THESIS

ON

THERAPEUTIC USE OF NEUROPROTECTIVE NUTRACEUTICALS IN EXPERIMENTAL NEUROLOGIC DISORDER

This thesis is prepared for the partial fulfillment of the requirements for the degree of Master of Philosophy (M.Phil.) of University of Dhaka, Bangladesh.

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Dedication

I dedicate this thesis to my beloved family for their earnest support, phenomenal inspiration and unequivocal love.

Certificate

This is to certify that the thesis entitled 'Therapeutic Use of Neuroprotective Nutraceuticals in Experimental Neurologic Disorder' has been completed sincerely and satisfactorily by Sheikh Khalid Saifullah Sadi, registration no. 12, session 2012-2013, enrolled in University of Dhaka, Dhaka-1000, Bangladesh, for the degree of Master of Philosophy (MPhil) in Nutrition and Food Science, is an original record and was supervised by me and can be submitted to the examination committee for evaluation.

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THERAPEUTIC USE OF NEUROPROTECTIVE NUTRACEUTICALS IN EXPERIMENTAL NEUROLOGIC DISORDER

is submitted for the degree of the Master of Philosophy (M.Phil.) in accordance with the rules and regulations of the University of Dhaka. The research described herein was conducted under the supervision of Prof. Dr. M. Akhtaruzzaman, Institute of Nutrition and Food Science, University of Dhaka, from September 2016 to December 2016.

This work is to the best of my knowledge original, unpublished. No similar dissertation has been or is being submitted for any other degree, diploma or any other qualification at any other university.

Date:

_____

Sheikh Khalid Saifullah Sadi

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Author

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Abstract

Background

Neurologic disorder refers to dysfunction in any part of the brain or nervous system. Depression is a neurologic disorder characterized by low mood with physical and psychological defects. Stress induced depression is common and it is induced by psychosocial stress. Omega-3 fatty acids are nutraceuticals that have potential benefit in neurologic disorders for their antioxidant, anti-inflammatory and proregenerative effects. Ascorbic acid and zinc have also neuroprotective properties due to their antioxidant activity.

Research objective

The aim of this study is to evaluate the neuroprotective potential of nutraceuticals (Omega-3 fatty acids, ascorbic acid and zinc) in stress induced neurological disorder in experimental animal model.

Research Methodology

The study was conducted on rat models during September -December 2016. A total of fifty (50) healthy Wistar albino rats, weighing between 100-120 grams of age ranging 50-60 days were included in this study. Neurologic disorder (depression) was induced by physical and chemical stress techniques. Physical stress induced depression was made in rat model through confinement and light exposure for 21 days. Chemical stress induced depression was made by administering chemical stressor (reserpine) for 21 days. Each model was divided into three groups, where they were administered nutraceuticals (Omega 3 fatty acids, ascorbic acid and zinc), antidepressant (clomipramine) and placebo accordingly for 21 days. Seven rats were taken as baseline control group. In order to assess the change in stress induced depression, behavioral tests- Forced Swim Test (FST) and Tail Suspension Test (TST) was conducted, oxidative stress marker Malondialdehyde (MDA) and fasting blood glucose levels were estimated. Adrenal gland and brain was extracted and weighed. Data analysis was done by SPSS v22 and Microsoft Excel 2013. ANOVA and Tukey post hoc test were done to analyze the data.

Results

In physical stress model, behavioral tests showed that the period of climbing ($p<0.001$) and swimming ($p<0.01$) were significantly higher and period of immobility was significantly lower ($p<0.001$) in forced swim test in nutraceutical treated group in comparison to that of depressed control group and were almost similar to antidepressant treated control group and baseline control group. In tail suspension test, period of immobility was also significantly lower ($p<0.001$) in nutraceutical treated group. Analysis of biochemical stress indicators showed that malondialdehyde ($p<0.01$) and fasting blood glucose level ($p<0.001$) were significantly lower in nutraceutical treated group than the depressed control group. The percent (%) change of body weight was significantly higher ($p<0.01$), adrenal gland weight was significantly lower ($p<0.001$) and brain weight was significantly higher ($p<0.001$) in nutraceutical treated group compared to that of depressed control group.

In chemical stress model, behavioral tests analysis of biochemical stress indicators and estimation of percent change of body weight, adrenal gland and brain weight revealed similar findings in the nutraceutical treated group.

Conclusion

In physical and chemical stress models, it was evident that therapeutic use of nutraceuticals led to a decline of depressive symptoms which was also revealed in laboratory findings. It can be concluded that nutraceuticals (omega 3 fatty acid, ascorbic acid and zinc) have neuroprotective potential.

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ACRONYMS

STRID:	Stress Induced Depression
WHO:	World Health Organization
LNA:	α-Linolenic Acid
DHA:	Docosahexaenoic Acid
EPA:	Eicosapentaenoic Acid
ROS:	Reactive Oxygen Species
YLD:	Years Lived with Disability
DALY:	Disability Adjusted Life
MAO:	Monoamine Oxidase
FST:	Forced Swim Test
TST:	Tail Suspension Test
MDA:	Malondialdehyde
FBS:	Fasting Blood Sugar
OD:	Optical Density
ACTH:	Adrenocorticotropic hormone

CHAPTER I
INTRODUCTION

1.1 Introduction

Neurologic disorder refers to dysfunction in any part of the brain or nervous system. The causes of neurological problems may include genetic disorders, congenital abnormalities, infections, lifestyle or environmental health problems such as stress, malnutrition and also injury to the brain, spinal cord or nerves. Structural, biochemical or electrical abnormalities in the nervous system can cause a range of symptoms such as paralysis, muscle weakness, poor coordination, loss of sensation, seizures, confusion, pain and altered levels of consciousness; thus causing disorders.[1]

Depression is a neurologic disorder characterized by low mood with physical and psychological defects. [2] It is a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings, and sense of well-being. Several types of major depressive disorders are present, but major depressive disorder or unipolar depression is most common. Sleep disturbances, weight gain or weight loss, feelings of guilt, psychomotor agitation, indecisiveness and suicidal tendencies are often associated with depressive disorders. Major causative factor for depression is inflammation, autoimmune tissue damage and prolonged psychological stress, which eventually leads to oxidative stress.[3, 4] Depression has been associated with increase in oxidative stress.[4, 6]

Stress is a state of disturbed homeostasis inducing somatic and mental adaptive reactions. Stress can be external (environment, psychological) or internal (illness, medication). It triggers a series of neuroendocrine events in the hypothalamic pituitary adrenal (HPA) axis leading to a rise in stress hormone (cortisol in human and corticosterone in rodents). Sustained or chronic stress leads to decrease in monoamines (serotonin, noradrenaline, dopamine), which have been linked to depression.[5] This type of depression is known as 'stress-induced depression (STRID).

The term "nutraceutical" combines two words – "nutrient" (a nourishing food component) and "pharmaceutical" (a medical drug) and is used to describe any product derived from food sources with extra health benefits in addition to the basic nutritional value found in foods. They can be considered non-specific biological therapies used to promote general well-being, control symptoms and prevent malignant processes. Omega-3 fatty acids, ascorbic acid and zinc function as neuroprotective nutraceuticals.

Omega-3 PUFAs include α-linolenic acid (LNA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). LNA is the most common omega-3 fatty acid in terrestrial diets; found in flaxseed oil and walnuts. EPA and DHA can also

be directly obtained from food of marine origin, for example, oily fish such as salmon, mackerel or trout Omega-3 fatty acids have potential benefit in neurologic disorders for their antioxidant, anti-inflammatory and proregenerative effects.[7]

Vitamin C or ascorbic acid is a water soluble vitamin abundant in citrus fruits. It serves as a co-factor in several important enzyme reactions. As an electron donor, vitamin C is a potent antioxidant in humans. It exerts neuroprotective function by altering oxidative stress. [8]

Zinc is a micro mineral found in oysters, red meat, poultry, legumes and nuts. It plays essential roles in the central nervous system across the lifespan from early neonatal brain development through the maintenance of brain function in adults. It exerts its neuroprotective property by its antioxidant activity by being effective in decreasing reactive oxygen species (ROS). [9]

1.2 Rationale

Depression is a severe, life-threatening, and neuropsychiatric disorder having an incidence of about 350 million cases globally. It ranks fifth among leading causes of global disease burden including developing countries, and by year 2030 it is predicted to represent one of the three leading causes of burden of disease worldwide.[11] World Health Organization (WHO) states that depression is the leading cause of disability as measured by Years Lived with Disability (YLDs) and the fourth leading contributor to the global burden of disease. By the year 2020, depression is projected to reach second place in the ranking of Disability Adjusted Life Years (DALY) calculated for all ages.[12]

Nowadays many antidepressant drugs are available and when initially consumed, they generally cause individuals to feel relaxed and calm. But prolong use of antidepressants can drastically affect an individual's ability to function normally, and has many physical effects as well. People who take antidepressants for extended time periods generally experience loss of libido, inability to achieve orgasm, weight gain, migraine headaches and sleep deprivation.[13] Many individuals also claim that the symptoms of their depression have enhanced after long-term antidepressant use. Therefore demands for safer natural products are increasing in modern times.

Nutraceuticals (omega 3 fatty acids, ascorbic acid and zinc) are reasonably safer and readily available either in raw form in sources or in commercial preparations as drug supplements. Globally there has been numerous studies regarding these nutraceuticals. But there is no study data regarding their therapeutic use in neuroprotection in stress induced depression in Bangladesh. It is expected that the finding of this study will be beneficial for the physician as well as depressed patients for better management and thereby maintaining their quality of life.

1.3 Operational Definitions

Nutraceutical: A foodstuff (as a fortified food or a dietary supplement) that provides health or medical benefits in addition to its basic nutritional value.

Neurologic Disorder: Disease of the central and peripheral nervous system.

Depression: Persistent sadness or low mood accompanied by physical and psychological symptoms of at least two weeks in duration.

Stress: A physical, mental, or emotional factor that causes bodily or mental tension.

Oxidative Stress: an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.

Forced Swim Test: A rodent behavioral test used for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states.

Tail Suspension Test: An experimental method used in scientific research to measure stress in rodents. It is based on the observation that if a rat is subjected to short term inescapable stress then the rat will become immobile.

MDA: A highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. It is a marker of oxidative stress.

FBS: The amount of glucose dissolved in circulating blood, recorded irrespective of when food was last ingested; two consecutive RBG recordings >10 mmol/L are strongly indicative of diabetes mellitus.

Brain: An organ of soft nervous tissue contained in the skull of vertebrates, functioning as the coordinating center of sensation and intellectual and nervous activity.

Adrenal Gland: Endocrine glands that produce a variety of hormones including adrenaline and the steroids aldosterone and cortisol. They are found above the kidneys. Each gland has an outer cortex which produces steroid hormones and an inner medulla.

Ascorbic Acid: A vitamin found particularly in citrus fruits and green vegetables. It is essential in maintaining healthy connective tissue, and is also acts as an antioxidant.

Zinc: An essential micronutrient found seafood, meat and legumes. It is an essential element for metabolic processes in the body and normal growth and development during pregnancy, childhood, and adolescence. Has role as an antioxidant and anti-Inflammatory agent.

Optical Density: The degree to which a refractive medium retards transmitted rays of light. It is measured in a spectrophotometer.

1.4 Research Question

What is the neuroprotective potential of nutraceuticals (Omega-3 fatty acids, ascorbic acid and zinc) in stress induced neurological disorder (stress induced depression) in experimental animal model?

CHAPTER II

LITERATURE REVIEW

2.0 Literature review

Over several years many studies have been done about neuroprotective agents and animal depression models globally. I have summarized some of the research that I have gone through.

