EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 4
609-618
Year: 2022

DOWN-REGULATION OF BRAIN DERIVED NEUROTROPHIC FACTOR AND OXIDATIVE STRESS MARKERS IN INDUCED ANXIETY/DEPRESSIVE STATE IN RAT'S BRAIN

***[1]Olorunfemi O. J., [2]Okoseimiema S. C., [2]Ibeachu P. C.**

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba Nigeria.
[2]Department of Human Anatomy, College of Health Sciences University of Port Harcourt, Port Harcourt, Choba, Rivers State, Nigeria.

***Corresponding Author: Olorunfemi O. J.**
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba Nigeria.

Article Received on 23/02/2022 **Article Revised on 13/03/2022** **Article Accepted on 31/03/2022**

ABSTRACT
The prevalence of brain diseases due to depression caused by stress is alarmingly high and requires global attention. This work aimed at investigating brain derived neurotrophic factor (BDNF) in depressive states of stress-evoked Male Wistar rats. Hundred and fifty Male rats of about three-month old (180-200 g) were used for this study. The rats were grouped into A (control- normal rats, rats without any drugs or shock), B (rats injected celebrex drugs and left to stay for one week, seven weeks and fourteen weeks (acute, subchronic and chronic levels), C (rats given ear shock/foot shock and left to stay for one week, seven weeks and forteen weeks), D (rats given retro-inversion/head-down maneuver shock and left to stay for one week, seven weeks and fourteen weeks), E (rats given tail immersion/tail clip shock and left to stay for one week, seven weeks and fourteen weeks) and F (rats given mechanical shock and left to stay for one week, seven weeks and fourteen weeks). The celebrex drugs used were injected to the rats at 0.1 mg/kg bw. Brain neurotrophic factor levels and antioxidant status were determined using enzyme-linked immunosorbent assay (ELISA) kits. BDNF levels decreased significantly in rats in groups D, E and F at subchronic and chronic levels when compared with the control. Total antioxidant capacity (TAC) increased significantly in rats in groups D and F while catalase (CAT) activities, superoxide dismutase (SOD) and reduced glutathione (GSH) levels increased significantly in rats in groups B, C, D and F respectively. Levels of malondialdehyde (MDA) increased significantly in groups B and E. The levels of BDNF in depressive states of stress-evoked Male Wistar rats therefore decreased. There was an increase in time spent in the Elevated plus maze, Novel Object recognition and Barnes maze which revealed a decline in memory, cognition and depression. In the overall, depression becomes established in the brain whenever the concentration of BDNF decreased abysmally low but became insignificant in expression whenever there is concomitant increase in the level of BDNF implying that BDNF could be chief determinant of depression.

KEYWORDS: BDNF, depression, stress, anxiety, Antioxidant capacity.

INTRODUCTION
Stress can be defined as the non-specific response of the body to any demand placed on it be it environmental, mechanical, physical etc. It can alter memory functions, reward immune functions, metabolism and disease susceptibility. It is a state of threatened Homeostasis caused by Intrinsic or Extrinsic adverse forces (called Stressors) and is counteracted by an intricate response of physiologic and behavioral responses aimed at maintaining body equilibrium (Tsigos et al., 2016). Stress is a necessary mechanism for survival and has both Positive and Negative effects on brain functions. Its impact depends greatly on the type and duration of the stressor. It can be referred to its acute form in which the stress may be a natural mechanism for survival with only transient changes in the brain or chronic /severe/prolonged stress that causes overactivation and dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) Axis resulting to detrimental changes in Brainstructure and function (Karim Alkahdi., 2013). Stress has been established to elicit acute and chronic changes in certain brain areas which can cause long-term damage (Henckens *et al.*, 2009) as over-secretion of stress hormones most frequently impairs long-term delayed recall memory, but can enhance short-term, immediate recall memory. This enhancement is particularly relative in emotional memory. Such brain areas as the hippocampus, prefrontal cortex and the

amygdala are mainly affected (Oei *et al.,* 2007; *Henckens et l., 2009).* Cortisol and brain-derived neurotrophic factor (BDNF) have been reported to be typical stress responsive mediators in different brain, thus are good markers of emotional distress amongst others (de Quervain*et al.,* 1998; Dominique *et al.,*2000; Oei*et al.,* 2007; Schutte*et al.,* 2016).

2. MATERIALS AND METHODS

120 Male wistar rats weighing 100-120 were used for the experiment. The animals were obtained from a care facility in the University of Port Harcourt. They were randomly placed, fed with normal rat chow and clean water and allowed to acclimatize for 14 days prior to the commencement of the experiment. The research was done in three (3) phases; Acute, Subchronic and Chronic. Each of the phases has four test groups, a drug group and a control group.

All chemicals used in this work were of analytical grade and were obtained from British Drug House (BDH) Ltd., Poole, England through their sale representative in Ikeja, Lagos State, Nigeria. All kits used were obtained from BioAssays Technology, Shanghai Korain Biotech CO., Ltd. Animals used were procured from the Animal Facility Unit, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt.

The duration for this experiment was 14 weeks after which the animals were sacrificed. Blood was collected through Cardiac puncture and placed in properly labeled bottles soaked in ice. It was then taken to the laboratory for assay of cortisol using the microplate enzyme- linked immunosorbent assay (ELISA) methods. Assay was done using the rat Elisa kit for Cortisol obtained from BioAssays Technology, Shanghai Korain Biotech co., Ltd.

The brain was also collected, placed in properly labeled bottles soaked in ice and sent to the laboratory. The Hippocampus was isolated for Histopathological examinations.

