Journal of Recent Advances in Medicine

Original Article

Study of the biochemical, histological and cytogenetic effects of two different energy drinks (EDs); red bull and power horse; on brain of adult male albino rats and to determine the possible protective role of Omega-3 on the adverse effects of EDs

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ABSTRACT

Background: Energy drinks (EDs) became a worldwide phenomenon and popular especially among young adult and the brain is one of the most vulnerable organs to toxicity.

Objective: This work aimed to study the sub chronic effect of consumption of two different popular energy drinks (power horse and red bull) on the brain of adult male albino rats through biochemical, histological and cytogenetic parameters, and to determine the possible protective role of Omega-3 on the adverse effects of EDs. Also, to determine the effects of EDs withdrawal (recovery period).

Methodology: Forty-eight adult male albino rats were divided into 8 groups; control, omega 3, RB, PH, each of EDs combined with omega 3 and after 2 weeks of withdrawal. Blood samples, brains and femora from the rats were obtained for biochemical, histological and cytogenetic evaluation.

Results: The studied EDs resulted in elevation of the oxidative stress marker malondialdehyde, structural and numerical chromosomal aberrations. While they decreased the antioxidant markers (catalase and Superoxide dismutase), acetylcholinesterase activity, total content of DNA and RNA in brain tissue and the mitotic index with disturbance in the normal histology of the brain. Either treatment with omega 3 or EDs withdrawal improve these changes with better effect to omega 3.

Conclusion: EDs are toxic to the brain. Omega 3 or EDs withdrawal can ameliorate this toxicity with better effect to omega 3. Therefore, strict control of EDs consumption is urgently needed.

JRAM 2020; 1(2):55-66

Keywords: Energy, drinks, power horse, red bull, brain toxicity.

Submission Date: 9 November 2019 **Acceptance Date** 25 December 2019

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Please cite this article as: El-Sayed SBM, Afify AMH, Abd El-Wahab WA, Mohammed MA, Abd Al-Rahman RA. Study of the Biochemical, Histological and Cytogenetic Effects of two Different Energy Drinks (EDs); Red Bull & Power Horse; on Brain of Adult Male Albino Rats and to determine the Possible Protective Role of Omega-3 on The Adverse Effects of EDs. JRAM 2020; 1(2):55-66. DOI: 10.21608/jram.2019.19793.1019

INTRODUCTION

Energy drinks (EDs) are beverages mainly contain caffeine, with or without other supplements[1]. Energy drinks often contain additives such as stimulants of plant origin (guarana and yerba mate), amino acids (taurine, creatine or carnitine), sugars or their derivatives (glucuronolactone, sucrose, glucose, ribose and fructose),

herbal extracts (ginseng ,ginkgo biloba), vitamin B complex and others [2].

These drinks are used to increase energy in their consumers, promote wakefulness and improve cognitive performance and enhance the mood [3]. Manufacturers have recently shifted their consumer target from athletes to adolescents whom are attracted to EDs, affected by the marketing without knowledge of their health hazards **[4].** Energy drinks are marketed in areas popular with teens. Approximately, two thirds of its consumers are the adolescents and young adults between 13-35 years old [1]**.** In 2006, more than 500 different brands of EDs were released all over the world [5]. Their production is increasing, and the sales reached up to 12.5 billion dollars in 2012. Owing to increasing consumption and an increase related health impacts associated with their consumption, the general population and the scientific community concerns have been increased [6]**.**

Gunja and Brown [7] reported that side effects from EDs are expected to be due to the vast array of components forming them rather than caffeine alone. **Cornelis et al.** [8] stated that chugging a cold beverage results in higher peak plasma of caffeine concentration than a dose of hot drinks that sipped slowly and have more side effects even if the dose is smaller. While **Bigard** [9] claimed that their negative effects are due to the toxicity of certain ingredients, mixing with drugs, nicotine or alcohol or accompanying physical exertion. This risk is worse in cardiac patients, pregnant women, teenagers, children and caffeine-sensitive individuals.

Adverse effects of EDs consumption include neurological symptoms as tremors and restlessness, cardiac as tachycardia or heart palpitations and/or gastrointestinal symptoms, which may be serious in some cases [10]**. Azagba et al.** [11] found associations between energy drink consumption and smoking, violence and drug abuse especially in young adults and adolescents**.**

Omega-3 fatty acids (ω3 FAs) are important to human health. Acting as strong antioxidants and play protective role in the cardiovascular, liver and kidney diseases [12]. Moreover, affecting brain function, normal growth and development [13]**.** As energy drinks became a worldwide phenomenon and popular especially among young adult and the brain is one of the most vulnerable organs to toxicity [14]; therefore, this work aimed to study the sub chronic effect of consumption of two different popular energy drinks (power horse and red bull) on the brain of adult male albino rats through biochemical, histological and cytogenetic parameters and to evaluate the possible protective role of Omega-3 on these adverse effects. Also, to determine the effects of EDs withdrawal (recovery period).

