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Research Article

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Effects of Energy Drink (Red bull) on some neurotransmitters content and histological structure in the hippocampus region in male albino rats

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ABSTRACT

Energy drinks (Red bull) is the most consumed in Saudi Arabia by different groups ages in recent times, especially by young age. It is one of the substances that cause many damages such as neurotoxicity, damage of nerve cells and impact on memory and learning difficulties. This study aims to determine the impact of oral administration of red bull on hippocampus tissue. 3.1 ml of red bull and saline (control) were daily administered orally for 3 weeks to rats. Neurotransmitters levelsincluding (Norepinephrine (NE), Dopamine (DA) and Gamma-AminobutyricAcids (GABA)) were measured and hippocampus tissues were used for histological assay.The result of the study showed that the levels of neurotransmitter were decreased in red bull administered rat. The histopathological findings of the hippocampus brain show that there were degeneration of nerve cells and nuclei,sharp abnormalities in pyramidal cells. This change will affect the memory.

Key words:*Red Bull,Neurotransmitters, The hippocampus.*

INTRODUCTION

Energy drinks are considered the most consumed by the different age groups, especially teenagers, believing that they improvethe level of performance, give them more energy; therefore, they often drink a lot of it. The active ingredients that found in most energy drinks (caffeine and taurine, glucose and many vitamins) working to provide the body a great deal of metabolic and mental energy, manufacturers claim that their products increase the energy and activity, overcome sleepiness, provide concentration while studying and driving as well as reduce the symptoms of headaches resulting from alcohol (1). It was found that the energy drinks have side effects such as cardiac arrhythmia, hypertension and toxicity especially when overconsumption. They also have a significant effect on the functions of neurons, catechol amin concentration in the brain, linked to injury Parkinson's disease and low age related cognitive. Studies have shown that energy drinks cause insomnia and sleeplessness (1-2).

Despite the adverse effects of these drinks, some companies are still competing for the production of these drinks and put them on the market and the most brand names traded in Saudi Arabia are: Red Bull, Bison, Power Horse,

Cod Red and Boom Boom. The energy drinks are marketed to consumers as activators called attractive names express the strength, power and speed (3).

Most people consume red bull to compensate to improvement in higher cognitive functions such as memory. A consensus on red bull impact on memory and hippocampus tissue has not yet been reached (4).

It is important to reveal the risks of energy drinks and to know importance of it. The current study aimed to study the effect of red bull on some neurotransmitters content: norepinephrine (NE), dopamine (DA), gamma-aminobutyrice acids (GABA)and the histological study of the hippocampus in brains of young male albino rats treated with energy drink red bull.

MATERIALS AND METHODS

2. Materials and Method

2.1. Materials used

a.Animals used:

Experiments of this research was conducted on a group of male albino rats (30 rats) from the rank of rodents Order: Mammalia which ranges weights of (70g - 90g) and ages ranges roughly from 5-7 weeks, were obtained and all the tests conducted at King Fahd Medical Research Center that located at King Abdulaziz University in Jeddah, where they developed throughout the duration of the experiment in private rooms in metal cages with food availability (dry balanced diets for animals experiments), water and lighting (12 hours of darkness and 12 hours lighting), good ventilation and temperature ranging from 22-25 ° C.

b. Energy drink (Red Bull)

It was obtained from local markets in Saudi Arabia and give to experimental animals orally at a dose (3.1 ml / day) (5).

2.2. Experimental Design

2.2.1. Physiological study

The rats (24 rats) of this group divided into:

The first group (G1) :(6) rats were giving a normal saline by oral tube for a week and then killed at the end of the week (control group).

The second group (G2): (18)male rats weregiven a dose of red bull (3.1 ml / day) by oral tube for succession 3 weeks, and after that six of the rats were slaughtered at the end of 1, 2 and 3 weeks, then compared the results of this group to (G1).

2.2.2. Histological study

Young male rats of this group was divided (24 rats) to 2 main groups:

The first group (G1) :(6) rats were giving a normal saline by oral tube for a week and then killed at the end of the week (control group).

The second group (G2): (18) rats were given a dose of red bull (3.1 ml / day) by oral tube for 3 consecutive weeks and killed at the end of 1, 2 and 3 weeks, then compared the results of this group to (G1) for histological study.

2.2.3. The measurement of neurotransmitters in hippocampus region of the brain:

In this study, measurement of norepinephrine content (NE) and dopamine (DA) according to the method of (6) as well as the account of GABA content depending on the method of (7) were done. It has all the physiological measurements using a Jenway 6200 fluorometer.

2.2.4. Separations of hippocampus region in the brain:

Rats were killed suddenly at different times of the experiment and then taken brain carefully and cut lengthwise into halves on a cold plate glass and then separated the hippocampusregion accordingto (8).The sections of hippocampus were fixed in Bouin'sfluidand cut using Microtome at 5 microns and staining by Heamatoxylin and Eosin (H & E), then Examined by light microscope (9).

