

Histopathological and Neurotransmitter Changes in Albino Rats' Brain after Consumption of Energy Drink in Egypt

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Abstract

Background: Energy drinks are carbonated non-alcoholic beverages, used during exercise and different sports for increasing physical strength and mental alertness. Recently they are widely used by different ages of male and female. Visits to the emergency department due to energy drinks consumption were doubled from 2007 to 2011. Most of Energy drinks have attractive names as to express the speed, power, and strength. Energy drinks have a lot of ingredients and some of them are not approved by the food and drug administration (FDA). **Methods:** A total of eighty adult albino rats were divided randomly into four groups (20 per each). **Control group:** not exposed to any treatment and received tap water. Second, third and fourth group received a dose of (3.8ml\100g) of body weight for 3 weeks orally of three different energy drinks brands available in Egyptian markets (Power horse, Red bull, Sting) respectively. **Results:** Our results concerning energy drinks effects on brain disclosed significant increase of stimulant neurotransmitters of norepinephrine and dopamine with significant decrease of inhibitory neurotransmitter of Gamma-aminobutyric acid in response to high dose of energy drinks, which were consistent with findings of histopathological examination that showed severe degenerative changes and necrosis of neural cells of the brain on the cerebral cortex and hippocampus neurons, indicating toxic effect of energy drinks on neurons of the brain. **Conclusion:** Energy drinks consumption in high dose had marked hyper stimulating toxic effects on brain stimulatory neurotransmitters and degeneration of neurons in different regions of the brain.

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Key words

Energy drink, histopathology changes, biochemical alteration, brain tissue

Background

Energy drinks appeared firstly in Europe and Asia. In 1962, a type of energy drink was produced by the Japanese company of Taisho Pharmaceuticals. In 1987, Austria and Europe started the production of Red Bull energy drink then it became available in more than 140 countries (Osaba et al., 2019). In 1997, it was introduced in the American market (Heckman et al., 2010).

Energy drinks are so popular for consumption by athletes, secondary school students, and service members (Higgins et al., 2018). Energy drinks attract young adults because of their distinct taste, vigorous feeling, overcome sleep, improve mental and physical performance, and increase concentration during the study and driving (Hasan et al., 2020).

Most of energy drinks contain caffeine, taurine, glucuronolactone, carbohydrates, vitamins, and other herbal extracts as ginseng and guarana. Guarana and ginseng are considered natural sources of caffeine, and their levels are often not labeled on the package labeling (Seifert et al., 2011). Consumption of 250ml of an energy drink can contains (80mg of caffeine, 27g of carbohydrate, 1.0g of taurine, 0.6g of

glucuronolactone, 20mg of niacin, 5mg of vitamin B6, 5mg of pantothenic acid, and 5 µg of vitamin B12 and other ingredients) (Heckman et al., 2010). Different brands of energy drinks contain caffeine in a range of 50mg to 550mg per can or bottle (Ishak et al., 2012).

Caffeine represents one of the essential components of energy drinks and recent research found synergistic interaction between components of energy drinks rather than the actions of caffeine alone (Peacock et al., 2013). Most of energy drinks contain 32 mg per 100 mL, but others could contain high levels up to 30 to 134 mg of caffeine per 100 mL, concentrations that greatly exceeds the FDA limit of 20 mg per 100mL of caffeine in traditional soda drinks (Higgins et al., 2018).

Habitual intake of caffeine dose of (100-150mg) will stimulate cerebral cortex, increasing alertness, awareness but lower motor reaction time of visual and auditory events. In higher doses, it stimulates the myocardium and results in tachycardia and rhythm irregularities (Guilbeau, 2012). It increases the secretion of pepsin and hydrochloric acid from parietal cells and decrease the pressure of the lower esophageal

sphincter resulting gastrointestinal irritation, esophagitis, gastroesophageal reflux, nausea, and dyspepsia. It also causes rising of circulating epinephrine, norepinephrine, plasma renin activity which cause increase in blood glucose, lipolysis, free fatty acids, metabolic rate, cortisol, and serum cholesterol (Benowitz, 1990).

Caffeine increases circulating catecholamines resulting in increased body temperature and heat production. High caffeine intake may increase the excretion of calcium in urine which causes a reduction in bone mineral density (Bryant and Knights, 2014). Persons used to ingest 600mg/day of caffeine suffer from withdrawal syndrome (headache, fatigue, yawning, depression, irritation, and weakness) after cessation of caffeine (Bryant and Knights, 2014).

Also, Taurine is an essential ingredient for energy drinks. It is a sulphonated amino acid (2-aminoethyl sulphonic acid) (Peacock et al., 2013). Taurine increases systolic ejection and has detrimental effects on Sodium channels in the cellular membrane which cause increase in blood pressure, coronary vasospasm, development of arrhythmias (Hammond et al., 2018), and decrease the platelets aggregation (Granum et al., 2015).

Energy drinks contain a mixture of different carbohydrates including monosaccharides as (glucose and fructose), disaccharides as sucrose, and oligosaccharides as maltodextrin. Glucose and sucrose decrease systemic vascular resistance and increases heart rate and cardiac output however fructose increase systemic vascular resistance which may affect blood pressure (Oprea et al., 2019).