MATERIALS AND METHODS

Place of the study:

I- Faculty of Medicine for Girls Al-Azhar University: Handling of animals and histopathological studies.

II- National Research Center in Dokki (Cairo): Biochemical and cytogenetic studies.

Materials:

I-Beverages

*The **Power horse (PH)** and **Red bull (RB) EDs** were bought from a local market.

II-Chemicals

***Omega-3 (ω3)** used in this study was in the form of fish oil-containing gelatinous capsules, called Omega-300 capsules purchased from El- Ezaby pharmacy and was manufactured by The Arab Co. for Gelatin and Pharmaceutical products for Montana Pharmaceutical.

*Each capsule contains 1000 mg fish oil (fish oil contains ~ 30% omega 3 [13], and was evacuated by syringe carefully and given to rats by oral gavage.

III-Reagents for the measurement of the different parameters. Reagents for malondialdehyde (MDA): MDA standard, buffer chromogen (thiobarbituric acid, detergent and stabilizer).

Reagents for catalase (CAT): chromogenic buffer, H2O2 (substrate and standard), CAT inhibition, peroxidase enzyme. Reagents for superoxide dismutase (SOD): phosphate buffer, nitrobluetetrazolium , nicotinamide adenine dinucleotide hydrogen (NADH) ,phenadinemethosulphate. Reagents for acetyl cholinesterase: acetylthiocholine iodide, phosphate solution. All used reagents were purchased from Sigma-Aldrich Chemie GmbH, Germany.

Experimental animals:

Forty-eight adult male albino rats, weighting 150 ± 10 gm were obtained from Helwan animal breeding farm, Cairo, Egypt, they were conditioned in standard metallic cages (6 rats per cage) and kept under the same environmental conditions regarding light, feeding and temperature with an alternating 12 h light – dark cycle. They were acclimatized to the laboratory conditions, fed standard rat chow and water was available ad libitum. The handling of animals followed the rules for the experimental research ethics approved by Research Ethics Committee at faculty of Medicine for Girls Al-Azhar University. The animals were divided into 8 groups of 6 rats each and treated as follows:

- **Group 1 (control group):** Normal healthy animals were received distillate water orally ad libitum for 4 weeks.
- **Group 2 (omega 3 group):** Rats were given omega-3 by gavage, at a dose of 300 mg/kg once daily for 4 weeks [13]**.**
- **Group 3 (PH group):** Rats were given power horse energy drink with oral dose (1.5ml/100 gm / day) once daily for 4 weeks [15].

- **Group 4 (PH+ recovery period group):** Rats were given power horse energy drink with oral dose (1.5ml/100gm/day) once daily for 4 weeks **[15]**, these rats will be kept on normal diet only for another 2 weeks (for recovery).
- **Group 5 (PH+ omega-3 group):** Rats were administered power horse energy drink+ omega-3 with oral dose (1.5ml/100gm/day, 300 mg/kg/day) respectively once daily for 4 weeks [15; 16].
- **Group 6 (RB group):** Rats were given red bull energy drink with oral dose (1.5ml/100gm/day) once daily for 4 weeks [15].
- **Group 7 (RB+ recovery period group):** Rats were given red bull energy drink with oral dose (1.5ml/100gm/day) once daily for 4 weeks [15], then rats will be kept on normal diet only for another 2 weeks (for recovery).
- **Group 8 (RB+ omega-3 group):** Rats were administered red bull energy drink+ omega-3 with oral dose (1.5ml/100gm/day, 300 mg/kg/day) respectively once daily for 4 weeks [15;16].

Specimens' collection

i. Twenty-four hours after the last dose of treatment, all animals were lightly anaesthetized with diethyl ether inhalation. Blood samples were obtained from the retro orbital sinus puncture into heparinized capillary tubes from each rat before killing. Blood samples were collected in clean dry test tubes and centrifuged at 2000 rpm for 15 minutes. Sera were then separated and kept frozen at -20 °C for estimation of antioxidant markers.

ii. After collecting blood samples, animals were sacrificed by cervical dislocation while they were under anesthesia. Brain and both femora were dissected immediately for histopathological studies and cytogenetic analysis.