The biomarkers for oxidative stress were also evaluated. GSH, CATALASE, Superoxide Dismutases (SOD), Malonyldialdehyde (MDA) and Total Anti Oxidant Capacity (TAC) was determined.

Experimental Animal Groupings

Test Group	No of Animals	Stressors
1 (Control)	5	Rats without shock nor drugs-) No stress)
2	5	(Rats given celebrex drugs only)
3	5	Ear Shock/Foot Shock
4	5	Retroinversion/Head Down Maneuver
5	5	Tail Immersion/Tail Clip
6	5	Mechanical Stress (ECT)

Collection of Blood Samples and Separation of Serum

Five mills of blood samples were collected from the rats via orbital sinus in the morning between 7:30 am to 8: 30 am after the rats have fasted overnight. The blood samples collected were allowed to clot for 50 min without shaking them in a fixed position at 25 ^{O}C. These were then centrifuged at 1000 rpm for 15 min. The clear serum was carefully separated and stored in a refrigerator (LG, Model MEZ64816601, China) for onward analysis.

Determination of Serum Brain Derived Neurotrophic Factors (BDNF)

Serum BDNF levels were determined according by using commercially available enzyme-linked immunosorbent assay (ELISA) kit obtained from BioAssays Technology, Shanghai, Korain Biotech CO., Ltd. It was performed according to the manufacturers' directions.

Serum Antioxidant Assay

Serum SOD, catalase activities, TAC, GSH and MDA levels were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kit obtained from BioAssays Technology, Shanghai, Korain Biotech CO., Ltd. It was performed according to the manufacturers' directions.

Barnes Maze Test- it is a visual- spatial learning and memory task designed for rats. it consists of an elevated circular surface with holes around the edge.

Principles – It is a dry-land based rodent's behavioral paradigm for assessing spatial learning and memory. The animal is placed in the center of the platform at the start of each trial and given a defined period of time to find the Target Escape Hole. If an animal enters the Target Escape Hole before time runs out, the experiment ends. Animals that do not enter the Target Hole in time are led to it by the experimenter and allowed to briefly remain in the tube before being returned to their home cage. The location of the Target Escape Hole is moved daily while other components remain the same. Subjects receive a total of four trials. The rats use extra-maze visual cues to locate an escape hole that allows them to escape from open space and bright light into a dark box beneath the maze. The time it takes to locate the escape hole into the dark box beneath the maze should be recorded.

Elevated Plus Maze

The elevated plus maze is a widely used behavioral assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents and steroid

hormones, and to define brain regions and mechanisms underlying anxiety-related behavior.

Procedure: Rats are placed at the junction of the four arms of the maze; facing an open arm and entries/duration in each arm are recorded by a video-tracking system and observer simultaneously for 5 min. Other ethological parameters (i.e., rears, head dips and stretched-attend postures) can also be observed. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior.

Navigational Maze

Maze navigation tests are utilized in the assessment of exploration, path planning, and navigation which rely on learning and memory capacities to form cognitive maps.

Morris water maze

The Morris water maze (MWM) is a test of spatial learning for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform (Morris, 1984). The concept behind it is that the animal must learn to use distal cues to navigate a direct path to the hidden platform when started from different, random locations around the perimeter of the tank.

Statistical Analysis of Results

Results obtained were expressed as M ± SD of triplicate determinations. All results were analyzed by the Statistical Package for Social Sciences (SPSS), version 23 (IBM Corp., Armonk, New York). Testing of hypotheses was done by testing for significant difference at $P < 0.05$ level of significance.

3. RESULTS

Table 3.1: Assessment of BDNF, TAC and GSH levels in the test and control groups at various stages of stress exposure in rats.

Groups	Level of Toxicity	BDNF (ng/ml)	TAC FRAP (µmol/L)	GSH (mmol/L)
Normal Control (Negative Control)	Acute	5.21±0.85	1241.98±133.25	3.59±0.45
	Sub-Chronic	5.22±0.85	1241.98±133.25	3.59±0.45
	Chronic	5.21±0.85	1303.53±126.41	3.38±0.43
Celebrex Drugs (Positive Control)	Acute	4.85±0.24	1706.66±165.23	3.57±0.17
	Sub-Chronic	4.88±0.23	1611.27±165.23	3.56±0.17*
	Chronic	4.85±0.24	1687.58±156.75	3.87±0.16*
Ear/Foot shock	Acute	3.06±.360	1514.58±67.14	5.40±1.00
	Sub-Chronic	2.66±0.21*	1651.27±357.12	4.32±0.53*
	Chronic	3.06±0.24*	1663.78±636.42	5.51±0.60*
Retro inversion/ Head down Maneuver	Acute	3.22±1.28	2137.50±309.15	5.23±0.25*
	Sub-Chronic	2.51±0.41*	1445.09±245.18*	6.95±1.85*
	Chronic	3.05±0.51*	2634.80±414.64*	5.78±1.10
Tail Clip/Immersion	Acute	3.41±0.70	1660.50±10.66	3.99±0.16
	Sub-Chronic	3.30±0.58	2032.86±527.69	2.92±0.05
	Chronic	3.16±0.77	2069.79±394.94	3.98±2.00
Analgesymeter (Mechanical Shock)	Acute	4.43±0.28	1907.71±106.17	4.84±0.51
	Sub-Chronic	3.35±0.22*	1534.33±191.89*	3.98±0.12
	Chronic	5.15±0.13*	2206.43±157.00*	4.47±0.004

Values are presented in mean ± sem. N=5. * means values are statistically significant when compared to the control group.

Table 3.1b: Assessment of oxidative stress markers in the test and control groups at various stages of stress exposure in rats.

