress rats providing support that alter in the glomeruli pattern and their number is a determining aspect for hypertension.[131]

Fall off male fertility is one of the identified outcomes of psychological stress [132] and stress appears to be a possible threat aspect for reproductive function. In males, physical and psychological stressors could inhibit reproductive task primarily through the suppression of hypothalamus-pituitary-gonadal (HPG) axis and activation of HPA axis.[133] In connection to this, other studies have been revealed that stress causes impaired spermatogenesis, decline in levels of serum testosterone and luteinizing hormone (LH),[134,135] increases the apoptotic

index in the seminiferous tubules of the rat testes.[87] In connection this, other studies revealed so as to stress causes endocrine disorders in testes and injures in testicular morphology, furthermore, psychological strain in humans decreases the sperm progress and excellence that escorts to infertility[136] ; immobilization or restraint strain distress spermatogenic and endocrine testes role [134,137-140] and affects male reproductive deed resulting enhances the apoptotic index in tubules of testes.[87] In parallel with these studies, we observed widely tubule damages and developed apoptosis or different size of vacuolation in testes of rats exposed to restrained stress rats.

Vitamin E, is an influential lipophilic antioxidant, utterly essential meant for the preservation of mammalian spermatogenesis.[141] Vitamin C as well adds to the spermatogenesis preserve at least partially through its ability in diminished α-tocopherol and preserving this antioxidant in an active condition.[142] It is well established that deficiencies of antioxidant vitamins C or E escorts to condition of oxidative stress in the testes that interrupts both spermatogenesis as well as testosterone production.[141,142] In the present study, exposure to restraint stress induced alter in the testicular function as reflected by noteworthy decline in the Vitamin C and E levels in blood serum results correlate well with histological damage as shown the spermatogenic cells and degeneration of seminiferous tubules and lumen devoid of spermatozoa.

Further, antistress drugs groups or withdrawal group designed for residual 21 days in continuance through stress suggesting that the effects of the treatments are recovered to normal level in stress induced. A recovery period resulted in normal spermatogenesis indicating that means of drugs exploit could be also direct otherwise indirect or else it may assume that regulation of stress might consequence as of arbitrator entailed in conveying the

drugs cause. Though, testicular tissue of alprazolam drug treated rats, exhibited retention of noticeable vacuolation in their cytoplasm. These vacuoles could be attributed to as a general early response to a restrained stress or might be phagocytic vacuoles remaining after digestion of necrotic germ cells.[143] In contrast, the histopathological changes were recovered to normal level in stress induced alprazolam convince the male reproductive function even though results of retention of intraepithelial vacuoles provide no perfect justification of the mechanism of action of this drug.[144] Studies have revealed to relative organ like liver or kidney injure is not a direct consequence of taking buspirone and unfavourable affairs accounts on organ toxicity are exceptional for buspirone.[145,146] However, buspirone often suggested to remedial consequence are not because of its instantaneous act on the 5-HT system, other than adaptive alters that happen following long-standing conduct.[147] In present study, a consequential of restoration phase in normal spermatogenesis representing the means of buspirone exploit possibly either direct or indirect otherwise it could imagine that stress regulation of might consequence as of arbitrator entailed in expressing the drug's effect.

In the testicular tissue of fluoxetine treated rats, the size of seminiferous epithelium and individual area of Leydig cells, length of the seminiferous tubules and sperm production were observed which corroborates the increased testosterone concentration because testosterone levels are directly related to the size of Leydig cells.[148,149] Studies showed that an increase in brain serotonin may change the levels of LH and follicle stimulating hormone (FSH) because of the inhibition of gonadotropin releasing hormone (GnRH).[150] The major intracellular organelles entailed in steroidogenesis are the smooth endoplasmic reticulum, mitochondria and more than occupy fifty percent of Leydig cell mass[151]; thus, fluoxetine may perhaps impair Leydig cells and modify its size in the testis.[149] Hence, the improved

vicinity of Leydig cells examined in the present study might have enhanced the testosterone concentration to promise the efficacy of spermatogenesis.