Also, fructose in high concentrations affects metabolism and causes an increase in insulin resistance, dyslipidemia, and increase visceral fat. The high concentration of simple sugars stimulates pancreatic beta cells to secrete and release high insulin which may result in the development of type 2 diabetes (Oprea et al., 2019).

The high concentration of carbohydrates slows gastric emptying and reduces the absorption of carbohydrates. This makes energy drinks hyperosmolar and could cause dehydration (Bigard and Guezennec, 2017).

Each can of energy drinks of 250ml contains 360% of vitamin (B6), 120% of vitamin (B3), and vitamin (B12), at such a high level, the antioxidant effect of the vitamins may become a pro-oxidant effect which in turn will decrease performance and cause health hazards (Oprea et al., 2019).

Vitamin (B3) is known as niacin or nicotinamide. There are side effects for niacin include flushing mediated by prostaglandins, stomach ulcers, muscle damage, hypotension, heart arrhythmia, insomnia, and hepatic toxicity (Oprea et al., 2019). Prolonged supplementation of high concentrations of vitamin (B6) could result in the development of sensory neuropathy which could be irreversible (Wolk et al., 2012). The accumulation of pyridoxal phosphate in the brain causes inflammation and development of pathological changes causing dementia (Bender, 1999).

Rise in popularity and consumption of energy drinks with decreased the awareness of ingredients of energy drinks, increased concerns in the scientific community, the public, and governments about the health hazards of these products, especially among children and adolescents (Hashem et al., 2018).

Methods

Animals

Eighty (80) albino rats, weighting (150-200) gram were purchased and housed in well-ventilated rooms, in polypropylene cages with stainless steel wire-bar covers, using a woody dust free litter as a bedding material, with Ad Libitum feed twice a day using a commercial balanced diet and clean freshwater continuous adequate supply. The rats were allowed to accommodate for ten days before beginning the experiment. Temperature and relative humidity were measured using a digital hygro-thermometer, with the temperature ranging between 19-22.5°C and the relative humidity between 40-55%, and the lighting system was kept on a reversed 12hr light-dark cycle. The Institutional Animal Care and Use Committee of Beni-Suef University provided ethical guidelines for this work (BSU-IACUC) (approval No.:021-179).

Substances

- 1- Power Horse energy drink is produced by Power Horse energy drinks GmbH which is a Company based in Austria. Each 100 ml of POWER HORSE energy drink contains 45 kcal, 32 mg of caffeine, 0.4 gram of taurine, 10.7 gram of carbohydrates, 0.06 gram of riboflavin (B2), 8 mg of niacin (B3), 2 mg of pantothenic acid (B5), 2 mg of pyridoxine (B6) with no fat, fiber, or protein (Eltahir et al., 2020).
- 2- Red Bull energy drink is produced by the Austrian company of RED BULL GmbH, each 100 ml of RED BULL energy drink contain water, carbon dioxide, 30 mg of caffeine, 0.4 gram of taurine, simple sugars as sucrose and glucose, riboflavin (B2), 8 mg of niacin (B3), 2 mg of pantothenic acid (B5), 2 mg of pyridoxine (B6), 0,002 mg of cyanocobalamine (B12), citric acid, flavors, sodium citrate, and inositol.
- 3- Sting energy drink is produced by Rockstar Inc of the international company of PepsiCo. Each 100ml contain 59kcal, 15 grams of sugar, 29mg of sodium, 2.5mg of vitamin (B3), 0.3mg of vitamin (B6) with no fat or fiber.

Those beverages were obtained from local markets in Egypt (Abdelwahab et al., 2020).

Experimental design

A total of eighty albino rats were weighed and randomly distributed into 4 groups (20 rats for each group). Control group (n=20) not exposed to any treatments received tap water, and each rat of the energy drinks treated groups (II, III, IV) received a dose of (3.8ml/100g) of body weight for 3 weeks orally of energy drinks (POWER HORSE, RED BULL, STING) respectively. The dose is adjusted according to Almeahadi, (2017).

Blood samples were obtained from retro orbital plexuses for biochemical analysis, then all animals

were scarified, rats were sacrificed via decapitation under light ether anesthesia. Mortality was also recorded during the treatment of all groups. Autopsy was intended to be done if animals died during the experiment.

Biochemical Analyses

Brain Tissue Homogenates Preparation:

Each animal's brain was completely extracted, grossed, and get rid of residual blood. Tissue was cut after weighing it and homogenize it in PBS with a glass homogenizer on ice. The homogenates of brain tissues were then centrifuged for 5 minutes at 5000×g to get the supernatant. After that, the supernatant was collected and treated based on the protocols for neurotransmitters (Owolabi et al., 2017).

Estimation of neurotransmitters:

- Estimation of GABA in brain homogenates: GABA was estimated using Rat GABA ELISA Kits supplied by Fine Test, Wuhan (Jeon et al., 2015).
- Estimation of Dopamine in brain homogenates: Dopamine was estimated using Rat Dopamine (DA) ELISA KIT supplied by CUSAPIO according to the method described by (Jia et al., 2020).
- Estimation of Noradrenaline in brain homogenates: Noradrenaline was estimated using NA/NE (Noradrenaline/Norepinephrine) ELISA Kit supplied by Fine Test, Wuhan (Al-Balawi et al., 2018).