Groups	Level of Toxicity	MDA (µmol/L)	CAT (U/ml)	SOD (U/ml)
Normal Control (Negative Control)	Acute	15.97±1.54	199.87±6.77	59.46±13.45
	Sub-Chronic	15.97±1.54	199.87±6.77	59.46±13.45
	Chronic	15.87±1.52	200.44±6.59	57.70±12.55
Celebrex Drugs (Positive Control)	Acute	25.83±0.88*	213.33±1.15*	55.98±1.00
	Sub-Chronic	26.92±2.45*	218.97±1.95*	58.14±4.33
	Chronic	27.04±2.43*	220.96±1.95*	58.45±4.24
Ear/Foot shock	Acute	29.47±0.49	219.90±1.04	114.33±17.25
	Sub-Chronic	24.54±0.73	207.90±0.52	92.28±12.78
	Chronic	30.37±2.88	204.74±3.94	116.89±17.94
Retro inversion/ Head down Maneuver	Acute	31.42±3.85	212.33±13.49	91.60±1.52*
	Sub-Chronic	25.46±1.50	208.77±4.23	88.79±6.42
	Chronic	27.06±2.95	205.28±1.23	118.43±8.96*
Tail Clip/Immersion	Acute	29.78±1.03*	201.87±1.91	72.95±7.37*

	Sub-Chronic	26.70±1.54*	199.53±20.001	76.18±8.78
	Chronic	24.17±1.35*	218.62±12.20	81.74±0.92*
Analgesymeter (Mechanical Shock)	Acute	46.90±12.25	214.27±3.45	51.92±6.96*
	Sub-Chronic	28.80±0.47	216.90±4.20	54.88±9.51
	Chronic	30.47±1.15	194.920±9.15	100.50±12.89*

Values are presented in mean ± sem. N=5. * means values are statistically significant when compared to the control group.

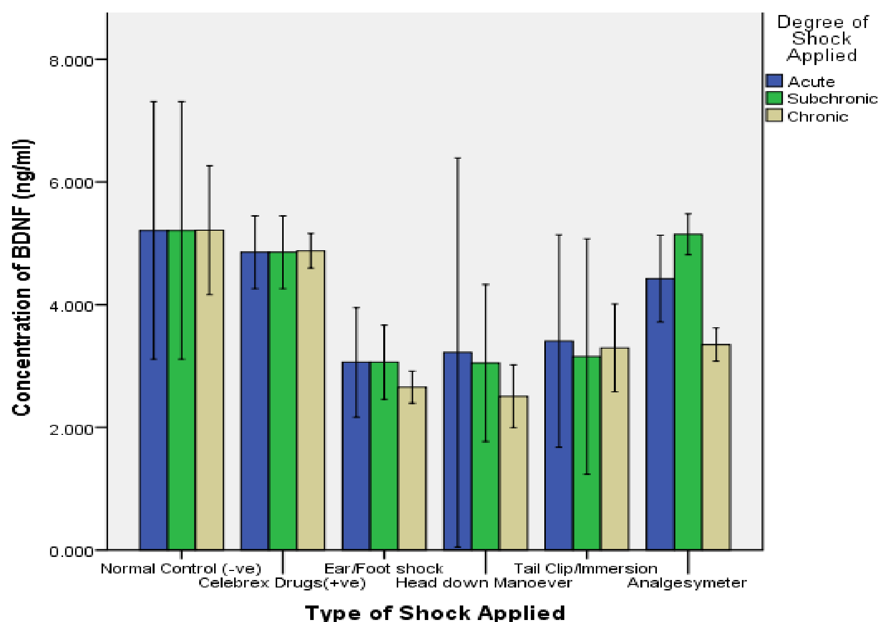


Figure 3.2: Variation of Brain Derived Neurotrophic Factor (BDNF) Levels of Albino Rats Induced Various Degrees of Shock. Values are means of triplicate determination ± standard error of mean (SEM). * (P < 0.05).

The results of brain derived neurotrophic factor (BDNF) levels of Albino-Male rats induced various degrees of shock are hereby presented in figure 3.1. The levels of BDNF were significantly lower (P< 0.05) in groups C, D, E and F at subchronic and chronic levels when compared with rats given celebrex drugs (Group B) and control rats at acute and subchronic phases as shown in figure 4.2. The concentration of BDNF showed a non-statistical decrease within the first 14 days of inducing stress (in the acute or sub chronic treatment) but showed a statistically significant increased afterwards to the 28 days of the study.

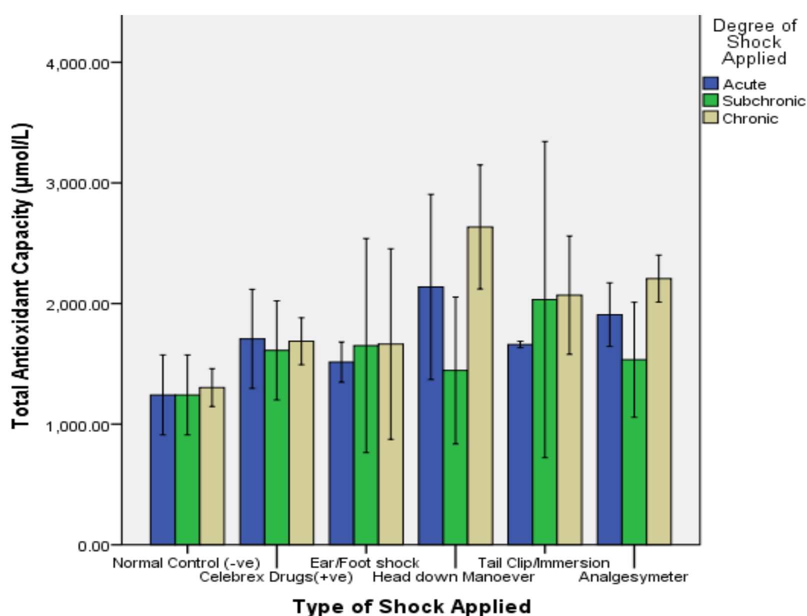


Figure 3.3: Total antioxidant capacity (TAC) levels of Albino rats induced various degrees of shock. Values are means of triplicate determination ± standard error of mean (SEM). * Significant at 0.05 level of significance.