Statistical analysis:

The expression of neurotransmitters content under study was an average arithmetic + standard error of the mean + S.E.M then compared to the groups treated with control using (Student's t -test) (10) and then calculated the percentage of difference% compared to the control group and this equation:

$$\% \text{ difference} = \frac{\text{Treated value} - \text{control value}}{\text{control value}} \times 100$$

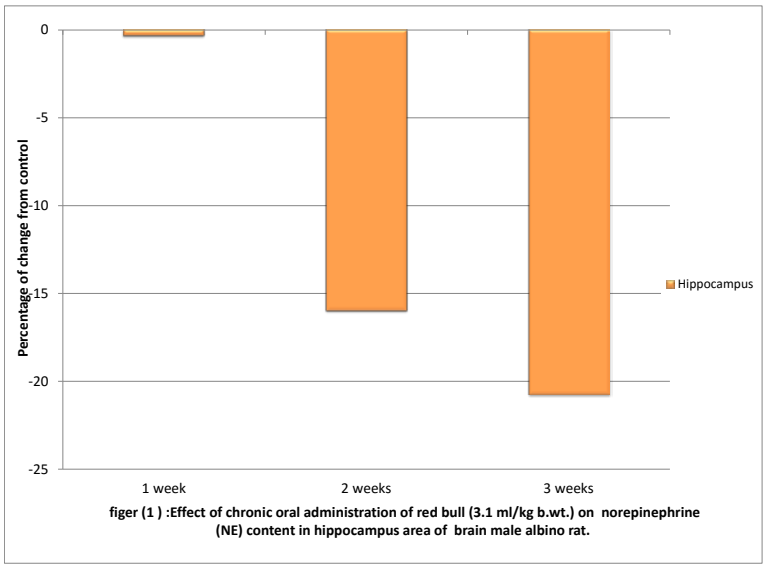
RESULT

3-1: neurotransmitters level in hippocampus region of the brain

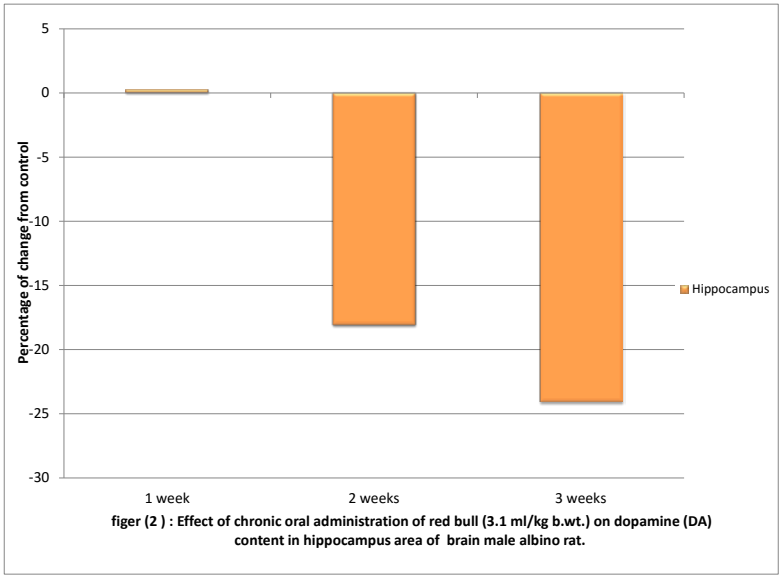
The daily intake of red bull (3.1 ml / day) causes significant decrease in the content of NE in the hippocampus area after the second and third week. The results recorded the highest significant decrease in the hippocampus (-20.72%) after the third week (Fig 1).

The daily intake of red bull (3.1 ml / day) causessignificant decrease in the content of DA in the hippocampus area after the second and third week. The results recorded the highest significant decrease in the hippocampus (-24.05%) after the third week (Fig 2).

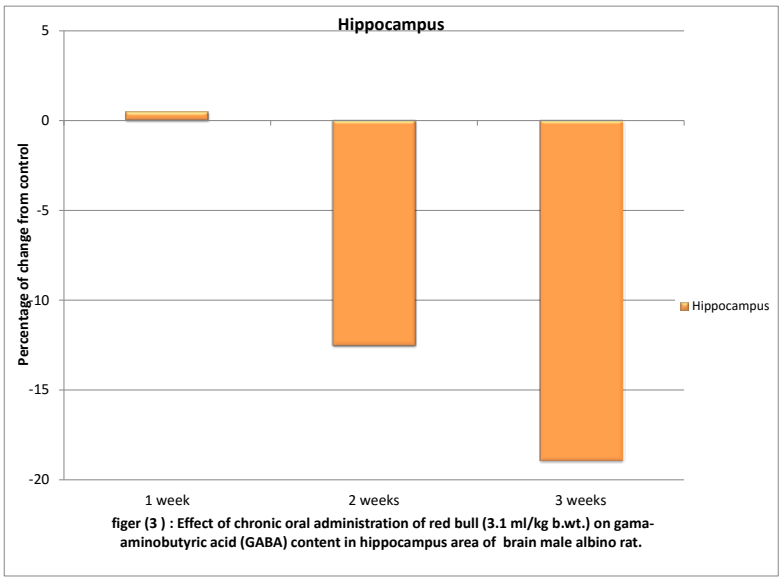
The daily intake of red bull (3.1 ml / day) causessignificant decrease in the content of GABA in the hippocampus area after the second and third week of treatment. It has been found the highest significant decrease of the GABA content in the hippocampus area (-18.91%) after the third week (Fig 3).



figer (1) :Effect of chronic oral administration of red bull (3.1 ml/kg b.wt.) on norepinephrine (NE) content in hippocampus area of brain male albino rat.



figer (2) : Effect of chronic oral administration of red bull (3.1 ml/kg b.wt.) on dopamine (DA) content in hippocampus area of brain male albino rat.