Histopathological study:

Autopsy samples were taken from the rats in the four previously mentioned experimental groups. Then, brain samples were fixed in 4% paraformaldehyde solution. All samples were washed in tap water then diluted serially with absolute ethyl alcohol for dehydration. Specimens were cleared in xylene and submerged in paraffin at 56°C in a hot air oven for twenty-four hours. Blocks of paraffin beeswax tissue were prepared for sectioning at four microns thickness sections by sledge microtome. The obtained sections were fixed on glass slides, cleared from paraffin, and stained with H & E stain for histopathological examination under the light microscope (Bancroft et al., 1996).

Statistical analysis:

Data was analyzed using SPSS version 25 for windows 10. Numeric scale variables in the study were normally distributed, so they were expressed as mean and standard deviation. One Way ANOVA test was used to compare the four groups. Tukey Post Hoc test was used to compare each 2 groups. P-value was considered significant at ≤ 0.05 (two sided) (McHugh, 2011).

Results

Results of biochemical parameters:

Table (1) and Figure (1) revealed that there was a significant difference between the four groups regarding the GABA level. Power horse group showed significant decrease in GABA levels, followed by Red Bull group then Sting group when compared to the control group. The mean GABA level was 47.89 ± 2.71 ,

24.67 ± 2.99 , 35.42 ± 2.59 and 41.85 ± 1.92 pg/ml for control group, Power Horse, Red Bull and Sting, respectively.

Table (2) and Figure (2) revealed that there was a significant difference between the four groups regarding the norepinephrine level. Power Horse group showed significant increase in norepinephrine levels, followed by Red Bull group then Sting group when compared to the control group. The mean norepinephrine level was 12.41 ± 1.63 , 41.41 ± 2.17 , 32.76 ± 1.39 and 24.88 ± 2.88 pg/ml for control group, Power Horse, Red Bull and Sting, respectively.

Table (3) and Figure (3) revealed that there was a significant difference between the four groups regarding the dopamine level. Power Horse group showed significant increase in dopamine levels, followed by Red Bull group then Sting group when compared to the control group. The mean dopamine level was 0.38 ± 0.05 , 1.56 ± 0.07 , 1.29 ± 0.04 and 0.75 ± 0.087 pg/ml for control group, Power Horse, Red Bull and Sting, respectively.

Results of Histopathological study

On histopathological examination of hematoxylin and eosin (H&E) stained brain sections of the control group showed in figure (4): a, b, there were preservation of normal histological structure of pyramidal cell neurons in cerebral cortex and normal structure of pyramidal and neuroglial cells. The pink stained background: the neuropil, was a mat of neuronal and glial cell and normal histological structure of the hippocampus showing well-organized pyramidal cells. Histopathological examination of brain sections of energy drinks received groups revealed the presence of pathological changes with variable degrees of degenerative changes in neurons of the cerebral cortex and the hippocampus areas.

Histopathological examination of Power Horse received group showed in figure (5): a, b. The examination showed severe degenerative changes on cerebral cortex neurons with moderate degenerative changes and necrosis in hippocampus neurons in form of shrunken pyramidal cells with small darkly stained pyknotic nuclei surrounded by empty space and dilated blood vessels. Histopathological examination of Red Bull received group showed in figure (6): a, b. There were mild to moderate degenerative changes in neurons of cerebral cortex accompanied by severe degenerative changes and necrosis of neurons of hippocampus in different areas. Degenerative changes are in form of small darkly stained pyknotic nuclei and surrounded with empty space in the neuron cells. Histopathological examination of Sting received group showed in figure (7): a, b. There were moderate to severe degenerative changes on cerebral cortex neurons with mild degenerative changes in hippocampus neurons. Degenerative changes are in form of shrunken pyramidal cells with small darkly stained pyknotic nuclei.

Table (1) Comparison between the studied groups (using Tukey post hoc test) regarding the Gamma aminobutyric acid level:

GABA(pg/ml)	Control group No=20	Power Horse group No=20	Red Bull group No=20	Sting group No=20	P-value
Mean±SD	47.89±2.71	24.67±2.99	35.42±2.59	41.85±1.92	<0.001*
	P1<0.001*	P2<0.001*	P3=0.010*		
	P4<0.001*	P5=0.006*	P6<0.001*		

*P-value is significant, P1: Group control (I) vs Power Horse (II), P2: Group control (I) vs Red Bull (III), P3: Group control (I) vs Sting (IV), P4: Group Power Horse (II) vs Red Bull (III), P5: Group Red Bull (III) vs Sting (IV), P6: Group Power Horse (II) vs Sting (IV)

Table (2) Comparison between the studied groups (using Tukey post hoc test) regarding the norepinephrine level:

NE (pg/ml)	Control group No=20	Power Horse group No=20	Red Bull group No=20	Sting group No=20	P-value
Mean±SD	12.41±1.63	41.41±2.17	32.76±1.39	24.88±2.88	<0.001*
	P1<0.001*	P2<0.001*	P3<0.001*		
	P4<0.001*	P5<0.001*	P6<0.001*		

*P-value is significant, P1: Group control (I) vs Power Horse (II), P2: Group control (I) vs Red Bull (III), P3: Group control (I) vs Sting (IV), P4: Group Power Horse (II) vs Red Bull (III), P5: Group Red Bull (III) vs Sting (IV), P6: Group Power Horse (II) vs Sting (IV)