The results of total antioxidant capacity (TAC) of Albino rats induced various degrees of stressors are hereby presented in figure 4.3. The results showed that the levels of TAC were significantly lower (P<0.05) in group D and group F when compared with group B (those given celebrex drugs) and group A (Control group-normal rats) at sub chronic and chronic levels. There were no significant differences in levels of TAC between other groups when compared with the negative control (Normal rats). There were also no significant difference in levels of TAC in group given celebrex drugs and the control.

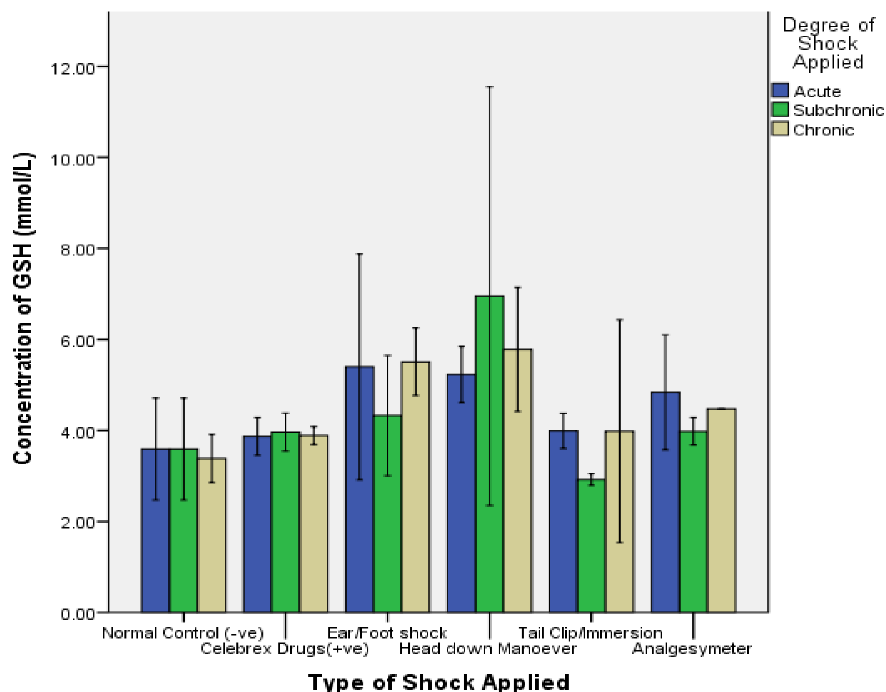


Figure 3.4: Reduced glutathione (GSH) levels of rats induced various degrees of shock. Values are means of triplicate determination ± standard error of mean.* Significant at 0.05 level of significance.

The results than of GSH assay carried out showed that levels of GSH in group C were significantly higher (P< 0.05) than those in positive and negative control groups at subchronic and chronic levels. In group D, the levels were significantly higher (P < 0.05) when compared those in positive and negative control groups.

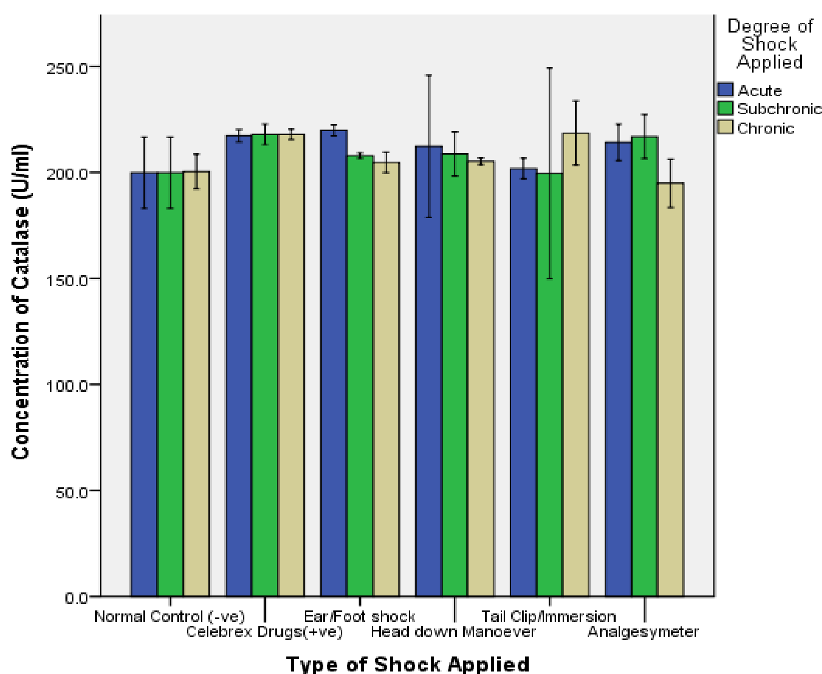


Figure 3.5: Catalase levels of rats induced various degrees of shock. Values are means of triplicate determination ± standard error of mean.* Significant at 0.05 level of significance.