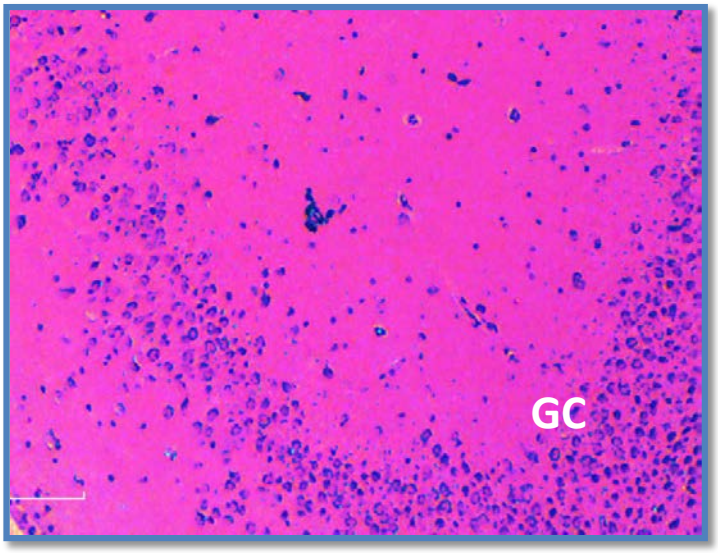
figer (3) : Effect of chronic oral administration of red bull (3.1 ml/kg b.wt.) on gama-aminobutyric acid (GABA) content in hippocampus area of brain male albino rat.

3-2: Histological examination result

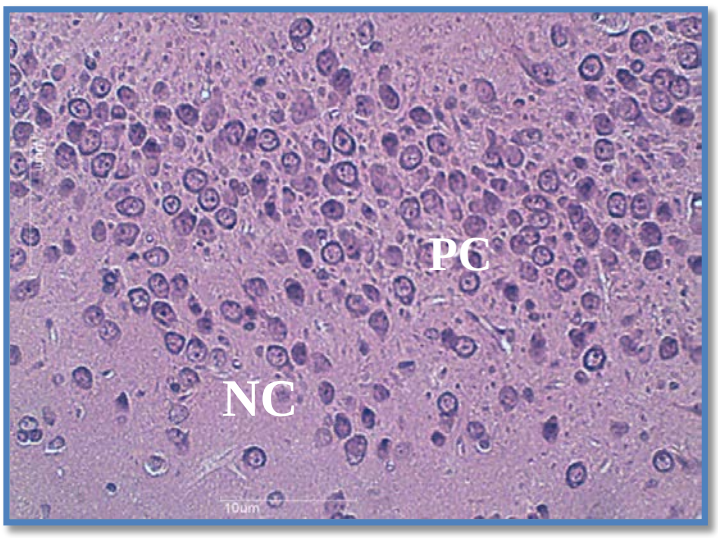
Control group

cross-section of light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the control group showing vesicular nuclei, few layers of large pyramidal cells in CA3 region, also with vesicular nuclei. Molecular layer (ML) shows many glial cells granular cells G with nuclei in dentate gyrus DG.

Fig (4a-b): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the control group



Figure(4-a): Section of the control group showing of granular cells(GC) of Dentate gyrus (H & E ×100).



Figure(4-b): Section from control group showing of vesicular nuclei, few layers of large pyramidal cells (PC) in CA3 region, also with vesicular nuclei . Molecular layer (ML) (H & E ×400).
PC = Pyramidal cells
NC = Neuroglial cells

Treatment group

Fig (5-a, b): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after one week showing distortion in the pyramidal cells, reduced pyramidal cells and neurodegeneration

Fig (5-a,b): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after one week

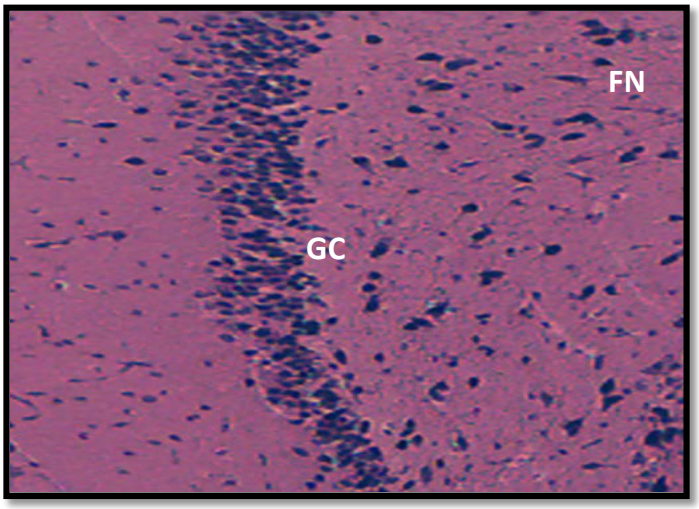


Fig (5-a): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after one week (H & E ×100).