Table (3) Comparison between the studied groups (using Tukey post hoc test) regarding the dopamine level:

DA (ng/ml)	Control group No=20	Power Horse group No=20	Red Bull group No=20	Sting group No=20	P-value
Mean±SD	0.38±0.05	1.56±0.07	1.29±0.04	0.75±0.087	<0.001*
	P1<0.001*	P2<0.001*	P3<0.001*		
	P4<0.001*	P5<0.001*	P6<0.001*		

*P-value is significant, P1: Group control (I) vs Power Horse (II), P2: Group control (I) vs Red Bull (III), P3: Group control (I) vs Sting (IV), P4: Group Power Horse (II) vs Red Bull (III), P5: Group Red Bull (III) vs Sting (IV), P6: Group Power Horse (II) vs Sting (IV)

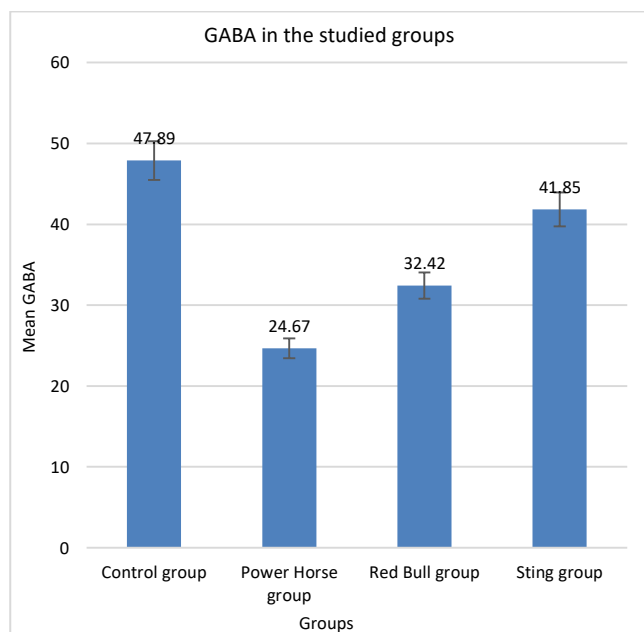


Figure (1) Comparison between the four groups regarding the Gama-aminobutyric acid (GABA) level (pg/ml)

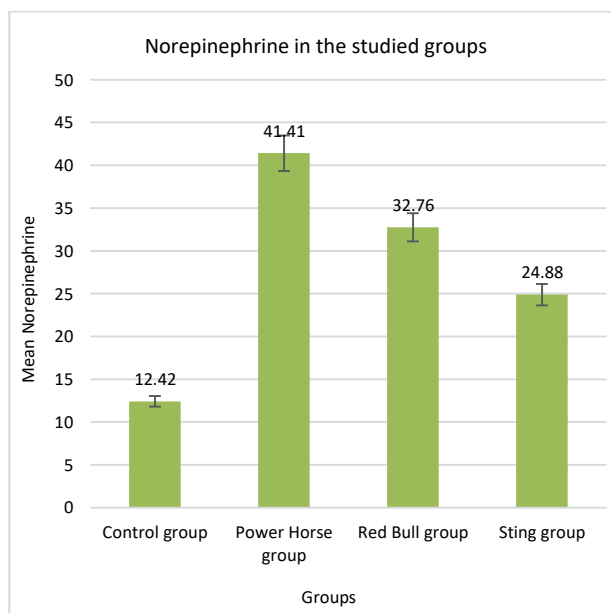


Figure (2) Comparison between the four groups regarding the norepinephrine (NE) level (pg/ml)

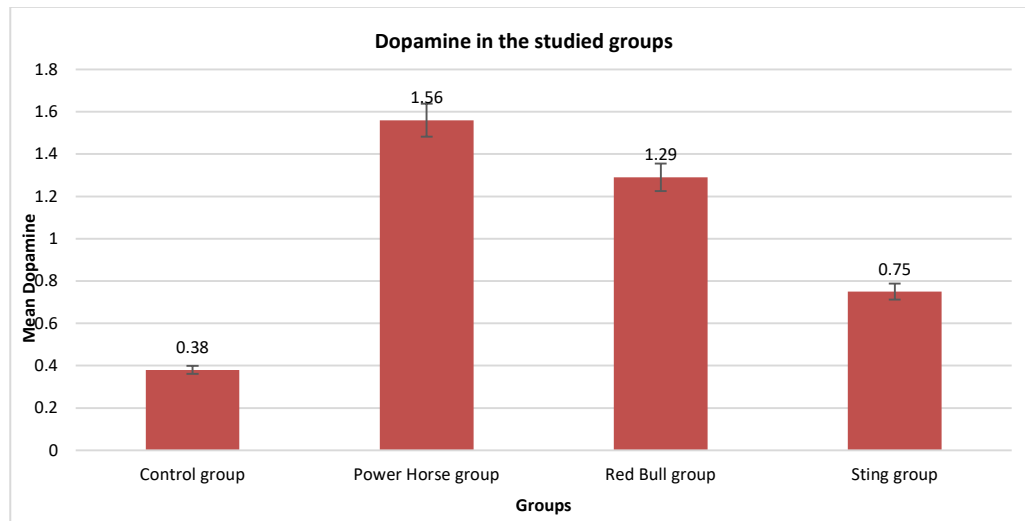


Figure (3) Comparison between the four groups regarding the dopamine (DA) level (ng/ml)