The levels in positive control group were significantly higher when compared with those in negative control group. There were no significant differences between other groups, positive and negative control groups.

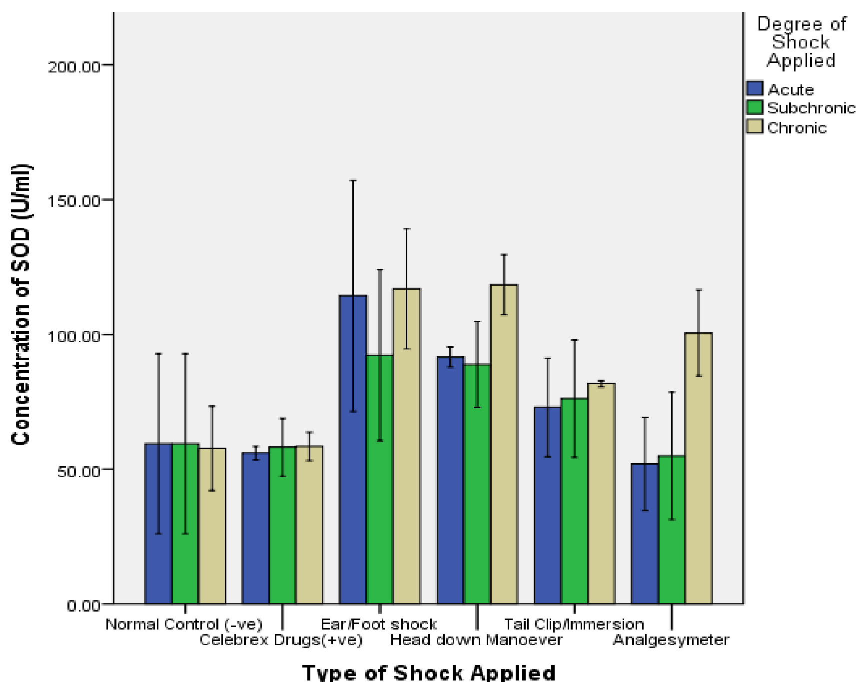


Figure 4.6: Superoxide dismutase (SOD) levels of rats induced various degrees of shock. Values are means of triplicate determination ± standard error of mean.* Significant at 0.05 level of significance.

The results showed that the levels of SOD were significantly higher ($P < 0.05$) in groups C-F when compared with those in control and group B at acute and chronic levels with the exception of group F whose level of SOD was lower significantly. There were no significant differences in levels of SOD in positive control group (those given drugs) when compared with those of control (Normal rats).

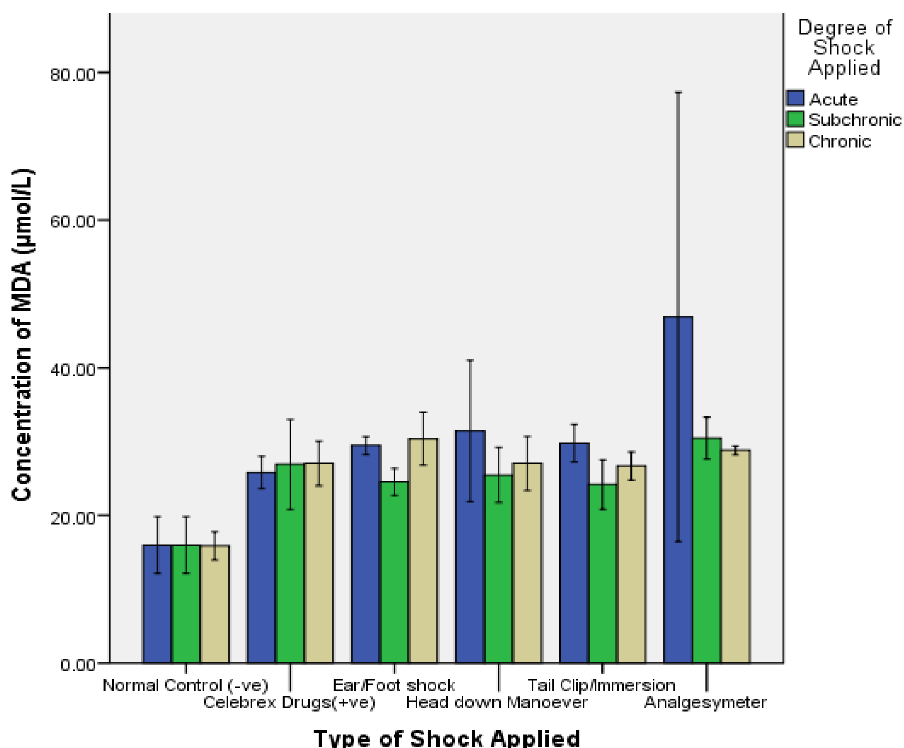


Figure 3.7: Malondialdehyde (MDA) levels in rats induced various degrees of shock. Values are means of triplicate determination ± Standard error of mean. *Significant at 0.05 level of significance.

The results of MDA assay of Albino rats induced various degrees of shock are hereby presented in figure 4.7. The results showed that MDA levels were significantly higher (P < 0.05) in rats given celebrex drugs and rats given tail clip or immersion shock (group E) at acute, subchronic and chronic levels.