Section of granular cells (GC) of Dentate gyrus of the hippocampus Showed granular cells of the Dentate gyrus of hippocampus with cell distortion and few Neurofibrillary tangle.
NF= Neurofibrillary Tangle

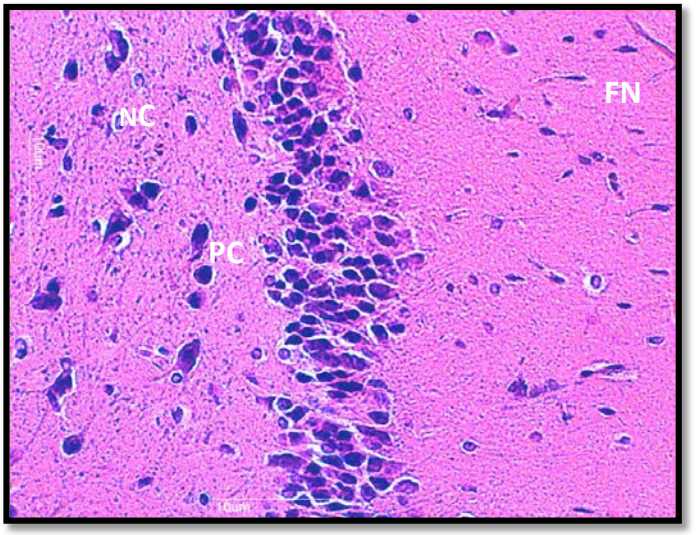


Fig (5-b): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after one week (H & E ×400).

Section showed distortion in the pyramidal cells , reduced pyramidal cells, neurodegeneration and Disintegration of nerve fibers

Fig (6-a, b,c): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after tow week.showinggranularcells retraction of processes with vacuolations showing apoptosis of large pyramidal cells, loss of small pyramidal cells, loss Neuroglial cells(NC) and separation of fiber.

Fig (6-a,b,c): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after tow week

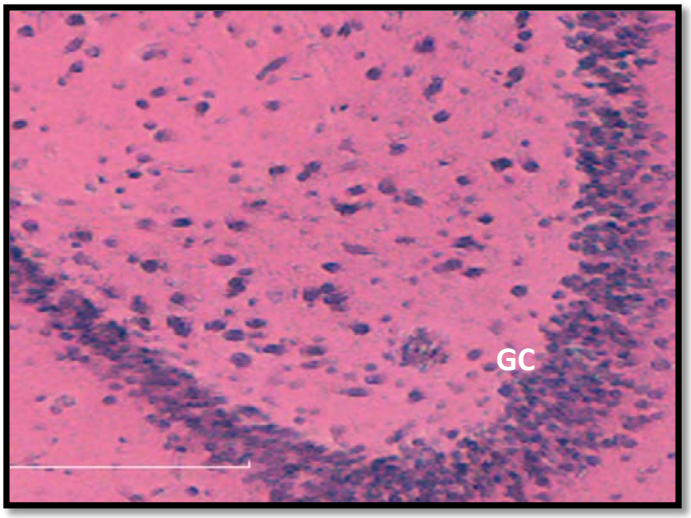


Fig (6-a): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after tow week (H & E ×100).

Section of loss of granular cells .

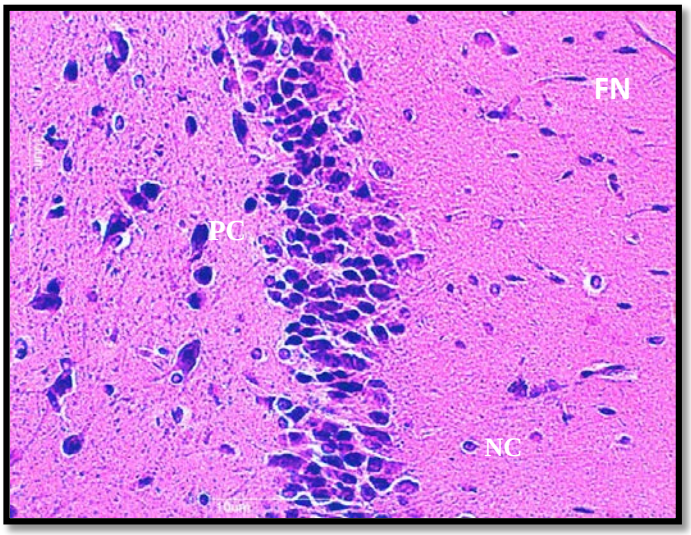


Fig (6-b): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after tow week (H & E ×400).

Section of distortion in the pyramidal cells ,reduced pyramidal cells and high level of neurodegeneration.