According to an experimental study conducted by Negreş et al. (2013) a model of depression was induced by administration of reserpine. Three doses of reserpine were tested (0.5mg/kg-bw, 0.75 mg/kg-bw, 1.5 mg/kg-bw). Depression onset evaluation was performed at 2 moments: after 11 and after 21 days of reserpine administration, both by classical pharmacological tests and also by determining the cerebral activity of monoamine oxidase (MAO). The experimental data revealed, after 21 days of treatment, for all doses used, modification of investigated parameters towards onset of depressive phenomenon. This way, compared to control group, for the animals submitted to forced swimming test, immobilizing time increased by more than 80% and MAO activity decreased dose-dependently (-39.92% for 0.5 mg/kg-bw dose; -40.31% for 0.75 mg/kg-bw dose; -40.61% for 1.5 mg/kg-bw dose). These effects were in correlation with a significant reduction of motor activity. [5]

Nasir and khan (2011) conducted a study on 15 adult albino rats (200-250gm) divided into control and experimental groups. First group was control, second group received acute depression (4 weeks) by immobilization method using rat immobilizer and third group received standard 4 week treatment (using Fluoxetine 1mg/kg body wt. orally) following acute depression. Total general activity was reduced markedly in depressed rats. At the end of the experiment animals were sacrificed and perfused with 10% formaldehyde. Brains were dissected and tissue blocks were processed for paraffin embedding. Observations were made on 10 micron thick H & E stained sections and neuronal density was estimated. Neuronal density was markedly reduced (100.3 cells/cubic mm) after acute depression, as compared to control (144.5 cells/cubic mm) and after standard treatment it improved to 121.1 cells /cubic mm. These results suggested that effect of short-term stress-induced depression on hippocampus is partly reversed by the pharmacological intervention of known antidepressants.[14]

Nishizawa et al. (2007) studied the antidepressant like effect of *cordyceps sinensis* by tail suspension test in 17 mice. In the study extract of mushroom was administered at different doses of 2.5 mg, 5 mg and 10 mg/kg body weight for 5 days. The researchers observed that the period of immobility in tail suspension test was gradually decreased as

the dose increased, and was dose dependent. Significant decrease was found in the latter higher doses. They suggested that the antidepressant like effect of *cordyceps sinensis* is due to some of its constituents which act like adrenoceptor and dopamine D_2 receptor agonists or noradrenaline/dopamine reuptake inhibitors thereby increasing levels of monoamines.[15]

Dhingra et al. (2012) conducted a study on antidepressant like activity of *Emblica officinalis* (amla) in 60 Swiss young male albino mice by forced swim test. They observed that administration of aqueous extract (200 mg and 400 mg/kg orally) of the fruits for 14 successive days decreased the immobility period in forced swim test but not in dose dependent manner. However, the lower dose (200 mg/kg) of the extract showed better antidepressant like action.[16]

Kenjale et al. (2007) conducted a study to see the effect of *chlorophytum borivilianum* on the weight of adrenal glands of 24 chronic stressed rats. In this study the extract was given in doses of 125 mg and 150 mg/kg body weight for 7 days. The researchers observed that the weight of the adrenal gland decreased as the dose increased and the maximum difference was found at the higher dose. It indicated that the effect was dose dependent and this may be due to presence of its active constituent alkaloids and saponins.[17]

Nayanatara et al (2013) conducted a study designed to elucidate the influence of acute and chronic stress on different brain tissues of Wistar albino rats. The animals were divided into two major groups as non-stressed group (n =10) and stressed group (n =10). The stressed groups were divided into acute (one day) stress groups and chronic (30 days) groups. The animals of the stressed groups were subjected to acute and chronic types of swimming stress and immobilization stress. Lipid peroxidation by MDA was estimated in cerebral cortex, hypothalamus and cerebellum. Acute swimming stress and chronic immobilization stress significantly ($P<0.001$) increased the lipid peroxidation level in the cerebral cortex and hypothalamus. Whereas, acute immobilization stress and chronic swimming stress increased ($P<0.001$) the cerebellar lipid peroxidation. The observed regional specific alterations in lipid peroxidation levels show that the nature of the stressors are probably capable of generating a different degree of cellular imbalance in between pro-oxidants and antioxidants which may provide some approach on variety of neurological and psychological processes as well as their treatment.[18]

A study conducted by Siraji et al (2004) assessed the effect of *Ocimum sanctum linn* (Tulsi) on body weight and some biochemical parameters in restraint stressed albino rats. The body weight in untreated stressed group was significantly lower ($p<0.001$) than those of the control group and Tulsi pretreated group. Serum levels of glucose, cholesterol, aminotransferases (ALT and AST) were significantly higher ($p<0.001$) in stressed group than those of control. Again in Tulsi treated group all these biochemical parameters were significantly lower ($p<0.001$) than those of stressed group. Prevention of stress induced changes in biochemical parameter by Tulsi pre-treatment indicates its anti-stressor effect. [19]

In another study done by Rabiei (2016), anti-depressant activities of *Mentha pulegium* was investigated. Six experimental groups (7 mice each) were used. Forced swim test was performed 30 min after essential oil injection. In the groups receiving *M. pulegium* essential oil (50, 75 and 100 mg/kg), immobility duration significantly decreased compared to the control group. Regarding the immobility duration in the FST, a significant difference was observed between the baseline controls, positive controls, negative controls and all the treated groups ($p<0.01$). *M. pulegium* (50 and 75 mg/kg) resulted in significant decrease in nitrate/nitrite content in serum compared to the baseline control group. Antidepressant effect that may be due to the inhibition of oxidative stress. The results showed that decrease in nitrate/nitrite content in serum and high anti-oxidant effects of *M. pulegium* essential oil. [20]

CHAPTER III

RESEARCH METHODOGY

3.1 Study Objectives

General objective:

To evaluate the neuroprotective potential of nutraceuticals (Omega-3 fatty acids, ascorbic acid and zinc) in stress induced neurological disorder in experimental animal model.

Specific Objective:

- To measure the body weight, weight of adrenal gland and brain of all the rats after the experiment.
- To perform Forced swim test, Tail suspension test of rats to observe their behavioral changes.
- To estimate fasting blood glucose levels of rats and assess their levels.
- To estimate Malondialdehyde (MDA) levels of rats and assess their levels.
- To compare the estimated parameters between groups.

3.2 Study Design

The study design was an experimental study based on animal model.

3.3 Study Site

Institute of Nutrition and Food Science, University of Dhaka.

3.4 Study Period

September 2016-December 2016

3.5 Sample Population

A Total of fifty (50) healthy Wistar albino rats, weighing between 100-120 grams (initial body weight) age ranging from 50-60 days were included in this study. Animals were collected from animal house of Zoology department, Jahangirnagar University, Savar.

3.6 Sampling Technique

Sample size for each experimental model was estimated using the resource equation method.[21]

3.7 Sample Size

According resource equation method,

E = Total number of animals (n x N) - Total number of groups (N)

Here n= number of animals in each group

N=Number of Groups

In our study

$$n = 7, N=4$$

$$E = 7 \times 4 - 4$$

$$= 28-4 = 24$$

Hence seven rats (n=7) per group for four groups can be considered as an appropriate sample size.

(Here $10< E< 20$ for optimum sample size. If a value of E is less than 10 then more animal should be included)

3.8 Inclusion &Exclusion Criteria

Inclusion criteria

- Wistar Albino rats
- 50-60 days old rats
- Weighing between 100 to 120 grams
- Healthy rats

Exclusion criteria

i. Unhealthy or diseased rats

3.9 Data Collection Tools

An experiment flow sheet based on the objectives and variables was used for conducting the tests in each experimental model. It consisted of behavioral tests- FST and TST, Biochemical tests- measurement of FBG and MDA; lastly weighing of brain and adrenal gland samples after dissection.

3.10 Data Management and Analysis

The data for this study was analyzed by using Statistical Package Social Science (SPSS) software version 22 and Microsoft Excel 2013. ANOVA and Tukey post hoc test were done to analyze the data.

3.11 Validation of the study

The experimental models were pre-tested via pilot experiments prior to commencement of the study to evaluate the effectiveness of each model.

3.12 Quality Control and Quality Assurance

All data processing work included registration of schedules, checking, re-checking, editing, coding and computerization, preparation of dummy tables and lastly analysis of data.

3.13 Ethical Considerations

Ethical permission was taken prior conducting the study from the office of the Dean of Biological Sciences, University of Dhaka.

CHAPTER IV

STUDY PROCEDURE

4.1 Study Procedure

After selection of rats on the basis of selection criteria they were acclimatized in the animal house of Institute of Nutrition and Food Science, University of Dhaka for 14 days prior to intervention. Total study period was 21 days for each experimental model. To observe behavioral changes Forced Swim Test (FST) and Tail Suspension Test (TST) of all animals were performed on day 22 of each experimental model. All the animals were sacrificed on day 23. Then blood samples were collected for estimation of fasting blood glucose (FBG) and Malondialdehyde (MDA). Then adrenal gland and brain samples were collected after meticulous dissection. All behavioral tests were done by using standard methods in the animal house of INFS, University of Dhaka. FBG was estimated using a standard glucometer and MDA was estimated by the thiobarbituric acid assay.[22] Adrenal gland and brain samples were weighed using electric balance analyzer.

4.2 Grouping

After acclimatization for 14 days rats were divided into groups. Each Model consisted of four groups

Baseline Control Group, A

This group consisted of seven (7) Wistar albino rats. They were given basal diet for 21 consecutive days. No stress or intervention was given.

Experimental Group (Nutraceutical Group, B)

This group consisted of seven (7) Wistar albino rats. They were given basal diet for 21 consecutive days. This group was also given Nutraceuticals (omega3 fatty acids 415 mg/kg, zinc 4.15 mg/kg and ascorbic acid 51.46 mg/kg body weight) orally daily in the morning.

Positive Control Group (Antidepressant clomipramine group, C)

This group consisted of seven (7) Wistar albino rats. They were given basal diet for 21 consecutive days. This group also received antidepressant drug clomipramine (12.65mg/kg) orally daily in the morning.

Negative Control Group (Stress induced depressed control group, D)

This group consisted of seven (7) Wistar albino rats. They were given basal diet for 21 consecutive days. Either physical stress by containing and light exposure or chemical stress by administering reserpine was applied daily, according to the model concerned. No nutraceutical or drug intervention was given.