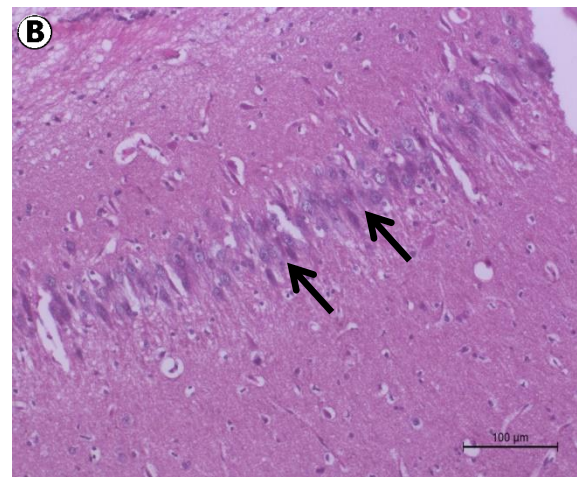
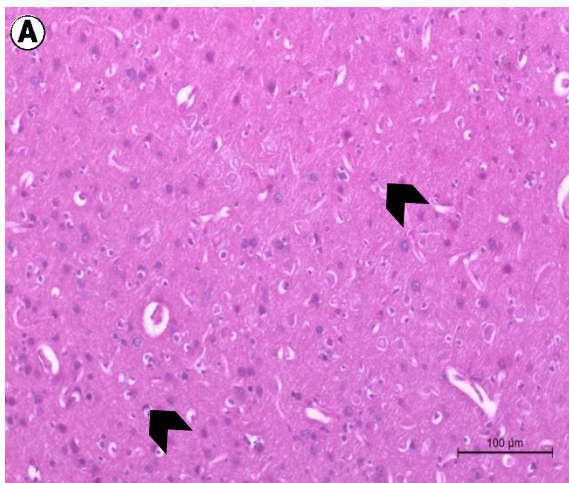


Figure (4, a): A photomicrograph of a section in the cerebral cortex of adult male albino rat of control group showing: normal histological structure of pyramidal cell neurons in cerebral cortex (arrow heads). Figure (4, b): A photomicrograph of a section in the hippocampus of adult male albino rat of control group showing: normal histological structure of pyramidal cell neurons in hippocampus (arrows).

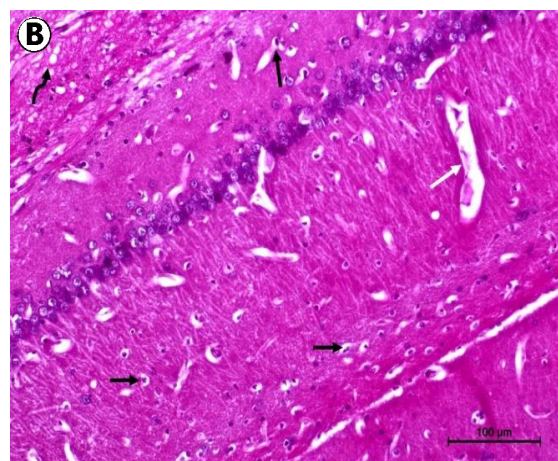
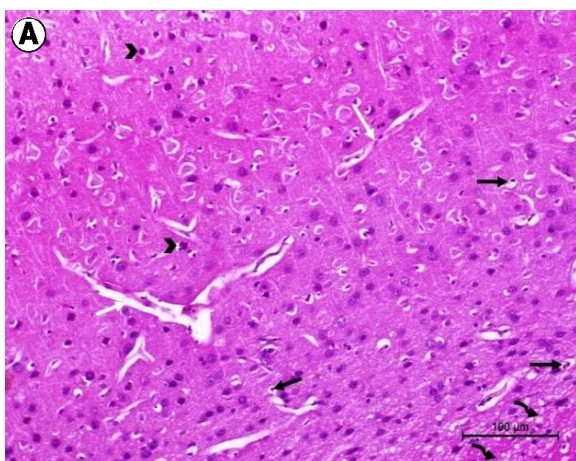


Figure (5, a): A photomicrograph of a section in the cerebral cortex of adult male albino rat of POWER HORSE group showing: many degenerated neurons (black arrow) having small darkly stained pyknotic nuclei and surrounded with empty space. Dilated blood vessels (white arrow) can be observed. Some pyramidal cells (arrowhead) appear shrunken and surrounded by empty space. Note the spongiform change of the cortex (curved arrow). (H&E X 200). Figure (5, b): A photomicrograph of a section in the hippocampus of adult male albino rat of POWER HORSE group showing: many degenerated neurons (black arrow) having small darkly stained pyknotic nuclei and surrounded with empty space. Dilated disorganized blood vessels (white arrow) can be seen. Note the spongiform change of hippocampus (curved arrow). (H&E X 200).