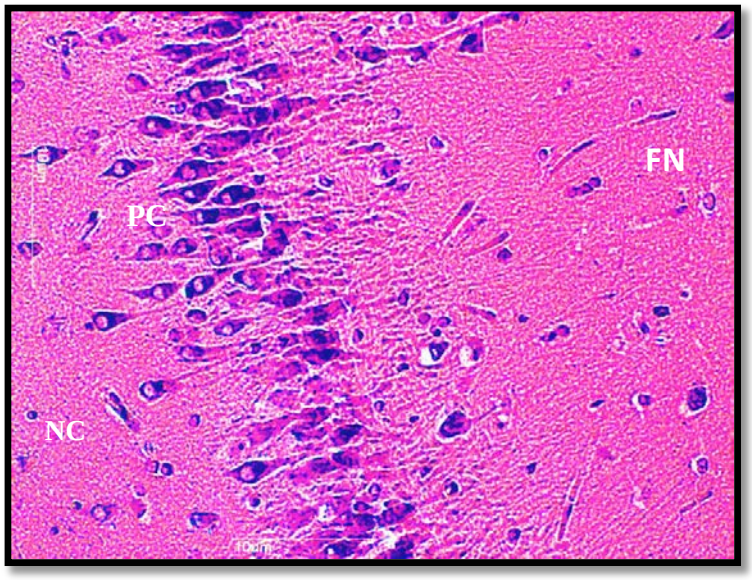


Fig (6-c): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after tow week (H & E ×400).
Section of apoptosis of large pyramidal cells , loss of small pyramidal cells, loss Neuroglial cells(NC) and Separation of fiber.

Fig (7-a, b,c,d):Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after three weeks. Showinga degeneration of nerve cells and nuclei, Sharp abnormalities in pyramidal cells, analyzed the nervous tissue, the expansion the blanks around nerve cells, lysis nerve tissue and atrophy neurons and the small number of nerve cells

Fig (7-a,b,c,d): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after three week

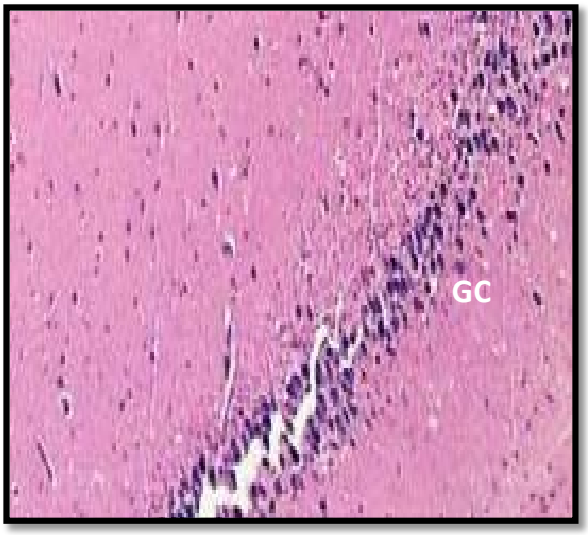


Fig (7-a): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after three week (H & E ×100).

Section of granular cells of the Dentate gyrus Showing degenerations with vacuolations, , cell distortion and few Neurofibrillary tangle with dilated vessels.

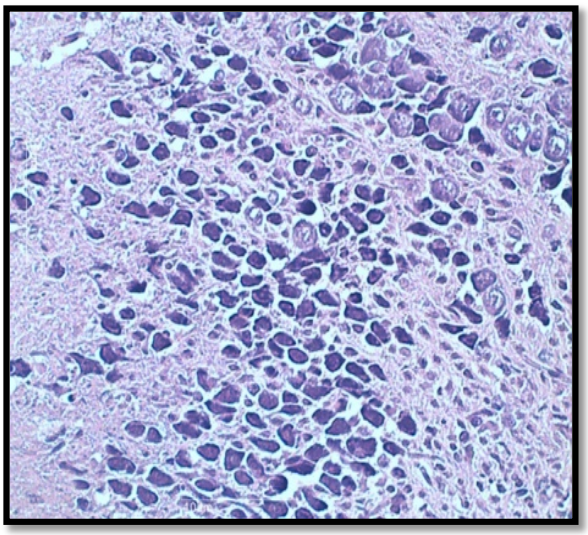


Fig (7-b): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after three week (H & E ×400).

Section of pyramidal cell layers of hippocampus Showing cell degenerations, , necrosis of pyramidal cells and few neurofibrillary tangle

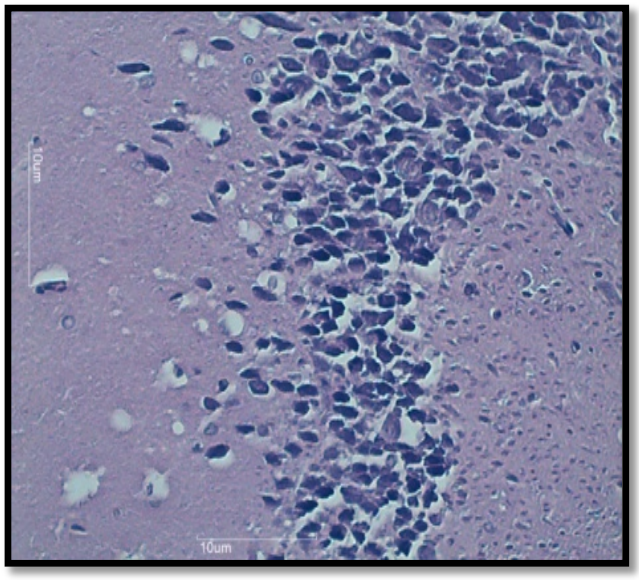


Fig (7-C): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after three week (H & E ×400).