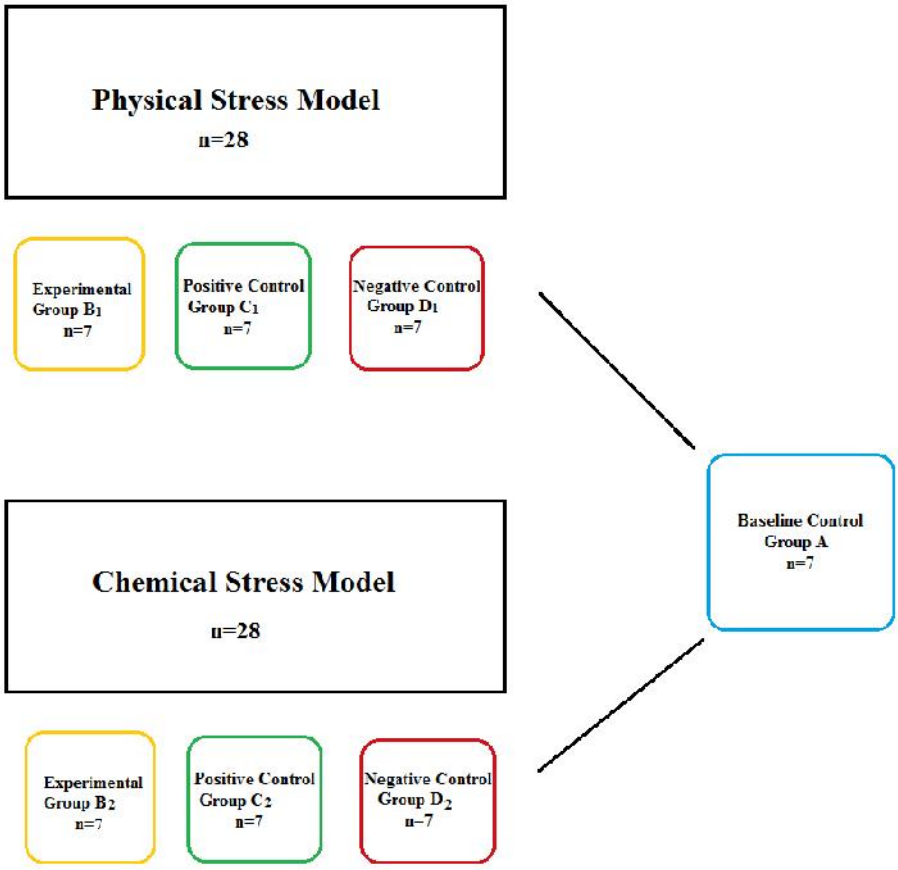

Fig 1: Grouping in Experimental Models

4.3 Stress procedure

All animals except baseline control group were exposed to stress. Two models were used for application of stress:

1. **Physical Stress Experimental Model** (rats were placed in special constrained cages and were exposed to light during night for 21 days. Each cage housed 3 rats in a 9”x 3”x 6” dimension space for each rat)
2. **Chemical Stress Experimental Model** (stress was induced by administering reserpine 0.38mg/kg body weight orally for 21 days)

4.4 Study parameters

i. Body weight, weight of adrenal gland and brain

ii. Behavioral tests
   - Forced Swim Test
   - Tail Suspension Test

iii. Biochemical tests
   - Estimation of MDA
   - Estimation of FBG

4.5 Behavioral tests

FST (Porsolt et al. 1977)

All rats were forced to swim individually in a cylinder (40 cm height and 15 cm diameter), containing fresh water (temperature 22^{o}C) up to a height of 30 cm for 5 minutes. The total duration of swimming, climbing and immobility in the last 4 minutes of the test session was recorded.[23]

TST (Steru et al. 1985)

All rats were individually suspended on the edge of a table, 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal was visually and acoustically isolated from other animals during the test. The total period of immobility was recorded manually for five minutes.[24]

4.6 Specimen collection

Collection of Blood Sample

On 21st day all rats were anaesthetized with the help of chloroform (30%) and sacrificed. From all rats, blood samples (approximately 3 ml) were collected from heart using sterile disposable syringes and were taken in separate clean and dry test tubes with proper identification numbers. One drop of blood was taken from each sample for FBG estimation. Then blood was centrifuged at a rate of 4000 rpm for 5 mins after that supernatant serum was collected in labelled Eppendorf tube and preserved in a refrigerator for biochemical analysis.

Collection of adrenal gland sample

From each rat, one adrenal gland was extracted and washed in saline, wiped in tissue paper, weighed and recorded. The weight was measured by electric balance analyzer. The gland samples were preserved in 10% formalin.

Collection of brain sample

From each rat, brain was removed by meticulous dissection and washed in saline, wiped in tissue paper, weighed and recorded. The weight was measured by electric balance analyzer. The gland samples were preserved in 10% formalin.

4.7 Biochemical tests

Fasting Blood Glucose Estimation

Normal range of fasting Blood glucose level is 3.5-5.5 mmol/l. Serum blood glucose level increases significantly in stressed rats.[19, 25]

Fasting blood glucose was done by using a standard glucometer.

Malondialdehyde (MDA) level estimation

It is an organic compound with the formula $CH_2(CHO)_2$. It his reactive species occurs naturally and is a marker for oxidative stress. Depression is associated with raised MDA levels.[6]

Malondialdehyde (MDA) level estimation was done by thiobarbituric acid assay. Here 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 N hydrochloric acid is used as reagent (TCA-TBA-HCl). 1.0 ml of biological sample is combined with 2.0 ml of TCA-TBA-HCl and mixed thoroughly. The solution is heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate is removed by centrifugation at 1000 g for 10 minutes. The absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the biological sample. Serum MDA is measured by using the following formula:

MDA concentration, $c = OD/b\ \varepsilon$

Here

Extinction coefficient $\varepsilon = 1.56 \times 10^5\ M^{-1}cm^{-1}$

Width of tube b = 1 cm

OD= Optical density at 535nm

CHAPTER V

RESULTS

5.1 PHYSICAL STRESS EXPERIMENTAL MODEL

Table 1.1: Body weight, weight of adrenal gland and brain in different groups of rats (n=28)

Groups	Body Weight		% change of Body Weight	Weight of Adrenal gland (gm)	Weight of Brain (gm)
	Initial	Final			
A(n=7)	106.14 ±3.53	116.57±3.77	9.84± 2.32	0.0138± 0.001	1.215±0.037
	(100-110)	(112-122)	(6.67-12.96)	(0.012-0.016)	(1.178-1.266)
B_1(n=7)	106.85±4.41,	114.57±5.12	7.23±2.83	0.0147±0.001	1.130±0.031
	(110-114)	(108-120)	(2.86-11.32)	(0.013-0.018)	(1.089-1.173)
C_1(n=7)	105.85±4.33	113.28±5.12	7.04±3.50	0.0156±0.003	1.123±0.022
	(112-110)	(105-118)	(2.94-12.0)	(0.012-0.022)	(1.092-1.152)
D_1(n=7)	106.28±3.86	109.85±4.09	3.36±1.19	0.028±0.007	1.043±0.017
	(100-110)	(104-115)	(1.82-4.76)	(0.018-0.038)	(1.023-1.066)

Statistical Analysis

ANOVA	F=0.075 F=0.973	F=2.665 P=0.071	F=7.329 P=0.001*	F=20.21 P=0.0001*	F=43.56 P=0.0001*
Tukey's Test					
A Vs B_1	P= 0.987	P=0.845	P=0.265	P=0.970	P=0.0001*
A Vs C_1	P=0.999	P=0.545	P=0.212	P=0.808	P=0.0001*
A Vs D_1	P=1.000	P=0.51	P=0.001*	P=0.0001*	P=0.0001*
B_1 Vs D_1	P=0.993	P=0.243	P=0.048*	P=0.0001*	P=0.0001*
C_1 Vs D_1	P=0.997	P=0.510	P=0.064	P=0.0001*	P=0.0001*
B_1 Vs C_1	P=0.967	P=0.952	P=0.999	P=0.970	P=0.956

*Significant<0.05

Control group

Group A (Baseline Control Group)

Group C_1 (Positive Control, antidepressant treated depressed control group)

Group D_1 (Negative Control, Depressed Control Group)

Experimental Group

Group B_1 (Nutraceutical treated depressed control group)

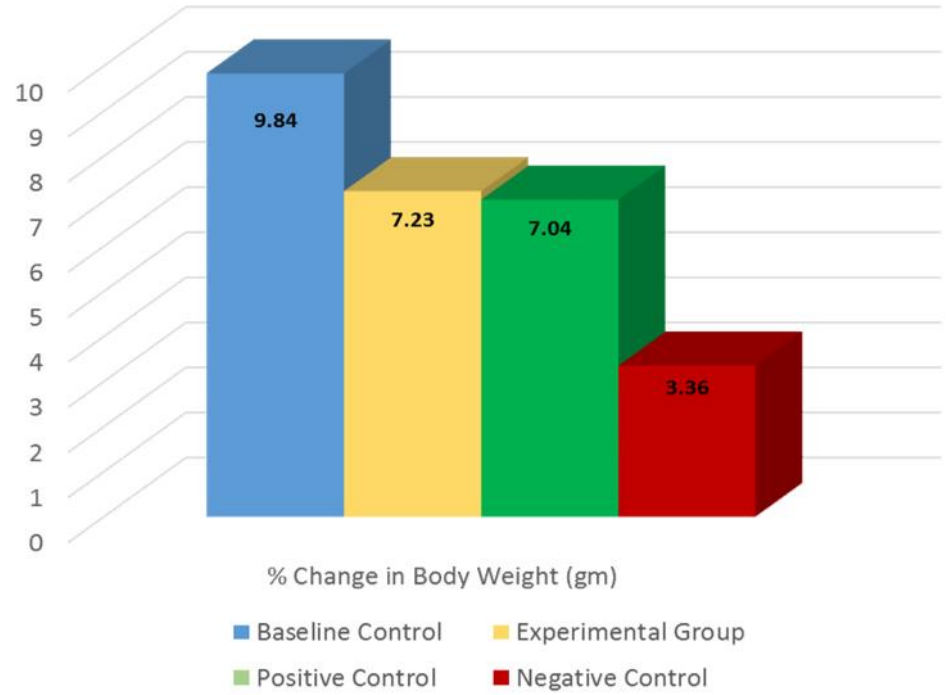

Fig 2.1: Percent change in body weight in different groups of rats (n=28)

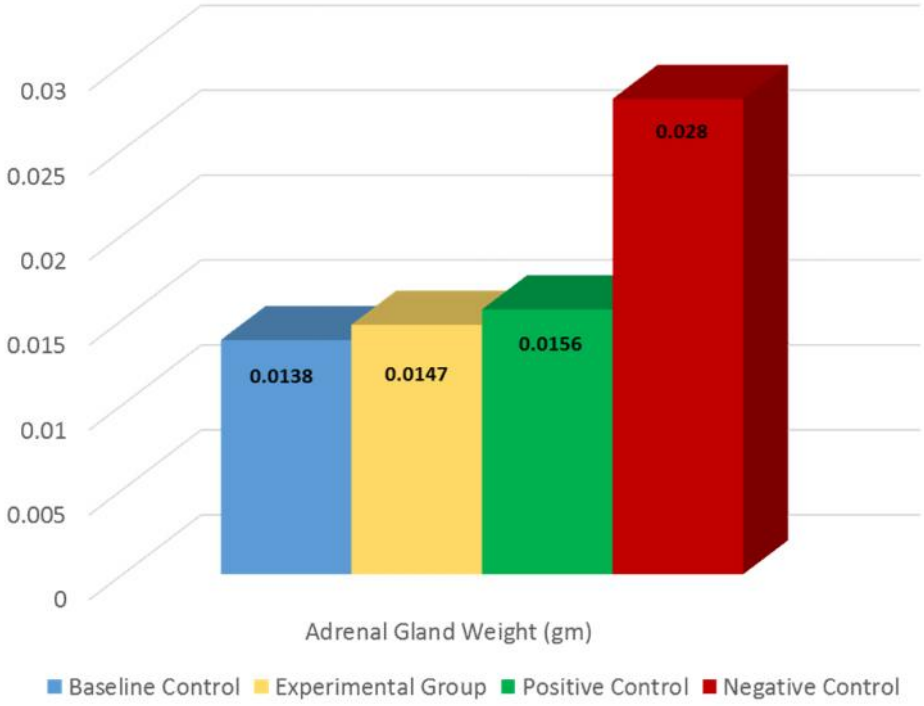

Fig 2.2: Weight of adrenal gland in different groups of rats (n=28)