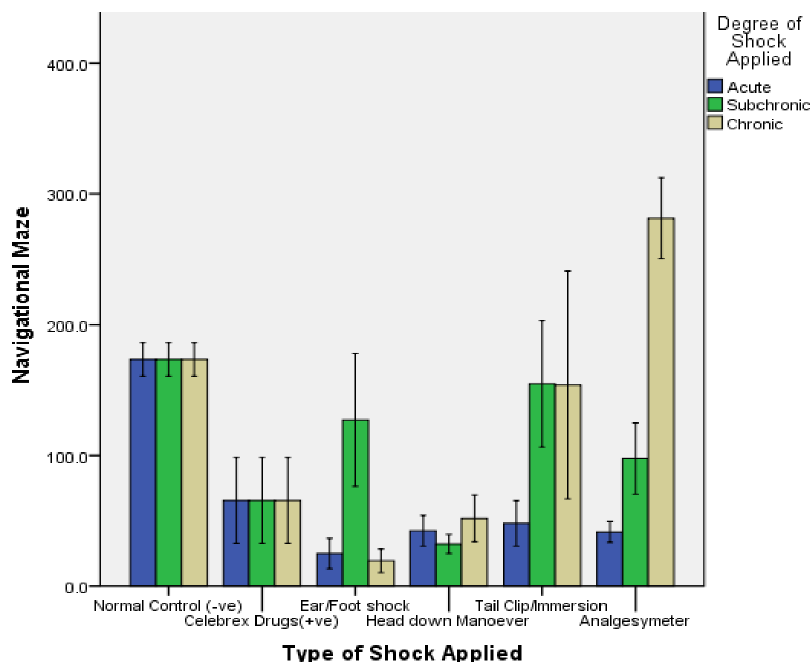


Fig 3.8: Navigational Maze Test between the three phases following exposure to various stressors.

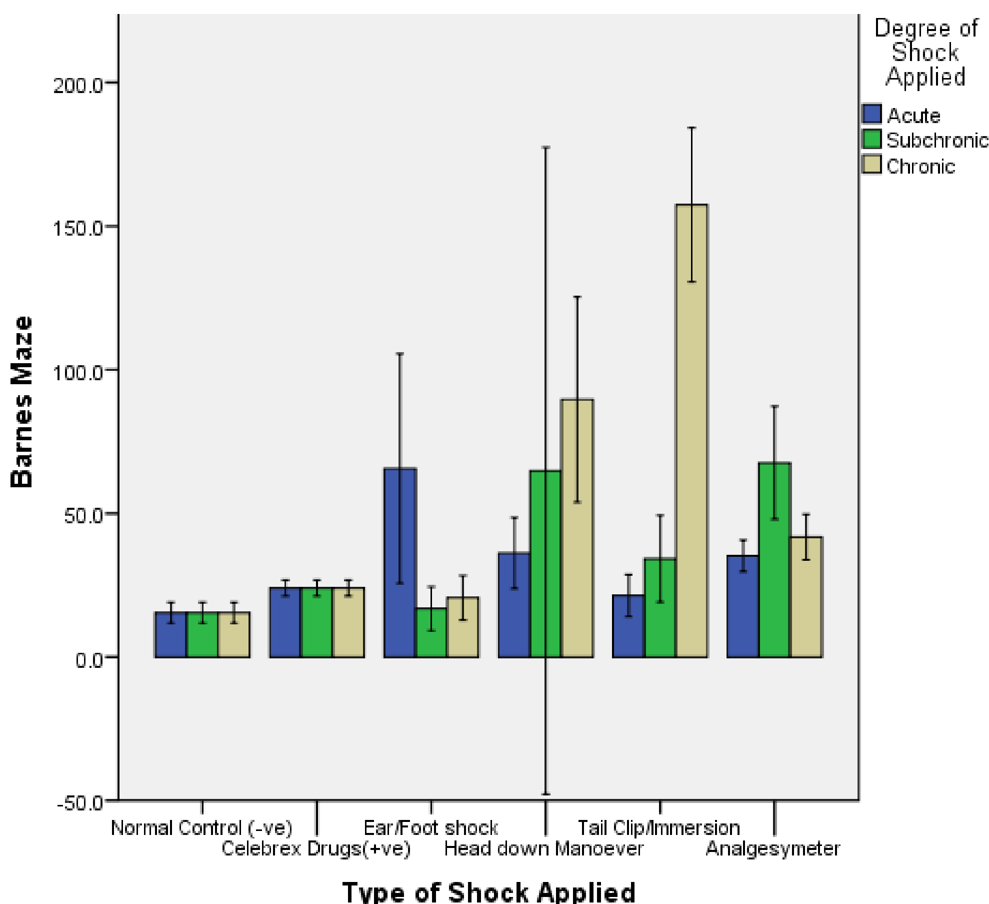
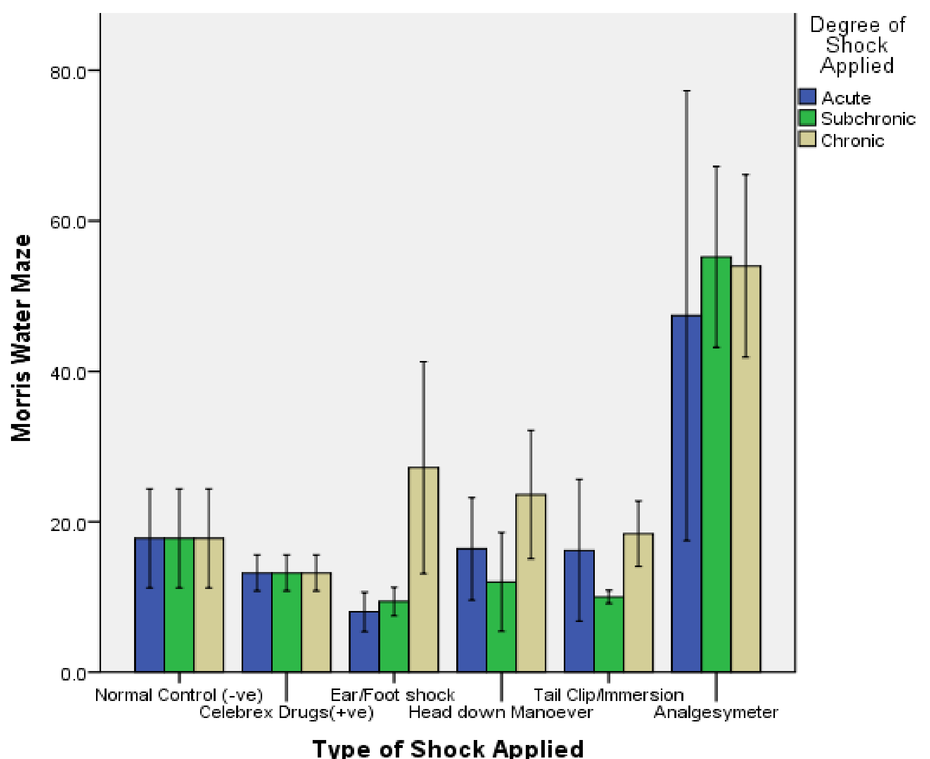