Section of pyramidal cell layers of hippocampus shows neurodegeneration,disorganization with apoptosis of large pyramidal cells with vacuolation

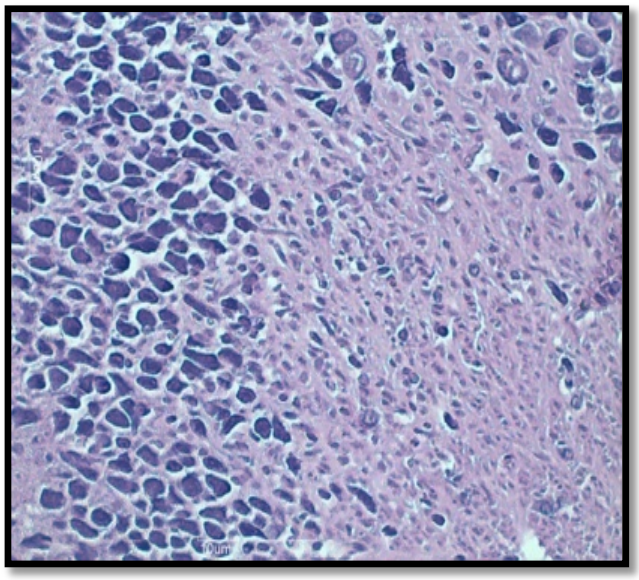


Fig (7-d): Cross-section of Light microscope (LM) sector accidental in the hippocampus area of the brain male albino rats of the treatment group after three week (H & E ×400).
Section of pyramidal cell layers of hippocampus Showing apoptosis of large pyramidal cells

DISCUSSION

Red bull contains many ingredients that have adverse effects on the brain such as anxiety, stress, hyper movement headache, fatigue (11-12) depression, sleep disturbance, mood change and irritable (13-14).

The results of the current study Explained that daily treatment withared bull dose (3.1ml / day)for 3 weeks succession led toobserve decrease in neurotransmitters content: nnorepinephrine,ddopamine and gamma-aaminobutyricacidsinhippocampus. This is consistent with previous studies which showed that caffeine activates nnoradrenergic neurons , adrenergic neurons , cholinergicneuron, serotonergic neurons and GABAergic neurons led to increase its liberation from producing neurons in different brain regions (15) and alsocaffeine affects the function of the dopaminergic neurons particularly in Mid brain and Pons, which works to increase nerve conduction of dopamine(16)by increasing the spread of dopamine fromthe pre-synaptic cells thus, an occurrence of locomotor activity (17-18).

Caffeine (the most important component) acts on the central nervous system which has a high ability to cross the blood-brain barrier because it's high soluble in lipids (19). Caffeine would lead to activate the central nervous

system and increase motor activity (20) by changing the enzyme activity of phosphodiesterase (21) and to increase the entry of calcium ions into the ends of nerve cells (22). It was found that the caffeine increases the oxidation of fatty acids, which increases the energy and the level of glucose in blood (23).

Some studies have shown that the caffeine lead to the closure of adenosine receptors in the central nervous system and reduces the level of serotonin to the level of DA in the brain, leading to delay fatigue (25-26),occurring stimulation neuron cell and liberation of neurotransmitter as a result of the closure adenosine receptors(27-28).

The study of (29)hasnotedto the excessive use amount of caffeine found in red bull lead to the release of dopamine-producing cells. Caffeine works to prevent GABA from the link receptors and performing his job as a moderator, leading to stress(29).

Caffeine works to close the Adenosine receptors (A2) in the striatum area (30). In the striatum area,thecaffeineworksto activate dopamine receptors,as a result, it reduces the activity of adenosine mono phosphate cyclic (AMP)which lied to increase the release of neurotransmitters, the occurrence of activity and alertness associated with caffeine abuse (31).

Nerve cells contain adenosine natural, which increases concentration in the brain during waking and decreases during sleep (32). Activating the adenosine receptor plays an important role in the neurotransmission in the brain (33) by preventing the release of neurotransmitters, including dopamine, acetylcholine, serotonin, NE, GABA and glutamate. This resultsin changing ofbehavioral and inhibition of motor activity, the change in content of dopamine and serotonin it is associate to behavior (34).

A2 receptors is Located in Dopaminergic neurons, such as the striatum (35) and A1 receptorsare located in all parts of the brain especially in the hippocampus, cerebral cortex, and hypothalamus (36).

It has been observed that caffeine is connected to adenosine A2receptors when take a low dose and it is associated with adenosine A1 receptors when take a high dose. Caffeine is associated with adenosine receptorswhichleads to increasetherelease of dopamine due to enhance the effect of caffeine in body (37).

Some studies showed that consumption of caffeine that affects the biological processes in the hippocampus is responsible for learning, memory and the occurrence of seizures, this is compatible with previous studies that showed the chronic consumption of caffeine causes neurological toxicity, damage to the nerve cells, having impact on memory and learning disability (38).