Biochemical studies:

a) Antioxidant markers (catalase and Superoxide dismutase):

- Catalase activity was measured in serum by monitoring the decomposition of H2O2 at 240 nm according to **Aebi** [17].
- Superoxide dismutase (SOD) activity in serum was assayed as described by **Nishikimi et al.** [18].

b) Oxidative stress marker:

Serum malondialdehyde (MDA) [the product of lipid peroxidation] was estimated according to **Ohkawa et al.** [19].

c) Acetylcholinesterase activity in brain tissue was determined according **Ellman et al.** [20].

Histopathological studies:

Brain specimens were fixed using Bouin solution. After proper fixation, the specimens were dehydrated in ascending grades of ethyl alcohol (70%, 90%, 100%), cleared in xylol, impregnated and then embedded in paraffin wax. 5-µm thick sections were cut using rotatory microtome. Brain sections were stained with routine haematoxylin and eosin **(H&E)** stain as a routine stain for studying the general histological structure of the brain [21].

Cytogenetic evaluation:

- **Estimation of total content of DNA and RNA in brain tissue:** according to **Dische and Schwartz** [22] and **Dische** [23] respectively.
- **b) Chromosomal aberrations study:** from bone marrow cells was prepared according to **Yoshida and Amano** [24]. It consists of:

I-Structural aberrations

II- Numerical aberrations and mitotic index

Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version (20). All data were presented as mean standard deviation (SD). For statistical analysis of the effects of different treatments, independent t- test was done P-value ≤0.05 was considered significant.

RESULTS

1-Biochemical results:

a) Antioxidant markers (catalase and SOD):

There was no statistically significant difference between the **control group and omega 3 (ω3) group** as regards the studied antioxidant markers. But there was statistically high significant decrease in catalase activity and increase in SOD activity in all other treated groups as compared to control group. Sub chronic toxicity with **(PH)** energy drink produced more reduction in catalase activity and more increase in SOD activity than **(RB)** energy drink. Statistically high significant increase in catalase and decrease in **SOD** activities were observed in **recovery and ω3 groups** as compared to their toxic groups. Treatment by **ω3** produced more improvement in catalase and SOD activities than the recovery period (Table 1).

b) The serum malondialdehyde (MDA):

There was no statistically significant difference (p>0.05) between the **control group and ω3 group** as regards serum **MDA** level. However, there was statistically significant increase in **MDA** level in the serum of other all treated groups as compared to control group except **PH+ ω3 group**. There was no significant difference between **(PH)** and **(RB)** groups as regards MDA level in serum. Statistically highly significant (P ≤ 0.000) decrease in MDA level was observed in **recovery group**s and **ω3 group**s as compared to their toxic groups (**PH or RB groups**). Treatment with **ω3** showed more reduction in **serum MDA** level than the recovery period (Table 1).

c) Acetylcholinesterase activity:

There was no statistically significant difference between the **control group and ω3 group** as regards

cholinesterase (p=0.3). But there were statistically high significant (p≤ **0.000**) decrease in acetylcholinesterase activity in all other treated groups as compared to control group. Sub chronic toxicity with **(PH)** energy drink showed more reduction in acetylcholinesterase activity than **(RB)** energy drink. Statistically high significant (p ≤ **0.000**) increase in cholinesterase level was observed in **recovery and ω3 group**s as compared to **their toxic** groups. The treatment by **ω3** resulted in more improvement in cholinesterase level than the recovery period (Table 1)**.**

2-Histopathological results of brain tissue stained with H&E:

The control and (ω3) groups showed normal histological structure of cerebral cortex with pia matter covers the molecular layer followed by external granular layer and external pyramidal layer (fig.1a). Also, the pyramidal cells and granular cells with rounded open face nuclei are also seen. The pink stained background is the neuropil (fig.1b, 1c). In (PH) and (RB) groups showed many apoptotic cells having small darkly stained nuclei and surrounded by empty space. Also, dilated congested blood vessel can be observed (fig.2a, ,3a). Recovery groups showed no improvement (fig.2b,3b). While in (ω3) groups moderate improvement was observed. The pyramidal cells have normal histological structure. Some pyramidal cells appear shrunken and surrounded with empty space (fig.2c, 3c).