Fig 3.9: Barnes Maze Test between the three phases following exposure to various stressors.



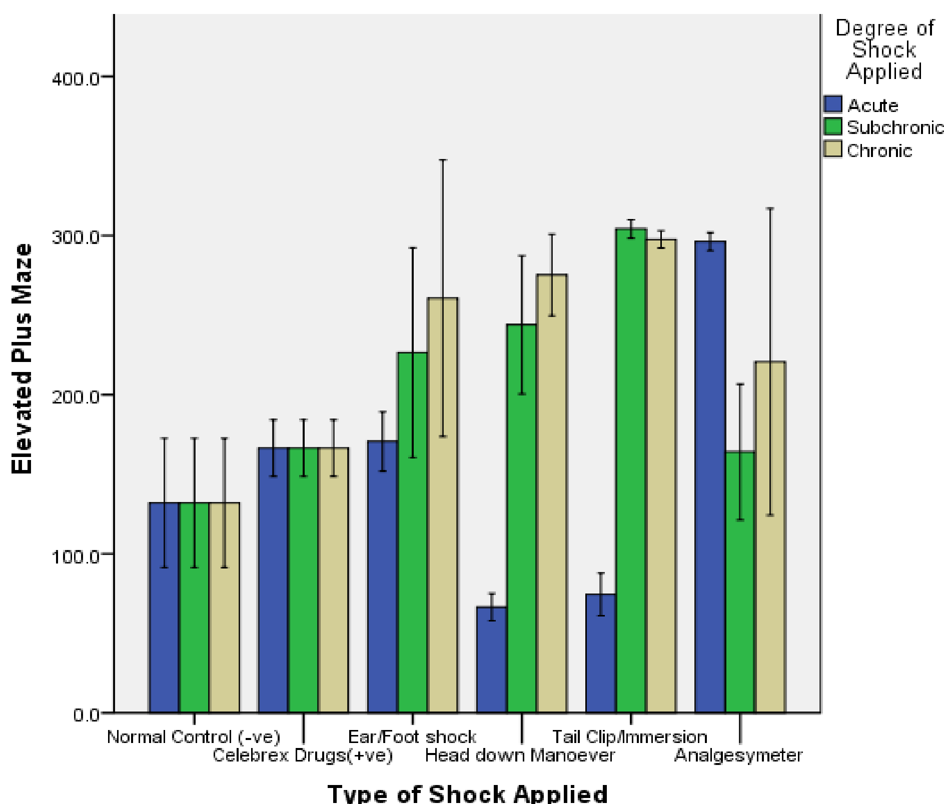


Fig 3.14: Elevated Plus Maze between the three phases following exposure to various stressors.

3.2 DISCUSSION OF FINDINGS

The increased risk of stress-induced neurological disorder in brain due to exposure to various types of stress has led to many researches on markers of oxidative stress in brain neurons, development of several preventive and therapeutic agents. This work has investigated the levels of BDNF, TAC, GSH, Catalase, SOD, and MDA in Male Wistar rats exposed to various degrees of shocks using various types of stressors.

This study has further investigated the patterns of BDNF in anxiety and depressive states of stress evoked rats via

different degrees of shock. Antioxidant status was also investigated.

There was a decrease in the concentration of BDNF in the Acute, Sub chronic and Chronic stages in all the groups when compared to the control. However, this decrease was significant ($P < 0.05$) at the sub chronic stages of groups C, D and F. Consequently, neurodegeneration decreases as the activation and repair of neural cells decrease. This agrees with the work of (Miao et al., 2020) who reported that an increase in stress leads to a decrease in BDNF as the severity of stress increases from acute to chronic. Also, reported that neurodegeneration is associated with the impairment in the synthesis and action of BDNF.

There was an increase in TAC across the various stages (Acute, Sub chronic and Chronic) in all the groups compared to the control. However, this increase was significant ($P < 0.05$) at the sub chronic and chronic stages of groups D & F. This indicates the increase in antioxidants to fight ROS.

There was a significant increase in the concentrations of reduced glutathione (GSH), Malondialdehyde (MDA) and Catalase across the various stages in all groups. This could possibly be as a response to the production of ROS from biotransformation (Surai *et al*., 2019).

The results also showed that the non-enzymatic antioxidants are first mobilized to arrest reactive oxygen species in the first 15 days (within the sub chronic phase), after which the enzymatic antioxidants are employed. This is due to the metabolic cost and the duration of synthesising the enzymatic antioxidants from their specific genes.