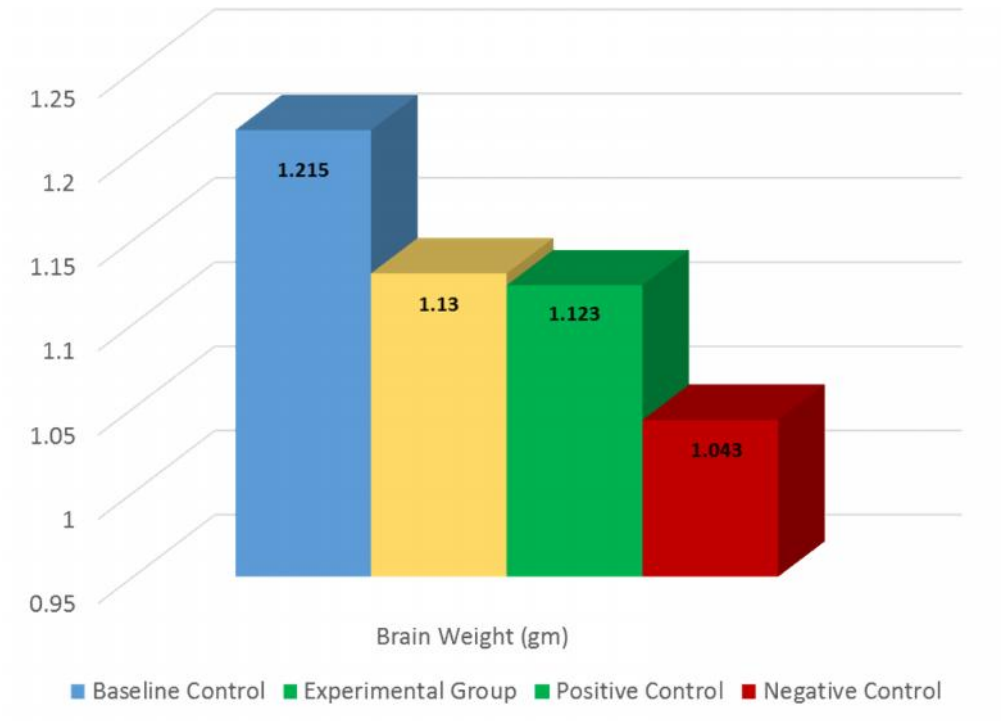

Fig 2.3: Weight of brain in different groups of rats (n=28)

The mean (±SD) initial body weights (day 1) were 106.14 ±3.53, 106.85±4.41, 105.85±4.33 and 106.28±3.86 and the final body weights (day 21) were 116.57 ±3.77, 114.57 ± 5.12, 113.28± 5.12 and 109.85±4.09 in group A, B_1, C_1 and D_1 respectively. Again, the percent (%) change in body weights from final to initial were 9.84± 2.32, 7.23±2.83, 7.04±3.50 and 3.36±1.19 respectively.

The weight of the adrenal glands were 0.0138± 0.001, 0.0147±0.001, 0.0156±0.003 and 0.028±0.007gm and the weight of the brains were 1.215±0.037, 1.130±0.031, 1.123±0.022 and 1.043±0.017 in group A, B_1, C_1 and D_1 respectively.

The mean (±SD) percent (%) change in body weight was significantly lower in group D_1 ($P<0.01$) compared to that of group A and B_1.

The mean (±SD) weight of adrenal gland was significantly higher in group D_1 ($p<0.001$) compared to that of group A, B_1 and C_1. The weights were almost similar and the difference was not statistically significant between groups A, B_1 and C_1.

The mean (±SD) weight of brain was significantly lower in group D_1 ($p<0.001$) compared to that of group A, B_1 and C_1. Again, the weights were similar and difference was not statistically significant between groups B_1 and C_1.

Table 1.2: Parameters of behavioral changes in different groups of rats (n=28)

Groups	Tail Suspension Test (second)	Forced Swim Test (second)		
		Climbing	Swimming	Immobility
A(n=7)	13.14±3.97	135.14±6.69	87.14±4.41	17.29±3.45
	(8-19)	(125-145)	(82-93)	(13-22)
B_1(n=7)	11.71±2.81,	123.43±9.48,	87.43±5.79	29.29±5.15
	(8-15)	(108-135)	(81-97)	(22-35)
C_1(n=7)	14.14±2.41	118.71±7.45	80.71±5.28	36.86±7.77
	(11-18)	(105-128)	(74-88)	(28-50)
D_1(n=7)	26.29±4.60	84.14±8.49	76.57±7.06	97.86±13.27
	(20-34)	(68-92)	(70-88)	(82-116)

Statistical Analysis

ANOVA	F=24.88 P=0.0001*	F=51.28 P=0.0001*	F=5.93 P=0.004*	F=131.4 P=0.0001*
Tukey's Test				
A Vs B_1	P=0.876	P=0.056	P=1.0	P=0.56
A Vs C_1	P=0.952	P=0.005*	P=0.181	P=0.001*
A Vs D_1	P=0.0001*	P=0.0001*	P=0.010*	P=0.0001*
B_1 Vs D_1	P=0.0001*	P=0.0001*	P=0.008*	P=0.0001*
C_1 Vs D_1	P=0.0001*	P=0.0001*	P=0.539	P=0.0001*
B_1 Vs C_1	P=0.587	P=0.70	P=0.153	P=0.342

*Significant<0.05
Control group
Group A (Baseline Control Group)
Group C_1 (Positive Control, antidepressant treated depressed control group)
Group D_1 (Negative Control, Depressed Control Group)
Experimental Group
Group B_1 (Nutraceutical treated depressed control group)

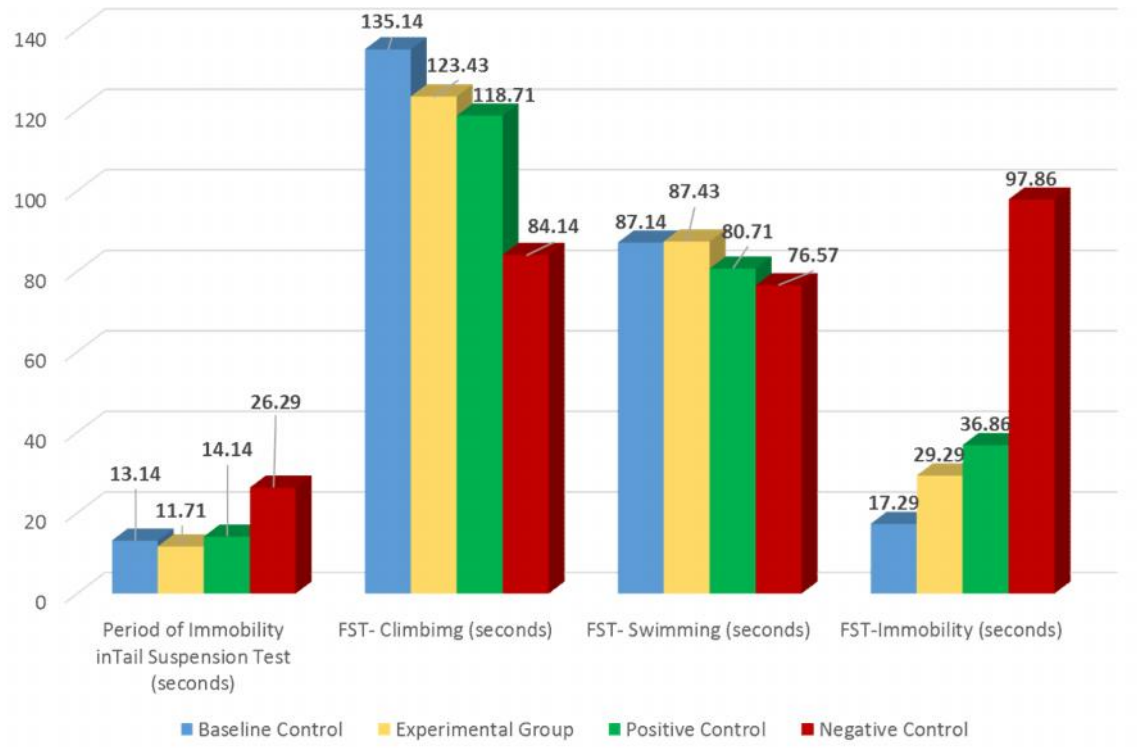

Fig 2.4: Parameters of behavioral changes in different groups of rats (n=28)

In FST the mean period (±SD) of climbing were 135.14±6.69, 123.43±9.48, 118.71±7.455 and 84.14±8.49 seconds; mean period of swimming were 87.14±4.41, 87.43±5.79, 80.71±5.28 and 76.57±7.06 seconds and period of immobility were 17.29±3.45, 29.29±5.15, 36.86±7.77 and 97.86±13.27 in group A, B_1, C_1 and D_1 respectively.

The mean (±SD) period of climbing in forced swim test was significantly lower ($p<0.001$) in group D_1 in comparison to that of group A, B_1 and C_1. Again, this period was almost similar and the difference was not statistically significant between groups A vs B_1 or B_1 vs C_1.

The mean (±SD) period of swimming in forced swim test was significantly lower ($p<0.01$) in group D_1 in comparison to that of group A and B_1. However, the period was similar and the difference was not statistically significant in groups A vs B_1, A vs C_1, C_1 vs D_1 and B_1 vs C_1.

The mean (±SD) period of immobility in forced swim test was significantly higher in group D_1 ($p<0.001$) in comparison to that of group A, B_1 and C_1. Again, this period was lower in group A and the difference was statistically significant ($p<0.01$) between group A vs C_1, but not statistically significant between groups A vs B_1 and B_1 vs C_1.

In TST the mean period (±SD) period of immobility were 13.14±3.97, 11.71±2.81, 14.14±2.41 and 26.29±4.60 seconds in group A, B_1, C_1 and D_1 respectively. The mean (±SD) period of immobility in TST was significantly higher in group D_1 ($p<0.001$) in comparison to that of group A, B_1 and C_1. Again, this period was almost similar and the difference was not statistically significant between groups A vs B_1 or A vs C_1 and B_1 vs C_1.

Table: 1.3 Parameters of biochemical changes in different groups of rats (n=28)

Groups	Fasting Blood Glucose (mmol/L)	MDA (nmol/mL)
A(n=7)	5.21±1.04	1.430±0.382
	(3.5-6.5)	(0.82-1.788)
B_1(n=7)	5.05±1.06	2.692±0.452
	(3.5-6.3)	(2.179-3.269)
C_1(n=7)	5.82±1.04	2.72±0.407
	(3.8-6.8)	(2.064-3.275)
D_1(n=7)	7.27±0.65	3.874±0.75
	(6.5-8.2)	(2.82-4.801)

Statistical Analysis

ANOVA	F=7.59	F=25.82
	P=0.001*	P=0.0001*
Tukey's Test		
A Vs B_1	P=0.990	P=0.001*
A Vs C_1	P=0.641	P=0.001*
A Vs D_1	P=0.003*	P=0.0001*
B_1 Vs D_1	P=0.001*	P=0.001*
C_1 Vs D_1	P=0.047*	P=0.002*
B_1 Vs C_1	P=0.459	P=1.0

*Significant<0.05

Control group

Group A (Baseline Control Group)

Group C_1 (Positive Control, antidepressant treated depressed control group)

Group D_1 (Negative Control, Depressed Control Group)

Experimental Group

Group B_1 (Nutraceutical treated depressed control group)

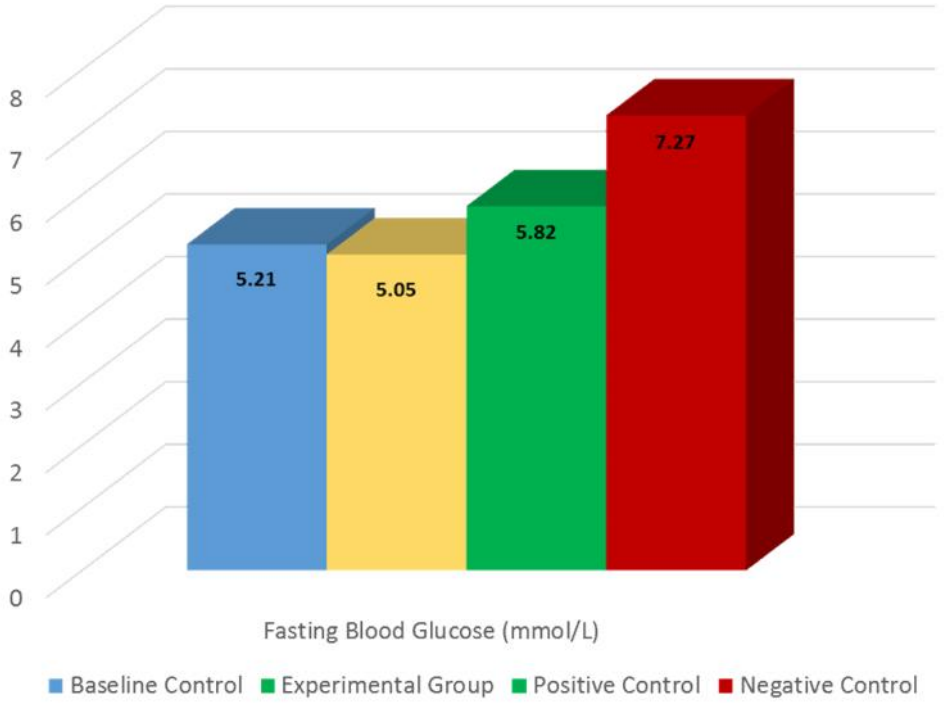

Fig 2.5: Fasting blood glucose level in different groups of rats (n=28)