The outcomes presented at this point guide us to conclude so as to prologue to restrained stress consequences alter the levels of serum cortisol, haematological parameters and antioxidant levels, food consumption, gravimetry, organ weights and histopathological in male albino rats. Withdrawal of stress or antistress drugs designed meant for residual 21 days in continuance through stress might possibly neutralize restrained stress provoked injure to escorting antioxidant equilibrium and modify hypothalamic-pituitary adrenal (HPA) axis. The exposure to restrained stress resulted in peripheral oxidative stress in male albino rats. These antistress drugs may perhaps a vital means connecting the defensive pharmacological consequences over restrained stress induced haematological, biochemical and histopathological adverse change.

The study therefore concluded that the treatment with these antistress drugs have a significant effect to counteract restrained stress induced foregoing alterations. Nevertheless, the level of alters is reliant on the stress period. Further research needs to be done with stress duration and recovery period to reveal the possible biochemical mechanism and other tissue damage by restrained stress. Further our particular interest experimentation needs to be done with these drugs to explore responsible for such activity and to elucidate the possible biochemical mechanism and at the level of ultrastructural of cerebellum particularly on the neuronal cells of cerebellar cortex which is one of the central areas where the organizational instances are principally evident.

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CHAPTER – 6

SUMMARY AND CONCLUSION

Stress has an impact to make changes in a range of anatomical, physiological, biological responses consist of psycho-physiological responses. In reaction to stressors, a sequence of behavioral, neurochemical, and immunological alterations happen that should to serve in an adaptive capability. It has been broadly studied on the stress effects on different organs of the body in both humans and animals and observed that severe stress lasting weeks or months can damage cell communiqué in the certain region of brain. Oxidative damage is a documented consequence of stress that has been involved in the pathogenesis of mood and anxiety disorders and resultant disturbances may differ depending upon type, intensity and the duration of a particular stressor and the strain/sex differentiation of the subjects.

Stress is able to be revealed as any inducement that creates an inequity in the homeostasis processes. The blood is one of the main homeostatic systems of the body preserving regular feasibility, integrity and adaptive responses. The efficient state of the blood systems changes animatedly according to the nature, effectiveness and period of exposure to exterior factors. Stress and emotional responses influence the immune system is by means of total blood counts, comprising the hematopoietic system, leukocyte outline or biochemical stress markers. In addition to this, other studies elucidated that serum cortisol levels and antioxidant vitamins C and E are a reliable indicator of stress responses in animals.

The antidepressants are a group of medicines employed to indulgence the signs of despair disorders by précising chemical inequities of neurotransmitters in the brain. The acute mechanisms of action of antidepressant drug's information guide to the common assurance to each effectual antidepressant medicines perform through boosting the activity

of serotonergic or noradrenergic system of brain. Alprazolam is a drug of choice for the management of anxiety disorder and panic disorders, on the other hand, high potency alprazolam defends against the unfavourable results from diverse types of stressors; relieves a few of stress consequences on the immune system and defends aligned with oxidative injure. Buspirone is an anxiolytic drug mainly or augment antidepressants employed to treat prevalent anxiety disorder. Reports revealed that buspirone perform like as antidepressant activities and commonly proposed that remedial effects of buspirone are not because of its instant action on the 5-HT system, but quite adaptive modification that happen following extended treatment. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), a first molecule of a new generation of antidepressants, effectively treats harmful effects of various types of stressors; attenuates effects of stress on haematological parameters and prevent from oxidative damage.

Based on the current available information on exposure to restrained stress standpoint, it was noticed that there was little information regarding the influence of antidepressants viz., alprazolam (benzodiazepine anti-anxiety agent), fluoxetine (a selective serotonin reuptake inhibitor) and buspirone (a non-benzodiazepine anxiolytic drug) on assessment of antistress effects in restrained rats. The majority of the studies were centered on their modifications of the immune function rather than the configuration. Thus, it was consideration to be of exacting attention to observe the antistress consequences of above said frequently employed drugs particularly on food consumption, gravimetry, organ weight, histopathology, haematological parameters, serum cortisol and antioxidant vitamin C and E levels in restrained stress albino rats at their maximum therapeutic doses.