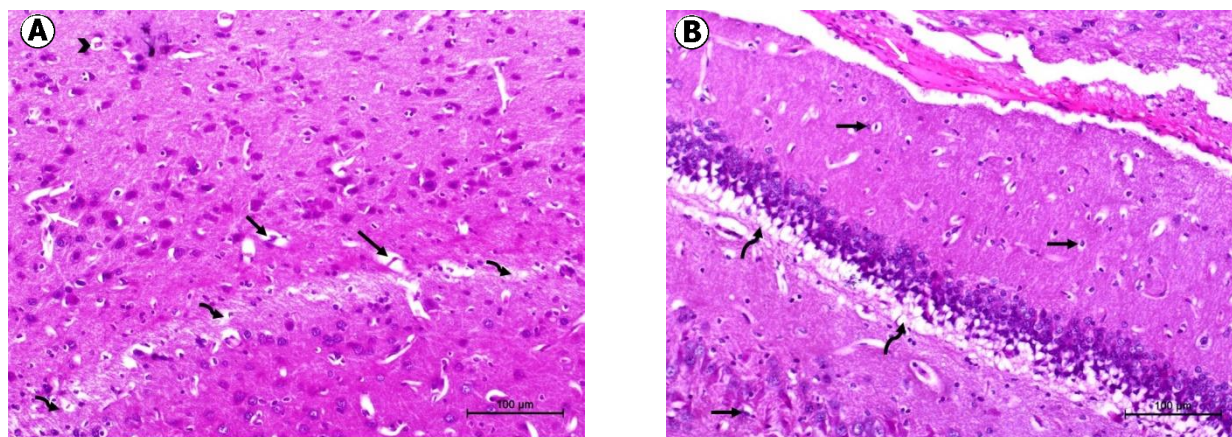


Figure (6, a): A photomicrograph of a section in the cerebral cortex of adult male albino rat of RED BULL group showing: Many degenerated neurons (black arrow) having small darkly stained pyknotic nuclei and surrounded with empty space. Dilated blood vessels (white arrow) can be observed. Note the spongiform change of the cortex (curved arrow). (H&E X 200). **Figure (6, b):** A photomicrograph of a section in the hippocampus of adult male albino rat of RED BULL group showing: Many degenerated neurons (black arrow) having small darkly stained pyknotic nuclei and surrounded with empty space. Dilated blood vessels (white arrow) can be observed. Note the spongiform change of the hippocampus (curved arrow). (H&E X 200).

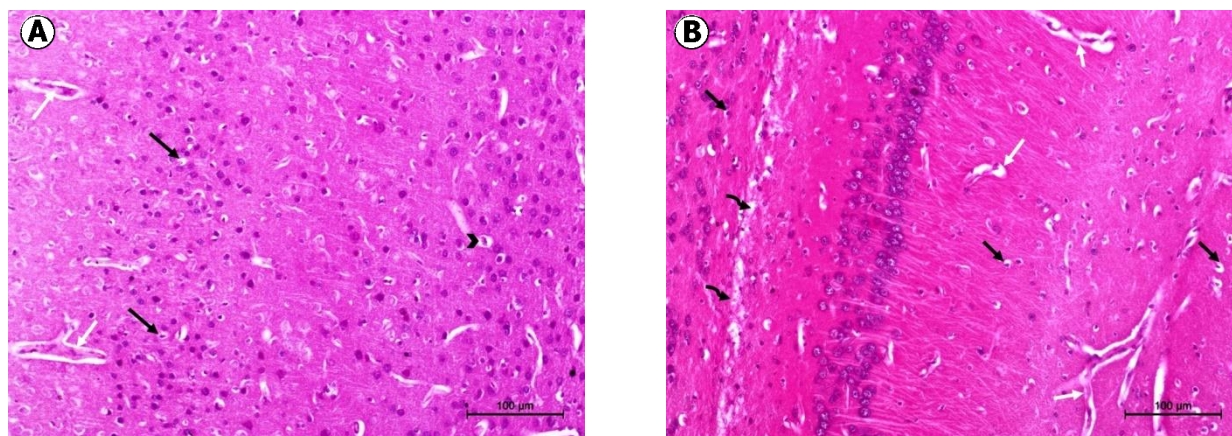


Figure (7, a): A photomicrograph of a section in the cerebral cortex of adult male albino rat of STING group showing: Many degenerated neurons (black arrow) having small darkly stained pyknotic nuclei and surrounded with empty space. Dilated blood vessels (white arrow) can be observed. Some pyramidal cells (arrowhead) appear shrunken and surrounded by empty space. (H&E X 200). **Figure (7, b):** A photomicrograph of a section in the hippocampus of adult male albino rat of STING group showing: Many degenerated neurons (black arrow) having small darkly stained pyknotic nuclei and surrounded with empty space. Dilated blood vessels (white arrow) can be observed. Note the spongiform change of the hippocampus (curved arrow). (H&E X 200).

Discussion

Energy drinks are nowadays become available in local markets everywhere and easily accessible for all people including children and young ones (Hasan et al., 2020). Also, concerns about energy drinks adverse effects are increased worldly as many adverse effects reported in different studies and poison centers of different countries (Hammond et al., 2018). Our study is helping to find out the toxicological effects of different energy drinks available at local market in a dose of (3.8ml/100g) for 3 weeks on biochemical and histopathological parameters of brain.

In this study, the results of biochemical parameters of the brain and degenerative changes of histopathology of the brain tissue showed the harmful and damaging effects of the 3 types of energy drinks

that used in this study. The results of neurotransmitters of brain tissue homogenates showed significant differences ($p < 0.001$) between all groups received energy drinks and control group. Our results showed significant decrease in GABA levels and significant increase in norepinephrine and dopamine in all energy drinks treated groups.