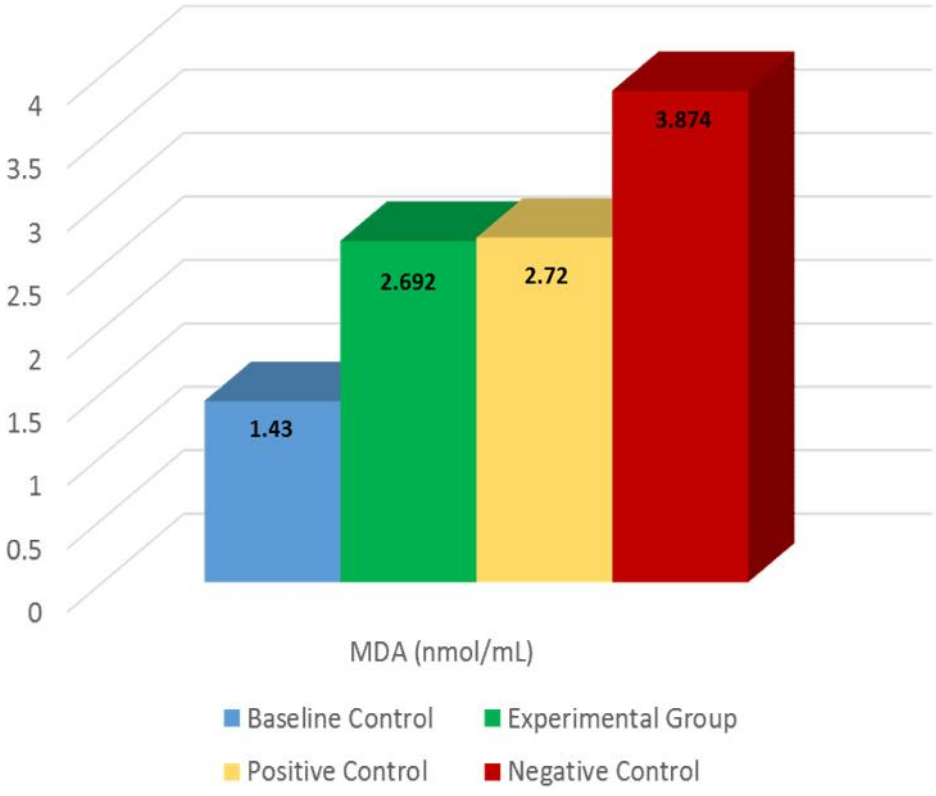

Fig 2.6: MDA level in different groups of rats (n=28)

The mean (±SD) fasting blood glucose levels were 5.21±1.04, 5.05±1.06, 5.82±1.04 and 7.27±0.65 mmol/L in in group A, B_1, C_1 and D_1 respectively.

The mean (±SD) blood glucose level was significantly higher in group D_1 in comparison to that of group A ($p<0.001$), B_1 ($p<0.001$) and C_1 ($p<0.01$). Again, this level was almost similar and the difference was not statistically significant between groups A vs B_1, A vs C_1 and B_1 vs C_1.

The mean (±SD) MDA levels were 1.430±0.382, 2.692±0.452, 2.72±0.407 and 3.874±0.75 nmol/ml in in group A, B_1, C_1 and D_1 respectively.

The mean (±SD) MDA level was significantly higher in group D_1 in comparison to that of group A ($p<0.001$), B_1 ($p<0.01$) and C_1 ($p<0.01$). This level was almost similar and the difference was not statistically significant between groups B_1 vs C_1. Again, this level was significantly lower in group A in comparison to that of group B_1 ($p<0.01$) and C_1 ($p<0.01$).

5.2 CHEMICAL STRESS EXPERIMENTAL MODEL

Table 2.1: Body weight, weight of adrenal gland and brain in different groups of rats (n=28)

Groups	Body Weight		% Change in Body Weight	Weight of Adrenal gland (gm)	Weight of Brain (gm)
	Initial	Final			
A(n=7)	106.14±3.53	116.57±3.78	9.84±2.32	0.0137± 0.014	1.215±0.037
	(100-110)	(112-122)	(6.67-12.96)	(0.012-0.016)	(1.266-1.178)
B_2(n=7)	105.86±4.91	114.57±4.27	8.28±2.0	0.0152±0.001	1.198±0.055
	(100-112)	(108-120)	(5.45-11.54)	(0.013-0.018)	(1.126-1.281)
C_2(n=7)	106.14±3.57	113.71±6.15	8.73±2.33	0.0165±0.002	1.145±0.028
	(100-110)	(104-120)	(3.85-11.32)	(0.013-0.020)	(1.098-1.178)
D_2(n=7)	105.29±3.94	108.71±3.20	3.28±1.17	0.021±0.003	1.086±0.037
	(100-110)	(104-112)	(1.82-5.00)	(0.017-0.028)	(1.026-1.142)

Statistical Analysis

ANOVA	F=0.921 P=0.446	F=3.872 P=0.022*	F=14.603 P=0.0001*	F=12.91 P=0.0001*	F=14.28 P=0.0001*
Tukey's Test					
A Vs B_2	P=0.535	P=0.838	P=0.480	P=0.633	P=0.847
A Vs C_2	P=0.458	P=0.639	P=0.734	P=0.158	P=0.017*
A Vs D_2	P=0.692	P=0.016*	P=0.0001*	P=0.0001*	P=0.0001*
B_2 Vs D_2	P=0.994	P=0.096	P=0.001*	P=0.001*	P=0.0001*
C_2 Vs D_2	P=0.980	P=0.187	P=0.0001*	P=0.006*	P=0.056
B_2 Vs C_2	P=0.999	P=0.984	P=0.974	P=0.761	P=0.10

*Significant<0.05

Control group

Group A (Baseline Control Group)

Group C_2 (Positive Control, antidepressant treated depressed control group)

Group D_2 (Negative Control, Depressed Control Group)

Experimental Group

Group B_2 (Nutraceutical treated depressed control group)

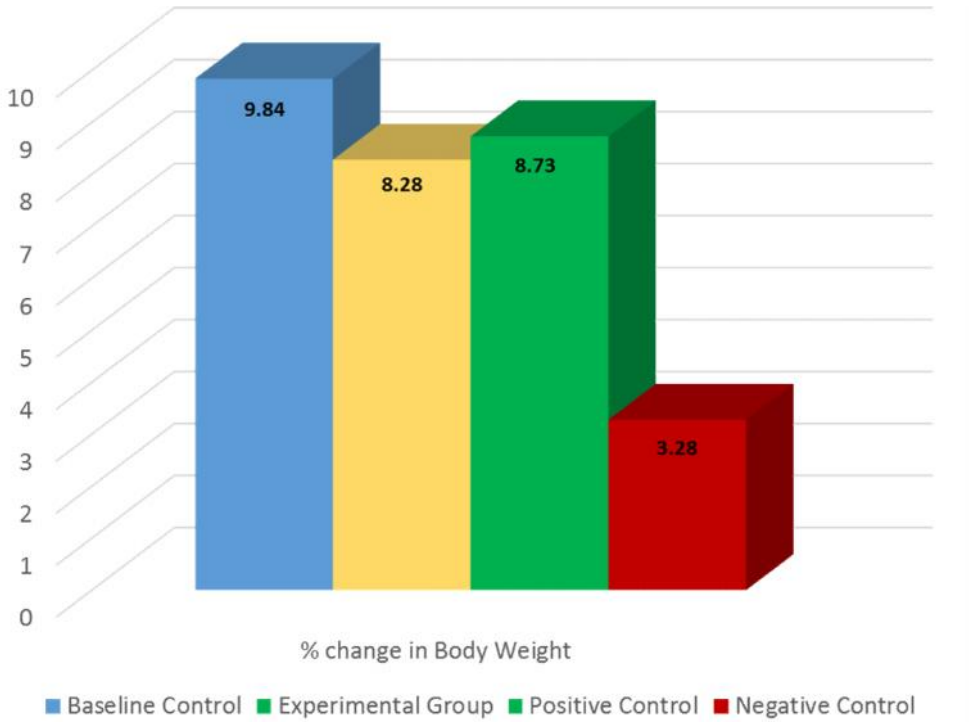

Fig 3.1: Percent change in body weight in different groups of rats (n=28)

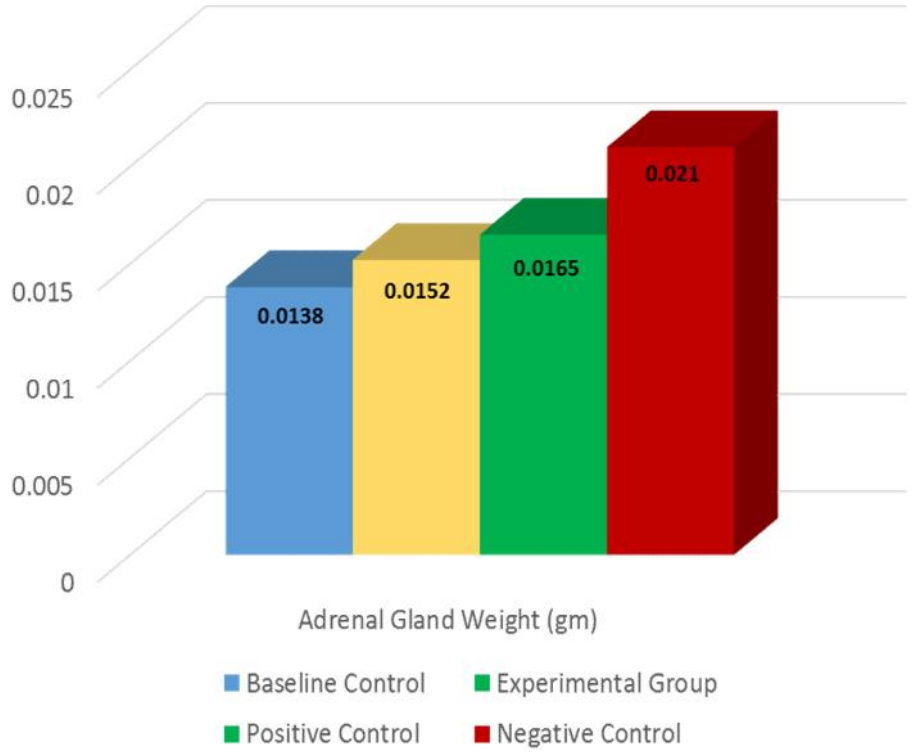

Fig 3.2: Weight of adrenal gland in different groups of rats (n=28)