Further, extensive research has been done on the consequences of stress on various hormones levels in the blood but information about reversible assessment of restrained stress after withdrawal in rats is lacking. Therefore, the current experimental studies have been designed to examine the consequence of restrained stress and its withdrawal possible alteration on gravimetry, organ weight, histopathology, haematological parameters and serum cortisol level in restrained stress rats. Thus our entire efforts have been directed for studying these novel commonly used antistress drugs to compare for their protective effects in restrained stress for 21 days in continuance with stress and, in contrast, the withdrawal of stress designed meant for residual 21 days in continuance with stress may probably neutralize such restrained stress provoked injure so as to escorting oxidant antioxidant equilibrium and change hypothalamic-pituitary adrenal (HPA) axis.

Acclimatized Wistar male albino rats weighing about 175 to 225g were obtained for the study and were randomly divided into six grouping of six animals each. Untreated control rats (Group I) reserved uninterrupted in the metabolic cage all through the experimental duration intended for 42 days; Stress induced rats (Group II) were strained daily for 6hrs in wire mesh restrainer intended for 42 days; Group III rats were stressed intended for 21 days by means of observance in mesh restrainer and then retaining animals in normal cages for residual 21 days designed for stress withdrawal and rest of other drug treated groups i.e., groups IV,V and VI rats were stressed intended for 21 days and then treated with alprazolam (5mg/kg body weight, intraperitoneally, BW, IP), buspirone (12mg/kg BW, IP) and fluoxetine drug (20mg/kg BW, IP), respectively for residual 21 days in continuation with stress. Drug doses and the testing time intervals were selected based on those reported in literature and conducted in our laboratory.

All animals were observed daily for mortality and morbidity, physical examinations and clinical observations like general appearance of the animal's behaviour; individual food consumption record of the animals; Gravimetry; using SYS MAX-35 automated cell counter machine, serum was then used for analyzing the haematological parameters; Biochemical parameters like assessments of vitamin C level in serum with the method of Roe and Koether, vitamin E level with modified Baker and frank method and cortisol level with the Enzyme-linked Immunosorbent Assay (ELISA) kit (DRG, USA) method. Finally, whole brain and relative organs of control and experimental rats subjected to histopathological evaluations.

From the data observed after the effect of restrained stress and its withdrawal and protective effects of antistress drugs for the possible alteration on remaining 21 days in continuation with stress following results are made:

1. Clinical observations: All the rats in groups continued active and well with normal behaviour during the entire period of experimentation.

2. Food consumption: A significant decrease in the final food intake ($p<0.05$) after 42 days of stress induced rats of group II (stress) though no alters in the final food consumption were observed in stress withdrawal group and antistress drugs as compared to control group rats.

3. Gravimetry: Stress induced rats found to be lethargic and had significant decrease in final body weight, however, administration of drug alprazolam, buspirone and fluoxetine showed noteworthy progress of BWs gain (%) contrast to stressed group.

4. Organosomatic index: There were no detectable abnormalities in relative organ weights (brain, liver, kidney, adrenal gland and testis) of control, withdrawal and antistress drugs

treated groups. However, stress induced group exhibit a major decrease ($P \leq 0.05$) in these virtual weight of organs when compare to the control and rest of treated groups.

5. Haematological parameters: A noteworthy decrease ($P \leq 0.05$) in Hb, total WBC and platelets and major increase ($P \leq 0.05$) in RBC, MCV, MCHC, neutrophils, lymphocytes, eosinophils and monocytes that stress induced group as compared to control and rest of groups. Stress withdrawal group and drug treated groups for remaining 21 day in continuation with stress showed remarkable improvement ($P \leq 0.05$) in lymphocytes count contrast to stress induced rats, however, some diversity in level of haematological parameters in withdrawal and drug treated groups, but the difference was insignificant against the control.