3-Cytogenetic results:

a) Estimation of total content of DNA and RNA in brain tissue

No statistically significant difference between the control and ω3 groups was found as regards total content of DNA and RNA in brain tissue. Also, differences were noticed between the control group and RB+ ω3 group as regards total content of DNA in brain tissue. However, there was statistically high significant decrease in total content of DNA and RNA in brain tissue in all other treated groups as compared to control group, more reduction was observed in (PH) group. The recovery and ω3 groups showed improvement of the total content of DNA and RNA in brain tissue with better effect in the ω3 group (Table 2).

b) Chromosomal aberrations study

I-Structural aberrations:

There was no statistically significant difference between the control and ω3 groups as regards (gap, break, C.A. and E.mitosis) forms of structural chromosomal aberrations. While, (del, frag and E to E) forms did not appear in the control and ω3 groups. But, there was significant increase in all forms of structural chromosomal aberrations in PH and RB groups as compared to control group. Both ω3 and the recovery period showed improvement in many forms of structural chromosomal aberrations with better effect to ω3 (Table3).

II- Numerical aberrations and mitotic index

All forms of numerical chromosomal aberrations (negative, positive and poly) did not appear in the control and ω3 groups. However, there were statistically high significant increase in all forms of numerical chromosomal aberrations (negative, positive and poly) in PH and RB groups as compared to control group with no significant difference between them. Both PH+ recovery period and PH+ ω3 groups showed improvement in forms of numerical chromosomal aberrations with no significant difference between them. The same findings were observed in RB+ ω3 group as compared to RB+ recovery period group (Table 4).

Regarding to mitotic index, no significant difference between the control group and ω3 group was found as regards mitotic index. Although, there were high significant decrease in mitotic index in all groups (except RB+ ω3 group showed non-significant decrease in mitotic index) as compared to control group with no significant difference between PH group and RB group. While recovery and ω3 groups showed high significant increase in mitotic index as compared to their toxic groups. The treatment by ω3 showed more increase in mitotic index than the treatment by recovery period (Table 4).

Table (1): Comparison between the eight treated groups by PH,RB and Omega3 as regards Catalase, SOD and MDA levels in the serum and acetylcholine esterase activity in the brain of adult male albino rats using independent T- test

Groups n=6 rats/ group	Catalase activity (U/L) Mean ±SD	SOD activity (U/L) Mean ±SD	MDA (nmol/ml) Mean ±SD	Acetylcholinesterase µmol/min/gm Mean ±SD
Control group	823.9±13.9	23.5±1.16 -	9.3±0.76	8008.9±123
ω3 group	830.1±14.5 [a]	23.3±1.15 [a]	8.9±0.68 [a]	8083±136.9 [a]
PH group	346.2±27.8 a**	64.4±1.11 a**	23.2±1.5 a**	3332.8±210.7 a**
PH+ recovery period group	699.4±14.6 a** b**	32.1±1.5 a** b**	13.3±0.92 a** b**	6545±148.2 a** b**
PH+ ω3 group	747±20.3 a** b** d**	26.8±0.87 a** b** d**	9.9±0.67 a** b** d**	7328.6±198 a** b** d**
RB group	410.9±16.6 a** b**	61.9±1.6 a** b**	21.8±1.5 a** b**	3897±167.6 a** b**
RB+ recovery period group	718.9±19.8 a** c**	30.4±1.13 a** c**	12.3±0.69 a** c**	6973±121.5 a** c**
RB+ ω3 group	784.8±20.2 a** c** e**	24.8±1.18 a** c** e**	10.2±0.79 a** c** e**	7633.8±139.2 a** c** e**

SD=standard deviation, **PH**=power horse, **RB**=red bull, **ω3**=omega 3, a= compared with control group, b= compared with PH group, c= compared with RB group, d= compared with PH+ recovery period group, e = compared with RB+ recovery period group.
$P \leq 0.05$ was considered to be significant= **

Table (2): Comparison between the eight treated groups by PH,RB and Omega3 as regards total content of DNA and RNA in the brain tissues of adult male albino rats using independent T- test

Groups n=6 rats/ group	DNA (mg/gm tissue) Mean ±SD	RNA (mg/gm tissue) Mean ±SD
Control group	0.61±0.02	0.3±0.02
ω3 group	0.62±0.02 [a]	0.33±0.01 [a]
PH group	0.23±0.01 [a**]	0.17±0.01 [a**]
PH+ recovery period group	0.4±0.03 a** b**	0.24±0.02 a** b**
PH+ ω3 group	0.6±0.01 a** b** d**	0.27±0.01 a** b** d**
RB group	0.27±0.02 a** b**	0.19±0.01 a** b**
RB+ recovery period group	0.4±0.02 a** c**	0.26±0.01 a** c**
RB+ ω3 group	0.6±0.01 a** c** e**	0.29±0.01 a** c** e**

SD=standard deviation, **PH**=power horse, **RB**=red bull, **ω3**=omega 3, a= compared with control group, b= compared with PH group, c= compared with RB group, d= compared with PH+ recovery period group, e = compared with RB+ recovery period group.
$P \leq 0.05$ was considered to be significant =**.