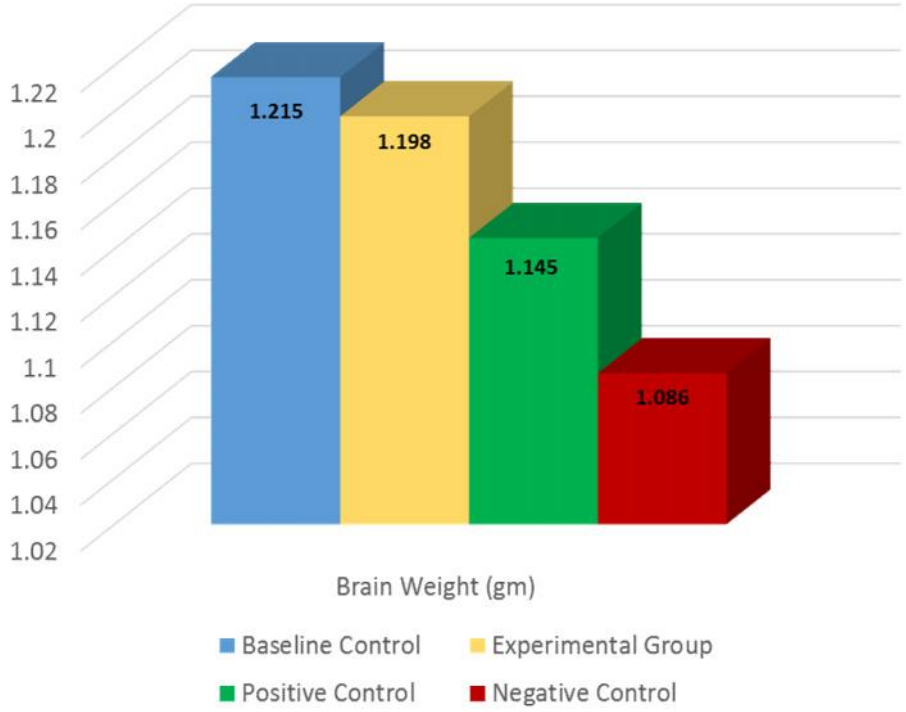

Fig 3.3: Weight of brain in different groups of rats (n=28)

The mean (±SD) initial body weights (day 1) were 106.14 ±3.53, 105.86±4.91, 106.14±3.57 and 105.29±3.94 and the final body weights (day 21) were 116.57 ±3.78, 114.57 ± 4.27, 113.43± 6.29 and 108.71±3.20 in group A, B_2, C_2 and D_2 respectively. Again, the percent (%) change in body weights from final to initial were 9.84± 2.32, 8.28±2.0, 8.73±2.33 and 3.28±1.17 respectively. The weight of the adrenal glands were 0.0137± 0.014, 0.0152±0.001, 0.0165±0.002 and 0.021±0.003 gm and the weight of the brains 1.215±0.037, 1.198±0.055, 1.145±0.028 and 1.08615±0.037 in group A, B_2, C_2 and D_2 respectively.

The mean (±SD) percent (%) change in body weight was significantly lower in group D_2 compared to that of group A ($P<0.001$), B_2 ($P<0.01$) and C_2 ($P<0.001$).

The mean (±SD) weight of adrenal gland was significantly higher in group D_2 compared to that of group A ($p<0.001$), B_2 ($p<0.01$) and C_2 ($p<0.01$). The weights were almost similar and the difference was not statistically significant between groups A, B_2 and C_2.

The mean (±SD) weight of brain was significantly lower in group D_2 compared to that of group A ($p<0.001$), B_2 ($p<0.001$); but not significant between groups A vs B_2, B_2 vs C_2 and D_2 vs C_2. However, the difference was statistically significant ($p<0.05$) between A vs C_2.

Table: 2.2: Parameters of behavioral changes in different groups of rats (n=28)

Groups	Tail Suspension Test (second)	Forced Swim Test (second)		
		Climbing	Swimming	Immobility
A(n=7)	13.14±3.97	135.14±6.69	87.14±4.41	17.29±3.45
	(8-19)	(125-145)	(82-93)	(13-22)
B_2(n=7)	17.57±3.97,	124.43±10.24	89.86±5.55	23.43±5.99
	(12-25)	(105-136)	(83-98)	(18-36)
C_2(n=7)	19.43±3.82	115±7.14	86.71±4.15	26.14±8.41
	(12-24)	(106-126)	(81-92)	(18-42)
D_2(n=7)	30.57±5.09	71.71±11.28	79.14±4.94	90.71±7.78
	(22-36)	(58-85)	(72-92)	(78-106)

Statistical Analysis

ANOVA	F=20.07 P=0.0001*	F=66.05 P=0.0001*	F=6.43 P=0.002*	F=185.01 P=0.0001*
Tukey's Test				
A Vs B_2	P=0.258	P=0.148	P=0.717	P=0.337
A Vs C_2	P=0.058	P=0.002*	P=0.998	P=0.872
A Vs D_2	P=0.0001*	P=0.0001*	P=0.022*	P=0.0001*
B_2 Vs D_2	P=0.0001*	P=0.0001*	P=0.002*	P=0.0001*
C_2 Vs D_2	P=0.0001*	P=0.0001*	P=0.033*	P=0.0001*
B_2 Vs C_2	P=0.857	P=0.235	P=0.617	P=0.872

*Significant<0.05

Control group

Group A (Baseline Control Group)

Group C_2 (Positive Control, antidepressant treated depressed control group)

Group D_2 (Negative Control, Depressed Control Group)

Experimental Group

Group B_2 (Nutraceutical treated depressed control group)

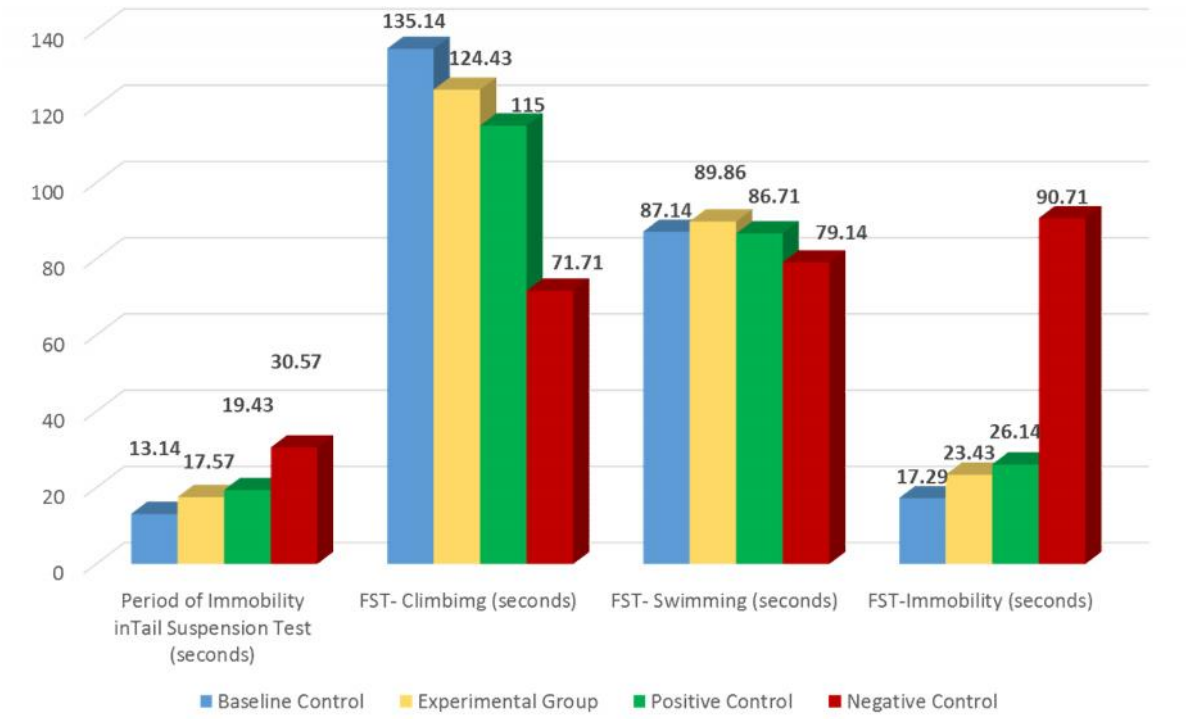

Fig 3.4: Parameters of behavioral changes in different groups of rats (n=28)

In FST the mean period (±SD) of climbing were 135.14±6.69, 124.43±10.24, 115±7.14 and 71.71±11.28 seconds; mean period of swimming were 87.14±4.41, 89.86±5.55, 86.71±4.15 and 79.14±4.94 seconds and period of immobility were 17.29±3.45, 23.43±5.99, 26.14±8.41 and 90.71±7.78 in group A, B_2, C_2 and D_2 respectively.

The mean (±SD) period of climbing in forced swim test was significantly lower ($p<0.001$) in group D_2 in comparison to that of group A, B_2 and C_2. Again, this period was almost similar and the difference was not statistically significant between groups A vs B_2 and B_2 vs C_2. However, the difference was statistically significant ($p<0.05$) between A vs C2.

The mean (±SD) period of swimming in forced swim test was significantly lower in group D_2 in comparison to that of group A ($p<0.05$), B_2 ($p<0.01$) and C_2 ($p<0.05$). Again, this period was almost similar and the difference was not statistically significant between groups A vs B_2, A vs C_2 and B_2 vs C_2.

The mean (±SD) period of immobility in forced swim test was significantly higher ($p<0.001$) in group D_2 in comparison to that of group A, B_2 and C_2. Again, this period was almost similar and the difference was not statistically significant between groups A vs B_2, A vs C_2 and B_2 vs C_2.

In TST the mean period (±SD) period of immobility were 13.14±3.97, 17.57±4.50, 19.43±3.82 and 30.57±5.09 seconds in group A, B_2, C_2 and D_2 respectively. The mean (±SD) period of immobility in TST was significantly higher ($p<0.001$) in group D_2 in comparison to that of group A, B_2 and C_2. Again, this period was almost similar and the difference was not statistically significant between groups A vs B_2, A vs C_2 and B_2 vs C_2.

Table 2.3: Parameters of biochemical changes in different groups of rats (n=28)

Groups	Fasting Blood Glucose (mmol/L)	MDA (nmol/mL)
A(n=7)	5.21±1.04	1.430±0.382
	(3.5-6.5)	(0.82-1.788)
B_2(n=7)	6.57±0.78	1.646±0.382
	(5.8-7.8)	(1.051-3.02)
C_2(n=7)	6.88±0.35	1.75±0.733
	(6.3-7.3)	(0.782-2.871)
D_2(n=7)	8.07±0.75	3.02.±0.68
	(7.2-9.3)	(2.01-4.179)

Statistical Analysis

ANOVA	F=16.11 P=0.001*	F=8.06 P=0.001*
Tukey's Test		
A Vs B_2	P=0.016*	P=0.930
A Vs C_2	P=0.003*	P=0.797
A Vs D_2	P=0.0001*	P=0.001*
B_2 Vs D_2	P=0.007*	P=0.004*
C_2 Vs D_2	P=0.040*	P=0.009*
B_2 Vs C_2	P=0.872	P=0.989

*Significant<0.05
Control group
Group A (Baseline Control Group)
Group C_2 (Positive Control, antidepressant treated depressed control group)
Group D_2 (Negative Control, Depressed Control Group)
Experimental Group
Group B_2 (Nutraceutical treated depressed control group)