6. Biochemical parameters: The serum levels cortisol and antioxidant vitamins (C and E) were considerably increased ($P \leq 0.05$) in stress induced group whereas in stress withdrawal this alter reversed by dropping the cortisol level. The administration of antistress drugs (alprazolam, buspirone and fluoxetine) treated groups for remaining 21 day in continuation with stress showed greatly major decrease ($P \leq 0.05$) in mean serum levels cortisol and antioxidant vitamins contrast to stress induced group.

7. Histopathological evaluations: Stress induced (Group II) illustrated

a) Brain sections exhibit cerebral cortex with neuronal cells, mild focal vacuolar degeneration and there were no features of necrosis, infarcts, inflammation or glial proliferation. Most neuronal cells were of normal appearance however, few distorted in shape and emaciated with intensely stained nuclei and enclosed by vacuolated regions most likely apoptotic cells.

b) Liver sections show mild distorted 'Lobular' architecture of liver parenchyma and hepatocytes and appears to be little swollen having ill-defined cell borders with

variation in cellular size and shape. The cytoplasm is microvesicular and there are foci of fatty alter, distending deterioration and hepatocytes necrosis in centrilobular regions. The sinusoidal spaces are mildly widened. Central vein shows features of dilatation and congestion.

c) Kidney sections exhibit mild congestion of the renal blood vessels and renal tubules showed mild degenerative changes with presence of eosinophilic debris in their Lumina. There were features of mild thickening of tubular basement membrane, focal cloudy swelling of tubular cells and slight glomerulus's shrunken with normal appearance of interstitium and vessels.

d) Testis sections exhibit deteriorated tubules and detained spermatogenesis in majority of the tubules. Spermatogenesis detained either at the primary spermatocytes or else the spermatogonial stages. Some of tubules exhibited cytolysis of the whole spermatogenic rudiments. The Sertoli cells also illustrated the vacuolization with cell debris. The intercellular spacing becomes wider, Leydig cells were decreased in number or the interstitium holds mainly fibroblasts. There was occurrence of fibroblasts like rudiments above the Leydig cells with emaciated nuclei.

e) These histopathological changes were remarkably reversed in stress withdrawal rats (Group III) and neutralized to normal level in stress induced alprazolam (Group IV), buspirone (Group V) and fluoxetine (Group V) drugs treated animals designed meant for remaining 21 days with continuation with stress.

The outcomes presented at this point guide us to conclude so as to prologue to restrained stress consequences alterations in the levels of serum cortisol, haematological parameters and antioxidant levels, food consumption, gravimetry, organ weights and

histopathological in male albino rats. Withdrawal of stress or antistress drugs designed meant for residual 21 days in continuance through stress might possibly neutralize restrained stress provoked injure to escorting antioxidant equilibrium and modify hypothalamic-pituitary adrenal (HPA) axis (Figure 6.1). The exposure to restrained stress resulted in peripheral oxidative stress in male albino rats. These antistress drugs may perhaps a vital means connecting the defensive pharmacological consequences over restrained stress induced haematological, biochemical and histopathological adverse change.

The study therefore concluded that the treatment with these antistress drugs have a significant effect to counteract restrained stress induced foregoing alterations. Nevertheless, the level of alters is reliant on the stress period. Further research needs to be done with stress duration and recovery period to reveal the possible biochemical mechanism and other tissue damage by restrained stress. Further our particular interest in experimentation needs to be done with these drugs to explore such activity and to elucidate the possible biochemical mechanism and at the level of ultrastructural of cerebellum particularly on the neuronal cells of cerebellar cortex which is one of the central areas where the organizational instances are principally evident.