Current result of GABA neurotransmitter goes along with results of Almehmadi, (2017) study which showed significant decrease in GABA levels in different brain regions after three weeks of a similar dose to our used dose, and this is explained by caffeine suppression of GABAergic pathways (Alasmari, 2020) through postsynaptic and presynaptic mechanisms, post-synaptically by caffeine blockage of GABAergic

inhibitory postsynaptic currents and pre-synaptically through decrease of GABA release by increasing intracellular calcium through activation of cyclic nucleotide-gated calcium permeable channels which activated by cyclic adenosine monophosphate, cyclic guanosine monophosphate that increased by caffeine (Isokawa, 2016)

This study results coincided with results of Hahn et al., (2015) which observed decrease of GABA release by measuring GABA levels by magnetic resonance spectroscopy after intake of caffeine in adolescents which sounded to be the cause for anxiety or excitement in adolescents following caffeine intake.

However, Ferreira et al., (2014) indicated that caffeine indirectly potentiate GABA release through blocking adenosine A1 receptors and increase cyclic adenosine monophosphate and protein kinase A which with Src kinase family work on phosphorylation of tyrosine residue of N-methyl D-aspartate receptor subtype 2B that when aspartate activate those receptors in presence of caffeine increase GABA release.

By this study results show significant increase in dopamine neurotransmitter in all groups received energy drinks, this result meets the findings of another study that measured dopamine level in The nucleus accumbens by micro dialysis after 4 weeks of voluntary consumption of RED BULL energy drink within a dose of 320ml/week and the study indicated that dopamine increase after consumption of energy drinks in non-adaptive mechanism in a similar way to its rise following administration of drugs of abuse (Vargiu et al., 2021). Previous studies demonstrated that caffeine induce dopamine release in a way similar to dopamine release in response to nicotine, tetrahydrocannabinol, or ethanol which in turn explain development of drug dependence (Solinas et al., 2002).

Vargiu et al. (2021), explained the rise of dopamine caused by the effect of 3 main ingredients of energy drinks that are found in high concentration (caffeine, taurine, sugars) as caffeine consumption increase dopamine levels in blood plasma of men on treadmill running exercise.

According to Lee et al., (2019), and Galvalisi et al., (2017) who realized significant rise in extracellular dopamine in the nucleus accumbens following pulmonary inhalation of caffeine and they explained this effect by antagonistic effect of caffeine on adenosine receptors of A1, A2a, which on adenosine binding to A1, inhibit release of excitatory neurotransmitters especially dopamine, therefore caffeine block that action increase dopamine release (Ferré, 2016).

Other studies of Bassareo et al., (2015), Rada et al., (2005) showed that increase of dopamine release in the nucleus accumbens with sucrose administration, supports role of dopamine in reward brain circuits following food administration for the first time.

In study of Rada et al., (2005), rats received intermittent sucrose every 12 hour for 21 days, have an increase in dopamine levels in the nucleus accumbens. Other study clarified that deprivation of food for 24hour can restore dopamine response to the same

level released in the first time for the same food on its administration again so intake of sucrose every 12 hour, help to refresh dopamine release at each time of sucrose intake (Bassareo and Di Chiara, 1999).

The study of Ericson et al., (2006) found out taurine perfusion in the nucleus accumbens is accompanied by increase in extracellular dopamine levels when measured by in vivo micro dialysis and explained such rise of dopamine by action of taurine on strychnine sensitive glycine receptors. When both taurine and strychnine given together, there was no increase in dopamine level due to antagonistic action of strychnine on those receptors. Ericson et al., (2006) assumed that taurine could be an endogenous ligand for strychnine sensitive glycine receptors and increase dopamine level in the nucleus accumbens through that mechanism.

So, all the previous studies revealed the role of increased dopamine in the development of substance abuse explain the potential abuse of energy drinks that as well increase dopamine levels.

In this study, levels of norepinephrine in the brain tissue homogenates of all groups treated with different types of energy drinks are significantly high when compared with control group, this could be explained by caffeine release of norepinephrine, dopamine, and serotonin in the brain and increases catecholamines in circulation correspondent with reversal of the inhibitory effects of adenosine on these systems.

These results are in agreement with results of Tkachenko et al., (2018) who revealed high blood levels of norepinephrine after 2 weeks of energy drinks intake by animals resulting in increased sympathetic activation. High Levels of plasma norepinephrine following consumption of energy drinks in young adults due to overproduction of catecholamines by adrenal glands (Svatikova et al., 2015).

The previous results concerning norepinephrine could be explained by stimulant effects of energy drinks because of their content of high concentration of caffeine but not as a result for sucrose component of energy drinks. Hajnal and Norgren (2004) measured levels of dopamine and norepinephrine in the nucleus accumbens following sucrose ingestion and found out that dopamine increase but norepinephrine decrease by 20%.

The high norepinephrine levels not caused by taurine component, as it inhibits norepinephrine and acetylcholine release in cerebral cortical slices in vitro by its suppressor effect at synapses. So, the most probable cause for increased norepinephrine levels is high concentration of caffeine in energy drinks as caffeine is an antagonistic of adenosine receptors, increase cyclic adenosine monophosphate and increase release of catecholamines including norepinephrine (Benowitz, 1990).