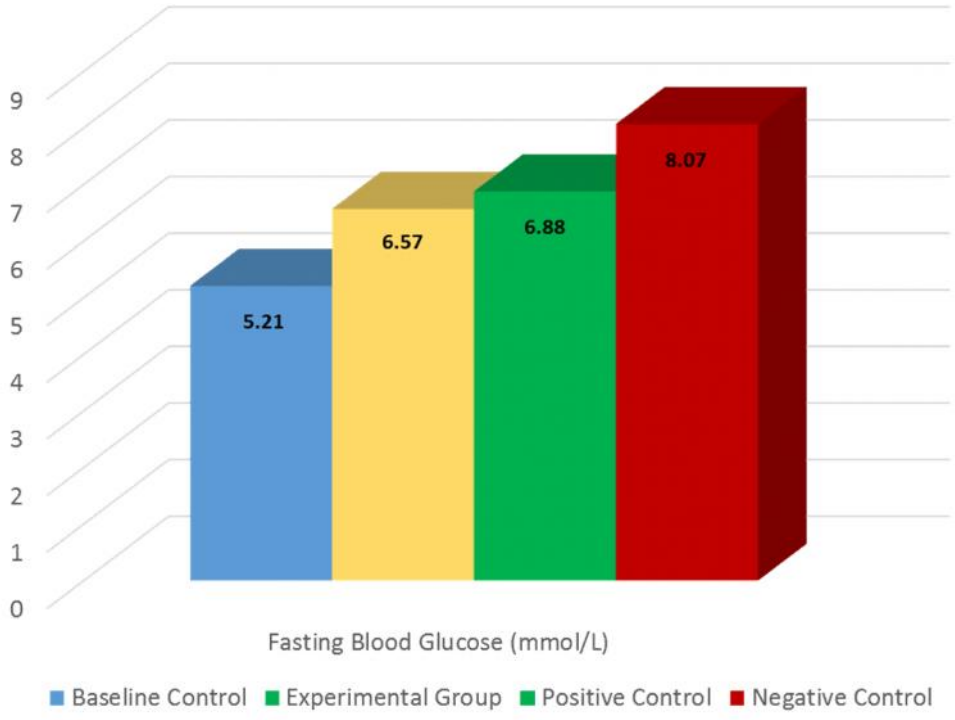

Fig 3.5: Fasting blood glucose level in different groups of rats (n=28)

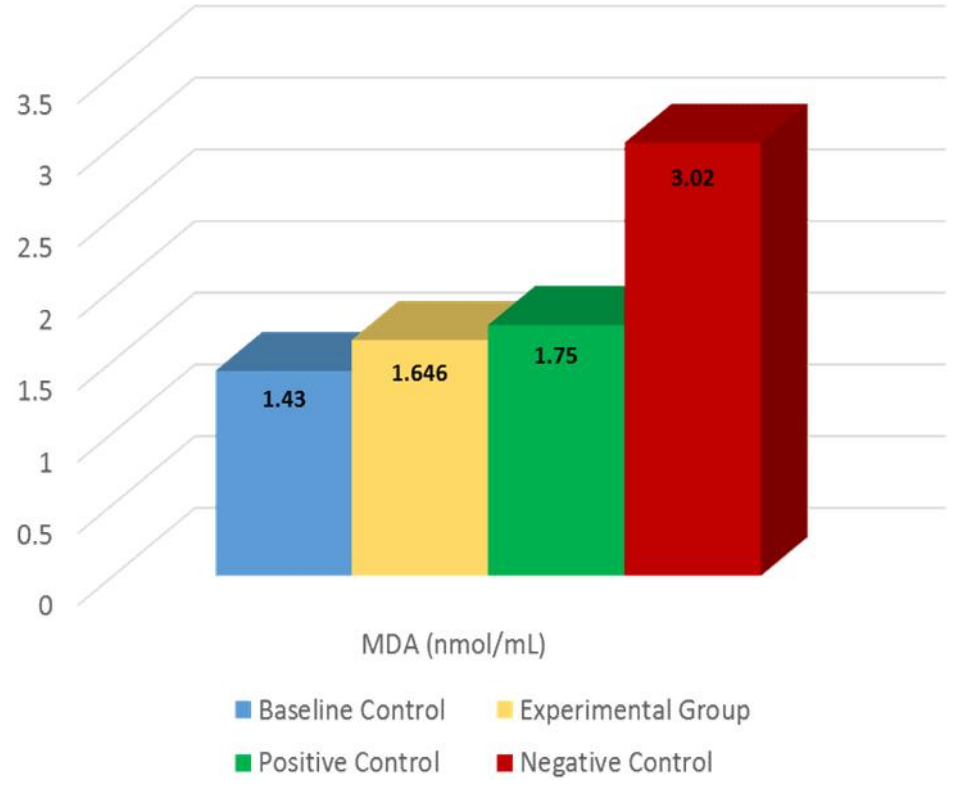

Fig 3.6: MDA level in different groups of rats (n=28)

The mean (±SD) fasting blood glucose levels were 5.21±1.04, 6.57±0.78, 6.88±0.35 and 8.07±0.75 mmol/L in in group A, B_2, C_2 and D_2 respectively.

The mean (±SD) blood glucose level was significantly higher in group D_2 in comparison to that of group A ($p<0.001$), B_2 ($p<0.01$) and C_2 ($p<0.05$). However, the difference was statistically significant between A vs B_2 ($p<0.05$) and A vs C_2 ($p<0.01$) and not significant between B_2 vs C_2.

The mean (±SD) MDA levels were 1.430±0.382, 1.646±0.382, 1.75±0.733 and 3.02.±0.68 nmol/ml in in group A, B_2, C_2 and D_2 respectively.

The mean (±SD) MDA level was significantly higher ($p<0.01$) in group D_2 in comparison to that of group A, B_2 and C_2. This level was almost similar and the difference was not statistically significant between groups A vs B_2, A vs C_2 and B_2 vs C_2.

CHAPTER VI

DISCUSSION

"Therapeutic use of neuroprotective nutraceuticals in experimental neurologic disorder" was an experimental study. The study population had 50 albino Wistar rats. Physical stress model and chemical stress model was used in this study to induce depression and the neuroprotective potential of nutraceuticals (omega 3 fatty acid, ascorbic acid and zinc) was observed.

The result of the study showed that, in physical stress model, the percent (%) change of body weight was significantly higher ($p<0.01$), adrenal gland weight was significantly lower ($p<0.001$) and brain weight was significantly higher ($p<0.001$) in nutraceutical treated group compared to that of depressed control group but was almost similar to baseline control and antidepressant treated control group (Table 1.1). previous studies have shown that repeated restraint stress alters some physiological phenomenon such as decreased body weight, adrenal hypertrophy and decreased brain weight.[19, 26, 27]

The adrenal gland is an essential stress-responsive organ that is part of both the hypothalamic-pituitary-adrenal axis and the sympatho-adrenomedullary system. Corticotrophin releasing hormone levels are increased in the hypothalamus as a stress response, which causes production of adrenocorticotropic hormone (ACTH) from the pituitary gland, which in turn causes increased production of cortisol from the adrenal glands. Several animal studies have shown hypertrophy of specific regions of the adrenal gland after exposure to chronic stress.[28] Toxicology studies have demonstrated that hypertrophy can arise from an acute stress response. Raised cortisol can also inhibit the formation of neurologic cells and reduces the size of the hippocampus, thus causing reduced weight of brain. Again, chronic stress causes anorexia and reduced appetite therefore causing reduced weight gain.

Investigation of behavioral tests showed the period of climbing in forced swim test was significantly higher ($p<0.001$), period of swimming was significantly higher ($p<0.01$) and the period of immobility in tail suspension test and forced swim test was significantly lower ($p<0.001$) in nutraceutical treated group in comparison to that of depressed control group but were almost similar to baseline control group and antidepressant treated control group (Table 1.2). It is well documented that exposure to chronic stress enhances depressive symptoms observed in forced swim test and tail suspension test.[29, 15] Again, use of nutraceuticals decreased these depressive symptoms.

Analysis of biochemical tests showed that, fasting blood glucose level and MDA levels were significantly lower ($p<0.001$) and ($p<0.01$) respectively in nutraceutical treated group in comparison to depressed control group and was similar to antidepressant treated depressed control group and baseline control group (table 1.3). It is well reported that chronic stress leads to increased serum glucose level and MDA levels.[4, 19] Raised stress promotes adrenal glucocorticoid levels to rise which in turn stimulates hepatic gluconeogenesis and raised glucose levels in blood. Stress causes production of reactive oxygen species which degrade polyunsaturated lipids, thus causing raised Malondialdehyde (MDA) levels. Again, in this study use of nutraceuticals led to a decline of these biochemical stress markers.

Similarly, in chemical stress model the percent (%) change of body weight was significantly higher ($p<0.01$), adrenal gland weight was significantly lower ($p<0.01$) and brain weight was significantly higher ($p<0.001$) in nutraceutical treated group compared to that of depressed control group but was almost similar to baseline control and antidepressant treated control group (Table 2.1). Evaluation of behavioral tests showed the period of climbing in forced swim test was significantly higher ($p<0.001$), period of swimming was significantly higher ($p<0.01$) and the period of immobility in tail suspension test and forced swim test was significantly lower ($p<0.001$) in nutraceutical treated group (table 2.2). Analysis of biochemical tests showed that, fasting blood glucose level and MDA levels were significantly lower ($p<0.01$) in nutraceutical treated group in comparison to depressed control group but was similar to antidepressant treated depressed control group and baseline control group (Table 2.3). Chronic stress causes body weight reduction, adrenal hyperplasia, increases depressive symptoms observed in behavioral tests and raised biochemical markers. Therapeutic use of nutraceuticals lead to a decline of depressive symptoms and laboratory findings.

Study findings in both stress models are consistent with other studies regarding depression animal models. In previous studies, depressive behavioral changes, adrenal gland hyperplasia, weight reduction and raised biochemical markers were alleviated through use of anti- depressant like experimental agents.[4, 15, 19, 20, 26, 27, 28]

CHAPTER VII

CONCLUSION

CONCLUSION

Among neurologic disorders depression is an important global health issue, both because of the relatively high lifetime prevalence and association with substantial disability. Control of depression and minimizing its effects in the society is therefore of utmost importance. Although antidepressants alleviate the symptoms of depression, the demand for safer substitutes are rising substantially. Nutraceuticals as neuroprotective anti-depressant agents may play a role in curtailing the disease burden. From this study it can be concluded that nutraceuticals (omega 3 fatty acid, ascorbic acid and zinc) have positive antidepressant effect. Depressive behavioral changes as well as increased fasting blood glucose level, MDA level and adrenal gland weights along with decreased body weight and brain weight are found in stress induced depressed control rats. Therapeutic use of nutraceuticals improved these parameters. Therefore, it can be suggested that nutraceuticals have neuroprotective potential through their anti-depressant effects.

LIMITATIONS OF THE STUDY

- Experiments required reasonable funding and procurement of instruments was difficult.
- Some rats died during feeding (five).
- Further behavioral tests and laboratory procedures could not be performed due to high expenditure and complex design.
- Sample collection required meticulous dissection which was hard to master.
- Histopathology of dissected samples could not be conducted due to unavailability of facilities regarding animal model.

RECOMMENDATIONS

- Further studies regarding anti-depressant effect of nutraceuticals should be conducted where measurement of serum cortisol and monoamines would be possible.
- Neuroprotective potential of other nutraceuticals can be studied based on this study.
- Omega-3 fatty acid preparations can be prescribed by physicians for patients with stress and depression.
- Clinical studies can be performed on the basis of this study.

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APPENDICES

APPENDICES

Appendix I
Experiment Flow Sheet **Date:**

Subject no.

Weight of Subject.

A. Behavioral tests:

Forced Swimming Test:

Climbing	Swimming	Immobility

Tail Suspension Test:

Period of immobility:	

B. Biochemical tests:

Fasting Blood Sugar:

MDA:

C. **Dissection:**

Adrenal Gland weight:

Brain weight:

Appendix II
Drug Procurement and Dosage

Drug Procurement

- Omega -3 fatty acids were procured from commercially prepared capsule Omesoft 1gm of Pacific pharmaceuticals which consists of EPA and DHA.
- Zinc was procured from commercially prepared tablet Xinc 20 mg of Eskayef pharmaceuticals
- Ascorbic acid was procured from commercially prepared tablet Cevit 250 mg of Square pharmaceuticals
- Clomipramine was procured from commercially prepared tablet Anfranil 25 mg of Novartis pharmaceuticals

Dose Calculation

- Rat dose was calculated from human dose by conversion coefficient of 6.2 (CDER, 2005)
- Nutraceuticals were administered in the following dose:
    i. Omega3 fatty acids 415 mg/kg body weight orally daily
    ii. Zinc 4.15 mg/kg body weight orally daily
    iii. Ascorbic acid 51.46 mg/kg body weight orally daily
- Anti-depressant clomipramine was given 12.65mg/kg body weight daily.
- Chemical stressor reserpine was given 0.38mg/kg body weight daily in chemical stress model.
- Gavage feeding needle was made from standard wide bore blood transfusion needles.
- Gavage dosage was estimated as 0.25 ml per rat.