The histological examination of the sectors in the hippocampus administration daily with red bull dose (3.1ml / day) for 3 week caused a degeneration of nerve cells, apoptosis of large pyramidal cells, necrosis of the nerve tissue, the expansion of the blanks around nerve cells, distortion granular cells of the Dentate gyrus with few Neurofibrillary tangle. and atrophy neurons. This damage may be back to the effect of caffeine (39).

The study showed that the daily ingestion of red bull dose (3.1 ml / day) for three weeks causes a significant decrease in the total content of neural connectors in all brain regions at different times of the study, and this is probably due to the effect of caffeine in energy drink which leads to the closure of adenosine receptors, which works to open calcium channels and increase calcium entry into the nerve cell and lack of the total content of the studied neurotransmitter.

The results of the current study and previous studies can be concluded in the daily administration of red bull dose (3.1ml / day) for three weeks succession led to a significant decrease in the total content of NE,DA and GABA in hippocampus area at different times of the study, and this may be back to caffeine affect that closes the adenosine receptors in brain which leads to increase influx calcium ions into the nerve cell which will lead to release neurotransmitters found in vesicles in pre synaptic cells through a special protein synapsin 1, to the cleft leading to decrease of the total content of neurotransmitters inside cells (40).

In conclusion, the findings of this study suggested that red bull may cause physiological and histological changes in rat hippocampus tissue, which could play an important role in memory dysfunction.

REFERENCES

1- Seifert, S. M.; Schaechter, J. L.; Hershorin, E. R. and Lipshultz, S. E. (2011): Health effects of energy drinks on children, adolescents, and young adults. Pediatrics. 127(3): 511-528.

2- Specterman, M.; Bhuiya, A.; Kuppuswamy, A.; Strutton, P.; Catley, M. and Davey, N. (2005): The effect of an energy drink containing glucose and caffeine on human corticospinal excitability. Physiol. behav. 83(5): 723-728.

3- Backer, W. S. and Baeissa, H. M. (2014): Effect of different energy drinks on liver and heart enzymes in rats. Int. J. Biotechnology. 3(1): 1-11.

4- Malinauskas, B. M.; Aeby, V. G.; Overton, R. F.; Carpenter-Aeby, T. and Barber-Heidal, K. (2007): A survey of energy drink consumption patterns among college students. Nutr. J. 6:35.

5- Bawazir, A.E. and Almehmadi, M. G. (2017): Effect of "Red Bull" Energy Drink on Some Neurotransmitters Content and Histological Structure of Cerebral Cortex in Male Albino Rats. Life Science Journal .4(1):63-73.

6- Chang, C. C. (1964): A sensitive method for spectrophoto metric assay of catecholamines. Int. J. Neuropharmacol. 3: 643-649.

7- Sutton, I. and simmonds, M. A. (1973): effects of acute and chronic ethanol on gamma-aminobutyric acid system rat brain. Biochem.pharmacol. 22: 1685-1692.

8- Glowinski, J. and Iversen, L.L. (1966) Regional studies catecholamines in the rat brain I. The disposition of [3H] norepinephrine, [3H] dopamine and [3H] dopa in various regions of the brain.Journal of Neurochemistry, 13,655-669.

9- Bancroft, J. D. and Steven, A. (1996): Theory and practice of hitologicaltecniques. 4th ed. Churchill Livingston.

10- Hill, H. B. (1971): Principles of medical statistic. 9th ed. Oxford Univ. press.

11- Guilbeau, J. R. (2012): Health Risks of Energy Drinks. Nursing for Women's Health. 16(5): 423- 428.

12- Buck, R.; Dixon, J.; Matjasich, L. and Petersen, R. (2013): Energy Drink Consumption among Adolescents and Young Adults: Health Effects and Implications for Practice. Westminster College School of Nursing and Health Sciences. 1- 58.

13- Tibrewal, P. and Dhillion, R. (2011): Caffeine induced psychotic exacerbation. Aust. N. Z. J. Psychiatry. 45(2): 179-180.

14- Ishak, W. W.; Ugochukwu, C.; Bagot, K.; Khalili, D. and Zaky, C. (2012): Energy drinks: psychological effects and impact on well-being and quality of life-a literature review. Innov.Clin.Neurosci. 9(1): 25-34.

15- Schuckit, M. A. (2000): Drug and Alcohol Abuse: A Clinical Guide to Diagnosis and treatment. 5th ed. Kluwer Academic/Plenum Publishers. New York.

16- Lorist, M. M. and Tops, M. (2003): Caffeine, fatigue and cognition. Brain Cogn. 53(1): 82–94.

17- Solinas, M.; Ferre, S.; You, Z. B.; Karcz-Kubicha, M.; Popoli, P. and Goldberg, S. R. (2002): Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. J. Neurosci. 22(15):6321–6324.

18- Kaasinen, V.; Aalto, S.; Nagren, K. and Rinne, J. O. (2004): Dopaminergic effects of caffeine in the human striatum and thalamus. NeuroReport. 15(2): 281–285.

19- Gepdiremen, A.; Sonmez, S.; Ikbal, M.; Düzenli, S.and Tuna, S. (1998): Response to nimodipine in caffeine-induced neurotoxicity in cerebellar granule cell culture of rat pups. Pharmacol. Res. 38(4):239–242.