The behavioural studies showed a marked decrease in time spent in the navigational maze in all the stages across the groups when compared to the control except at the chronic stage of group F exposed to Mechanical shock (Analgesymeter). This result showed that the rats exhibited neuro cognitive abilities after exposure to varying degrees of stress.

The study showed an increase in time spent in the Barnes maze in the three (3) phases across all groups. However, this increase was significant at groups D, E and F exposed to Retro inversion/Head down Manoeuvre, Tail Clip/Tail immersion, Analgesymeter (Mechanical shock) stressors. This implies that the learning process was impaired after prolonged exposure to stress.

Morris water maze results showed a decrease in time spent in all stages. Group E showed a significant increase in time spent to discover the exit platform. This could be as a result of the severity of pain experienced by this group. It reveals that pain can impair the process of learning and memory.

Prolonged exposure to stress impairs memory processes as observed from the results of Novel Object exploration. There was an increase at the chronic stages in the test groups when compared to the control.

There was a significant increase in time spent in the elevated plus maze in the test groups and across all stages when compared to the control. The elevated plus maze is widely used to assess the anti-anxiety effects of pharmacological agents. This study reveals that following exposure to various stressors, animals became depressed.

Results from the behavioural parameters in the study reveal an increase as the degree of stressor increases (mainly at sub chronic and chronic phase). The results validate the point that as the stressor increases from acute to chronic, the concentration of BDNF decreases in the hippocampus and prefrontal cortex. This translates to decrease in memory and cognition especially after prolonged exposure to stress. This learning activities controlled by the hippocamus were decreased as a result of BDNF decrease hence decrease cognition and it agrees with the work of (Rasmusson *et al*., 2002).

4. CONCLUSION

Brain derived neurotrophic factor (BDNF) patterns in anxiety/depressive states of stress-evoked wistar rats were investigated in the study using various stressor types and procedural tools. These factors respond to stress in different manners depending on the severity of the stress. BDNF declines in concentration as stress exposure increases.

As observed in the study, stimulations of different parts of the brain may either up regulate or down regulate cognition and memory. Regulation of memory is done through proteins which stimulates neural plasticity and protection. An increase in the severity of stress factors decreases the concentration of BDNF in the hippocampus which in turn reduces memory and cognition. The amygdala however, experience an increase in the concentration of BDNF which fosters responsiveness to threats and promotes anxiety. The reactive oxygen species produced from chronic stress induction may have led to an upsurge in the antioxidant capacity of the animal. Continuous stress stimulation will cause the reactive oxygen species to overwhelm the antioxidants causing neurodegeneration. Avoiding occasions of stress or removing the stress stimulus will protect neural cells from damage by the reactive oxygen species. Since BDNF regulation varies in different part of the brain, the molecule can be targeted for possible therapeutic gains against neurodegenerative conditions. In the overall, depression becomes established in the brain whenever the concentrations of BDNF decrease abysmally low but becomes insignificant in expression whenever cortisol level increases whether or not there is concomitant increase in the level of BDNF. It simply

means Cortisol and not BDNF is a chief determinant of depression.

5. REFERENCES

1. Tsigos, I. Kyrou, E. Kassi, G.P. Chrousos Stress, Endocrine Physiology and Pathophysiology Endotext, MDText.com, Inc, South Dartmouth (MA), 2016.
2. karim Alkadhi, K. H. Alzoubi, M. Srivareerat, T. T. Tran, "Role of 2-nAChRs in the neuroprotective effect of nicotine in stress-induced impairment of hippocampusdependent memory," (e International Journal of Neuropsychopharmacology, 2013; 16: 1–9.
3. Henckens MJ, Hermans EJ, Pu Z, Joels M, Fernandez G Stressed memories: How acute stress affects memory formation in humans. J Neurosci, 2009; 29: 10111–10119.
4. Oei, B.M. Elzinga, O.T. Wolf, M.B. deRuiter, J.S. D amoiseaux, J.P. Kuijer, D.J. Veltma, P. Scheltens, S. A. Rombouts Glucocorticoids decrease hippocampal and prefrontal activation during declarative memory retrieval in young men Brain Imag. Behav, 2007; 1: 31-41.
5. Henckens MJ, Hermans EJ, Pu Z, Joels M, Fernandez G Stressed memories: How acute stress affects memory formation in humans. J Neurosci, 2009; 29: 10111–10119.
6. De Quervain DJ-F, Roozendaal B, McGaugh JL Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature, 1998; 394: 787-790.
7. Schutte, S.S., Schutte, R.J., Barragan, E.V., O'Dowd, D.K. Model systems for studying cellular mechanisms of SCN1A-related epilepsy. J. Neurophysiol, 2016; 115(4): 1755—1766.
8. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. Pages 47-60 & pmid = 6471907, 1984.
9. Miao C, Pages A, Xu Z, Rodene E, Yang J, Schnable JC Semantic segmentation of sorghum using hyperspectral data identifies genetic associations. *Plant Phenomics,* 2020; 4216373.
10. Surai P.F., Kochish I.I., Romanov M.N., Griffin D.K. Nutritional modulation of the antioxidant capacities in poultry: The case of vitamin E. *Poult Sci.,* 2019; 072. doi: 10.3382/ps/pez072.
11. Rassmuson A, Morgan CA III, Wang S, Hoyt G, Hauger R Hazlett G NPY, cortisol and subjective distress in humans exposed to acute stress: Replication and extension of previous report. Biol Psychiatry, 2002; 52: 136-142.