Ref. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), July 2005

Appendix III
Illustrations

Illustration 1: Photograph showing procurement of Wistar albino rat

Illustration 2: Photograph showing confined rat cages

Illustration 3: Photograph showing rats of physical stress model

Illustration 1.4: Photograph showing rats of chemical stress model

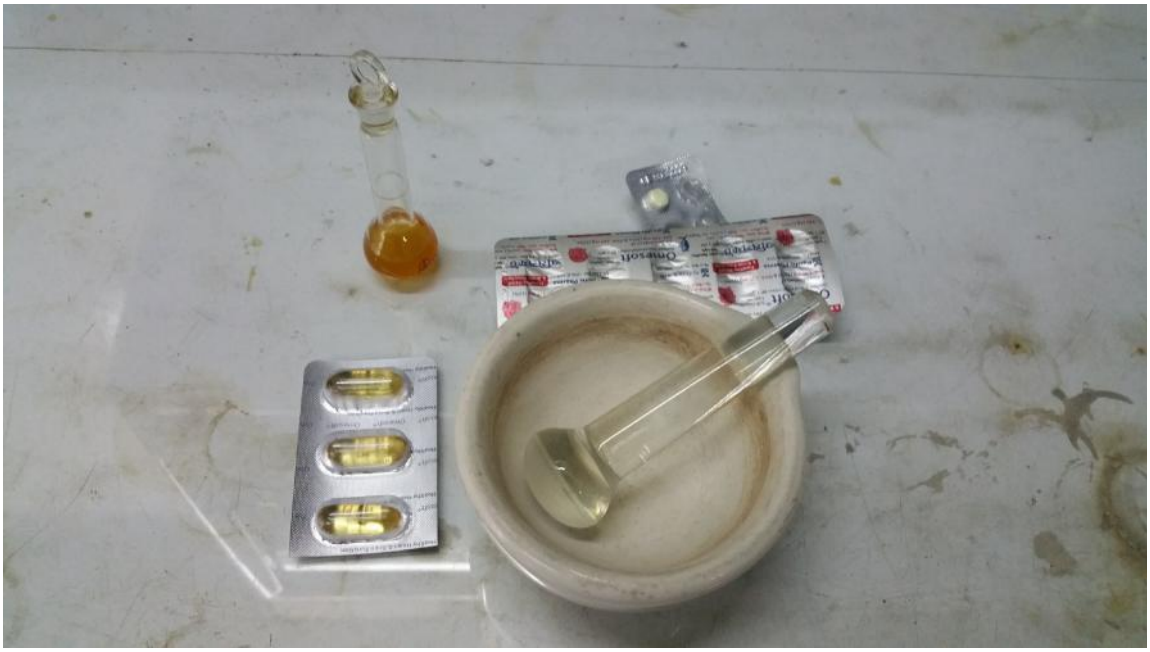

Illustration 5: Photograph showing Preparation of nutraceutical regimen

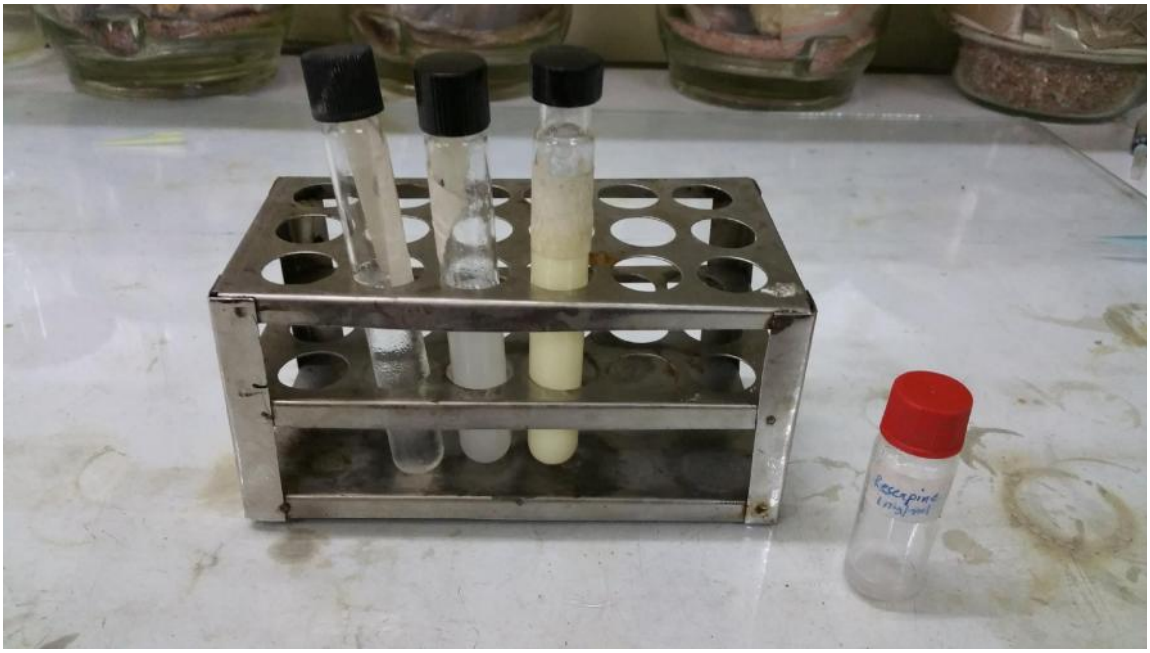

Illustration 6: Photograph showing Prepared therapeutic agents

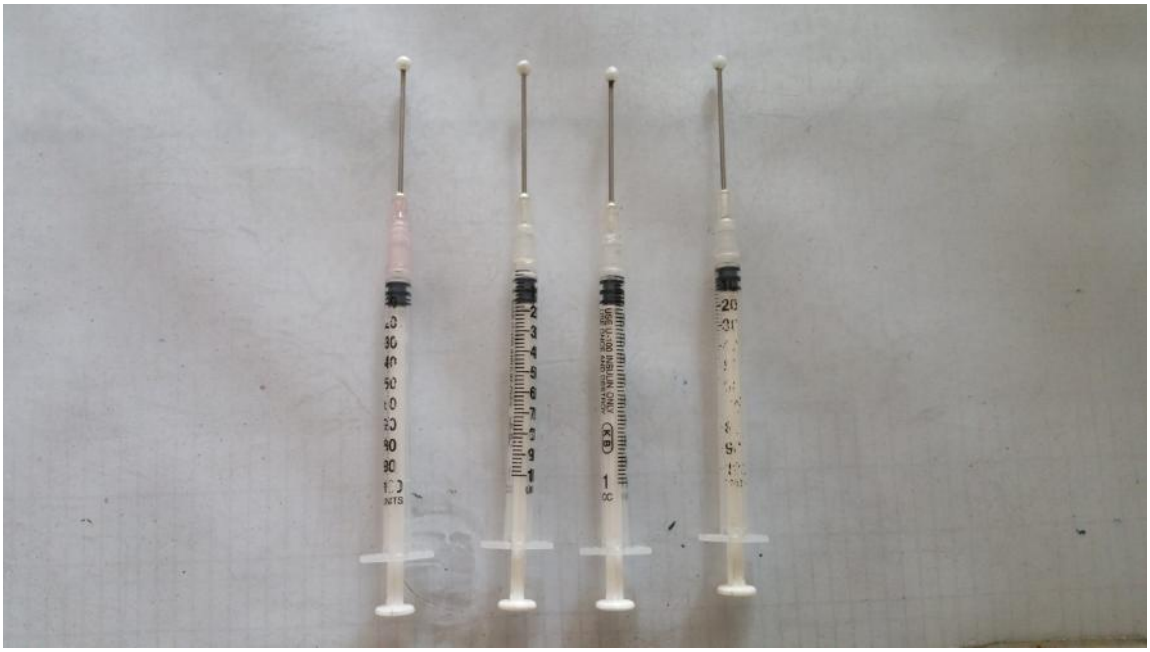

Illustration 7: Photograph showing wide bore feeding needle

Illustration 8: Photograph showing Feeding of rat

Illustration 9: Photograph showing behavioral test (tail suspension test) in rat

Illustration 10: Photograph showing behavioral test (forced swim test) in rat

Illustration 11: Photograph showing anesthetization of rat in chloroform

Illustration 12: Photograph showing collected blood samples of sacrificed rats

Illustration 13: Photograph showing measurement of fasting blood glucose of rat via standard glucometer

Illustration 14: Photograph showing dissection apparatus of rat

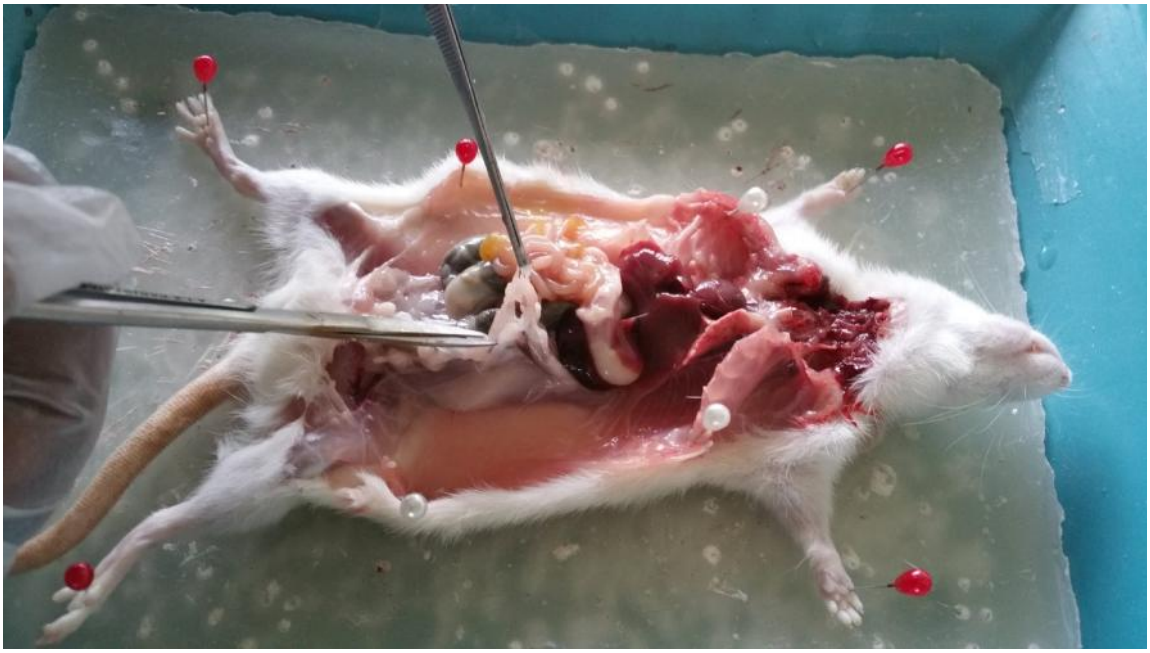

Illustration 15: Photograph showing dissection procedure of rat

Illustration 16: Photograph brain and adrenal gland specimen of rats

Illustration 17: Photograph working with micropipette in laboratory

Illustration 18: Photograph showing centrifuged serum of rat

Illustration 19: Photograph showing preparation of reagent during MDA estimation

Illustration 20: Photograph showing UV spectrophotometer