20- Fisone, G.; Borgkvist, A. and Usiello, A. (2004): Caffeine as a psychomotor stimulant: mechanism of action. Cell. Mol. Life Sci. 61(7–8): 857–872.

21- Choi, O. H.; Shamin, M. T.; Padgett, W. L. and Daly, J. W. (1988): Caffeine and theophylline analogues: Correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. Life Sci. 43(5):387–398.

22- Berridge, M. J. (1991): Caffeine Inhibits Inositol-Trisphosphate-Induced Membrane Potential Oscillations in Xenopus Oocytes. Proc. Biol. Sci. 244 (1309): 57-62.

23- Glade, M. J. (2010): Caffeine-Not just a stimulant. Nutrition. 26(10): 932-938.

24- Huxtable, R. J. (1992): Physiological actions of taurine. Physiol. Rev. 72(1): 101-163.

25- Davis, J. M.; Zhao, Z.; Stock, H. S.; Mehl, K. A.; Buggy, J. and Hand, G. A. (2003): Central nervous system effects of caffeine and adenosine on fatigue. Am. J. Physiol. Regul. Integr. Comp. Physiol. 284(2): 399-404.

26- Gillingham, R. L.; Keefe, A. A. and Tikuisis, P. (2004): Acute caffeine intake before and after fatiguing exercise improves target shooting engagement time. Aviat. Space Environ. Med. 75(10): 865-871.

27- Gervitz, L. M.; Luthere, L. O.; Davies, D. G.; Pirch, J. H.and Fowler, J. C. (2001): Adenosine induces initial hypoxic-ischemic depression of synaptic transmission in the rat hippocampus in vivo. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280(3): 639-645.

28- Okada, M.; Kawata, Y.; Kiryu, K.; Mizuno, K.; Wada, K.; Tasaki, H. and Kaneko, S. (1997): Effects of adenosine receptor subtypes on hippocampal extracellular serotonin level and serotonin reuptake activity. J. Neurochem. 69(6): 2581–2588.

29- Gurpegui, M.; Aguilar, M. C.; Martinez-Ortega, J. M.; Diaz, F. J. and De Leon, J. (2004): Caffeine intake in outpatients with schizophrenia. Schizophr. Bull. 30(4): 935-945.

30- Nehlig, A. and Boyet, S. (2000): Dose–response study of caffeine effects on cerebral functional activity with a specific focus on dependence. Brain research. 858(1): 71-77.

31- Rossi, S.; De Chiara, V.; Musella, A.; Mataluni, G.; Sacchetti, L.; Siracusano, A.; Bernadi, G.; Usiello, A. and Centonze, D. (2010): Effects of caffeine on striatal neurotransmission: focus on cannabinoid CB1 receptors. Mol. Nutr. Food Res. 54(4): 525–531.

32- Rossi, S.; De Chiara, V.; Musella, A.; Mataluni, G.; Sacchetti, L.; Siracusano, A.; Bernadi, G.; Usiello, A. and Centonze, D. (2010): Effects of caffeine on striatal neurotransmission: focus on cannabinoid CB1 receptors. Mol. Nutr. Food Res. 54(4): 525–531.

33- Latini, S. and Pedata, F. (2001): Adenosine in the central nervous system: release mechanisms and extracellular concentrations. J. Neurochem. 79(3): 463-484.

Porkka-Heiskanen, T. (1999): Adenosine in Sleep and Wakefulness. Ann. Med. 31(2): 125-129.

34- Yabuuchi, K.; Kuroiwa, M.; Shuto, T.; Sotogaku, N.; Snyder, G. L.; Higashi, H.; Tanaka, M.; Greengard, P.; Nishi, A. (2006): Role of adenosine A1 receptors in the modulation of dopamine D1 and adenosine A2A receptor signaling in the neostriatum. Neuroscience. 141(1):19–25.

35- Ferre, S.; Fuxe, K.; Von Euler, G.; Johansson, B. andFredholm B. B. (1992): Adenosine– dopamine interactions in the brain. Neuroscience. 51(3): 501– 512.

36- Winston, A. P.; Hardwick, E. and Jaberi, N. (2005): Neuropsychiatric effects of caffeine. Adv. Psychiatr. Treat.11(6):432–439.

37- Fredholm, B. B.; Bättig, K.; Holmén, J.; Nehlig, A. and Zvartau, E. E. (1999): Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 51(1):83–133.

38- Roy, M. and Sapolsky, R. M. (2003): The exacerbation of hippocampal excitotoxicity by glucocorticoids is not mediated by apoptosis. Neuroendocrinology. 77(1): 24-31.

39- Akande, I. S. and Banjoko, O. A. (2011): Assessment of biochemical effect of "Power Horse" energy drink on hepatic, renal and histological functions in Sprague dawley rats. Annual Rev. Res. Biology. 1(3): 45-56.

40- Bullock, J.; Boyle, J. and Wang, M. B. (1995): synaptic transmission in: physiology Middle East. 3rd ed. Chapter 3.Williams and wilkines. London. pp: 22-31.