Annexure-1

List of the papers published from this thesis:

- **S.D.Desai, Rohini S. Kori and Ravindranath H. Aladakatti**. Reversible Assessment of Restrained Stress After Withdrawal In Male Albino Rats. J Pharma Sci Res, Vol. 9(10), 2017,1738-1742.
- **S.D.Desai, Rohini S. Kori and Ravindranath H. Aladakatti**. Effect of Anti-Stress Activity Of Buspirone On Restrained Stress Induced Male Albino Rats. J Pharma Sci Res, Vol. 9(10), 2017, 2068-2071

List of the conference attended and presented papers from this thesis:

- "Anti-stress activity of Fluoxitine on restrained stress induced male albino rats: A study on hematological parameters and whole brain histopathology" at "National Conference of Physiological Sciences (ASSOPICON-2016)" held on September 15-17, 2016, organized by Department of Physiology, BLDE University's Shri B M Patil Medical College, Hospital & Research Centre, Vijayapura. Oral presentation.

- "Effect of Alprazolam drug on restrained stress induced alteration of serum cortisol and antioxidant vitamins in male albino rats" at "VII International Conference on "Advances in Laboratory Animal Science for Modeling Human Diseases" held on October 14-15, 2016, jointly organized by Laboratory Association Scientists Association (LASA) of India and Biocon Bristol-Myers Squibb Research & Development Center (BBRC), Bengaluru. Poster presentation.

- "Effect of anti-stress activity of buspirone on restrained stress induced male albino rats" in the World Congress on Reproductive Health with Emphasis on Family Planning and Assisted Reproductive Technology and 28th Annual Meeting of the Indian Society for the Study of Reproduction and Fertility (ISSRF-2018) and the held on February 23-25, 2018, at CSIR-Indian Institute of Chemical Technology, Hyderabad. Poster presentation.

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BLDEU'S DEPT. OF PHARMACOLOGY BIJAPUR-586103. OUTWARD No. 699/12 Date 8/10/12 SHRI B.M. PATIL MEDICAL COLLEGE

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ETHICAL CLEARANCE CERTIFICATE

The Institutional Animal Ethics Committee (IAEC) of this College met on 06.09.2012 at 10.30am to scrutinize the Research Project submitted by you.

The queries raised by the Ethics Committee have been satisfactorily answered by you, hence the Ethical Clearance is accorded for your Research project.

Title: Effect of antistress drugs (alprazolam, buspirone, fluoxetine) on stress induced changes of brain and other organohistopathology in male albino rats.

Principal investigator: Ms. Rohini.S.Kori.

Dr. R. S. Wali
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08.10.2012

BLDE (DEEMED TO BE UNIVERSITY)

Annexure -I

PLAGARISM VERIFICATION CERTIFICATE

1. Name of the Student: KORI ROHINI SHARANAPPA Reg No. 11PHD001
2. Title of the Thesis: EFFECT OF ANTISTRESS DRUGS (ALPRAZOLAM, BUSPIRONE AND FLUOXETINE) ON STRESS INDUCED CHANGES OF BRAIN AND OTHER ORGANOHISTOPATHOLOGY IN MALE ALBINO RATS
3. Department: ANATOMY
4. Name of the Guide & Designation: DR. S. D. DESAI PROFESSOR
5. Name of the Co Guide & Designation: DR. K. K. DAS PROFESSOR

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Name & Designation

Prof. Kusal K. Das PhD
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Verified by (Signature)
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Date: 28/8/2018

From,
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BLDE (Deemed To Be University)
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To,
The Registrar,
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SUB: SUBMISSION OF MY THESIS

(THROUGH PROPER CHANNEL)

Respected sir,

With respect to the above cited subject and reference and as per the University norms, I am herewith submitting six hard copies and four soft copies (CD) of my thesis with title "**Effect of antistress drugs (Alprazolam, Buspirone and Fluoxitine) on stress induced changes of brain and other organohistopathology in male albino rats**".

I kindly request you to accept this and do the needful.

Enclosure: Fees paid challan
Six hard copies of thesis
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Signature of Student

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