Current study regarding dopamine and norepinephrine results is unlike result of (Bawazir, 2017) study which indicated marked decrease in dopamine and norepinephrine levels in most brain regions after ingestion of a similar dose to our study

and explained that through continuous release of neurotransmitters from nerve cells lead to decrease their content in cells. This discrepancy might be related to chronic consumption leading to depletion of neurotransmitters.

Our histopathological findings in brain sections of the groups received energy drinks of POWER HORSE, RED BULL and STING showed variable degrees of degenerative changes and necrosis in the cerebral cortex in the form of shrunken pyramidal cells surrounded by empty space and small darkly stained pyknotic nuclei that surrounded with empty space and those degenerative changes were severe in POWER HORSE group, STING group and moderate in RED BULL group. There were similar degenerative changes in the pyramidal cells of hippocampus which were severe in RED BULL group, moderate in POWER HORSE group and mild in STING group.

Current histopathological changes match the findings of (Sayed, 2021) study in the form of extensive structural changes including severe neuropil degeneration in the cerebral cortex with darkly stained nuclei and pale cytoplasm in the groups treated with RED BULL in a dose of (7.5ml/day) for 4 weeks, and they demonstrated these histopathological changes resulted from oxidative stress which detected by the increased levels of thiobarbituric acid reactive substance that produced by lipid peroxidation of cortical tissue and down regulation of mRNA Expression Levels of Nrf2 proteins which have protective role against oxidative damage and their down regulation occur in case of exposure for chemical risk.

Our histopathological findings are consistent with findings of (Abdelwahab et al., 2020) study, as well, which showed alterations in brain tissue including cellular apoptosis, dark small nuclei, and congested blood vessels. These changes, considered a sign of toxicity, could be attributed to cellular damage by lipid peroxidation and DNA damage caused by preservatives and caffeine components of energy drinks.

Also, current findings are matching to the brain histopathological changes of (Salih et al., 2018) study which observed degeneration of cerebral cortex neurons in form of shrunken, pyknotic and darkly stained with small nuclei neurons, aggregation of focal eosinophilic plaques, and focal gliosis and reduction of neurons in hippocampus. These changes were evident in groups treated with RED BULL; those pathological alterations result from caffeine of energy drinks. Additionally (Bawazir, 2017) detected degeneration and necrosis of nerve cells, apoptosis of pyramidal cells and neural atrophy and these changes could be from caffeine of energy drinks.

On the contrary, both (Akande and Banjoko, 2011), (Reis et al., 2017) revealed no abnormality or irregularity on the histopathological analysis of brain tissues and they both attributed that to the short period of the studies which was for 14 days.

Conclusion

Energy drinks consumption in high dose had marked hyper stimulating toxic effects on brain

stimulatory neurotransmitters and degeneration of neurons in different regions of the brain.

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التغيرات التي تحدث في الأنسجة والناقلات العصبية في المخ للجرذان البيضاء البالغة نتيجة لاستخدام مشروبات الطاقة الموجودة في مصر

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الملخص العربي

المقدمة: مشروبات الطاقة هي مشروبات كربونية غير كحولية حيث يتم تناولهم اثناء ممارسة الانشطة المختلفة مثل الرياضة والتدريبات لزيادة القوة البدنية والتركيز العقلي. مؤخرا، يتم استخدامهم بواسطة الاعمار المختلفة من الرجال والنساء وتم تضاعف اعداد الحالات الواردة لقسم الطوارئ في الفترة الزمنية من ٢٠٠٧ الى ٢٠١١. يعد الباور هورس، الريد بول، من مشروبات الطاقة ذات الاسماء الرنانة التي توحى بالسرعة والقوة الطاقة وهي لها كثير من المكونات الغير مصرح بها من هيئة الغذاء والدواء العالمية. **طريقة البحث:** تم في هذه الدراسة توزيع ثمانون من الجرذان البيضاء البالغة بصورة عشوائية علي اربع مجموعات ، حيث تتكون كل مجموعة من عشرين جرذ. تم اعطاءهم مياه ، مشروب الباور هورس، مشروب الريد بول، مشروب الستينج علي التوالي بجرعة عالية قدرها (٣.٨ مللي لكل ١٠٠ جرام من وزن الجرذ) عن طريق الفم لمدة ثلاثة اسابيع. **النتائج:** اظهرت نتائجنا فيما يخص التأثيرات المصاحبة لتناول مشروبات الطاقة ارتفاع مستوي الناقلات العصبية المنشطة كالدوبامين والنورادرينالين وانخفاض مستوي الجابا الناقل العصبي المثبط، وهذه النتائج متوافقة علي ما تم وجوده في فحص انسجة المخ من التغيرات التنكسية الشديدة والتخرر بالخلايا العصبية في مناطق المخ المختلفة كمنطقة القشرة المخية مما يدل علي التأثير السمي لمشروبات الطاقة علي خلايا المخ. **الخلاصة:** تناول مشروبات الطاقة بجرعات عالية له تاثير سمي علي الناقلات المنشطة العصبية بالمخ وتاثير تنكسي علي الخلايا العصبية بالمناطق المختلفة بالمخ.

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