Table (3): Comparison between the eight treated groups by PH,RB and Omega3 as regards frequencies of structural chromosomal aberrations in bone marrow cells of adult male albino rats using independent T- test

Groups No=6 rats/ group	gap Mean ±SD	Break Mean ±SD	C.A Mean± SD	E. mitosis Mean ±SD	del Mean ±SD	frag Mean ±SD	E to E Mean ±SD
Control group	0.57±0.78	0.43±0.53	1±1.1	0.57±0.78	0.00±0.00	0.00±0.00	0.00±0.00
ω3 group	0.43±0.53 [a]	0.14±0.37 [a]	0.86±1.2 [a]	0.14±0.37 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
PH group	4.5±1.2 **a	3.2±1.6 **a	5.8±1.5 **a	4.7±1.3 **a	1.7±0.75 **a	1±0.8 **a	0.8±0.9 *a
PH+ recovery period group	2.7±0.75 **a**b	1.8±0.37* *a**b	3.3±0.75 **a**b	1.8±0.9 **a**b	0.57±0.78 a**b	0.4±0.7 a b	0.2±0.5 a b
PH+ ω3 group	1.4±0.53 a** b** d**	0.86±0.69 a**b**d	2.3±0.48 a** b** d**	1.3±0.95 a**b d	0.00±0.00 a**b d	0.00±0.00 a**b d	0.00±0.00 a**b d
RB group	4±0.81 **a b	3.4±1.5 **a b	5.5±1.7 **a b	4.1±1.2 **a b	1.4±0.7 **a b	0.8±0.9 *a b	0.6±0.5 **a b
RB+ recovery period group	2.1±0.69 **a**c	1.3±0.75 *a**c	2.7±0.75 **a**c	2±0.57 **a**c	0.29±0.48 a**c	0.00±0.00 a*c	0.00±0.00 a*c
RB+ ω3 group	0.7±0.48 a**c**e	0.57±0.53 a**c e	2±1 a**c e	1.5±0.53 **a**c e	0.00±0.00 a**c e	0.00±0.00 a**c e	0.00±0.00 a**ce

ω3=omega 3, **PH**=power horse, **RB**=red bull, **C.A**.= centromeric attenuation, **E. mitosis**= end mitosis, **del** = deletion, **frag**=fragments, **E to E** =end to end chromosome fusion **SD**=standard deviation a= compared with control group, b= compared with PH group, c= compared with RB group, d= compared with PH+ recovery period group, e = compared with RB+ recovery period group. $P \leq 0.05$ was considered to be significant =**

Table (4): Comparison between the eight treated groups by PH,RB and Omega3 as regards frequency of numerical chromosomal aberrations and mitotic index in bone marrow cells of adult male albino rats using independent T- test

Groups No=6 rats/ group	Numerical aberrations			Mitotic index
	Negative (2n-1)	Positive (2n+1)	Poly	
	Mean ±SD	Mean ±SD	Mean± SD	Mean ±SD
Control group	0.00±0.00	0.00±0.00	0.00±0.00	400±16.6
ω3 group	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	408.5±15.7 [a]
PH group	2.1±1.2 **a	1.8±1.1 **a	0.8±0.7 **a	231.7±15.4 **a
PH+ recovery period group	1±0.8 **a b	0.7±0.8 *a*b	0.3±0.5 ab	311±15 **a**b
PH+ ω3 group	0.7±0.7 *a*b d	0.4±0.5 **a**b d	0.00±0.00 a*b d	355.8±20.1 **a**b **d
RB group	1.5±1.3 **a b	1.3±1.7 **a b	0.7±0.8 *a b	246.1±13.7 **a b
RB+ recovery period group	0.4±0.5 *a* c	0.4±0.5 *a* c	0.00±0.00 a* c	323.7±15.3 **a** c
RB+ ω3 group	0.6±0.5 **a c e	0.4±0.5 *a *c e	0.00±0.00 a *c e	393.4±14.1 a **c **e

ω3=omega 3, **PH**=power horse, **RB**=red bull, **SD**=standard deviation; a= compared with control group, b= compared with PH group, c= compared with RB group, d= compared with PH+ recovery period group, e = compared with RB+ recovery period group. $P \leq 0.05$ was considered to be significant =**

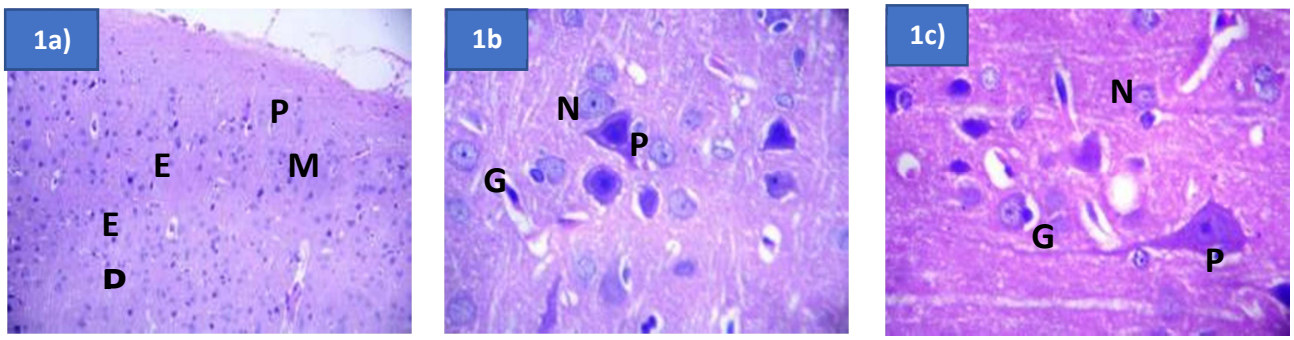

Fig. (1a&b): A photomicrograph of section in the cerebral cortex of adult male albino rat of control group (a) (H&E X 200), showing the pia matter (P) covers the molecular layer (M) followed by external granular layer (EG) and external pyramidal (EP). **(b): (H&E X 400)**, showing the pyramidal cells (P). Granular cells (G) with rounded open face nuclei are also seen. The pink stained background is the neuropil (N). **(c): A photomicrograph of section in the cerebral cortex of adult male albino rat of ω3 group (H&E X 400),** showing the pyramidal cells (P). Granular cells (G) with rounded open face nuclei are also seen. The pink stained background is the neuropil (N).

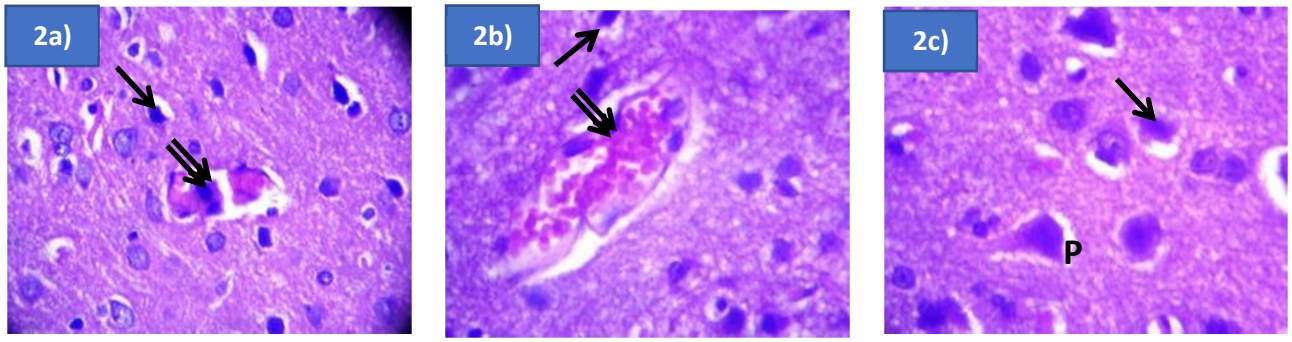

Fig. (2): (a) A photomicrograph of section in the cerebral cortex of adult male albino rat of PH group (H&E X 400), showing many apoptotic cells (arrow) having small darkly stained nuclei and surrounded with empty space. Dilated congested blood vessel (double arrows) can be observed. **(b): A photomicrograph of section in the cerebral cortex of adult male albino rat of PH+ recovery period group (H&E X 400),** showing no improvement with shrunken pyramidal cells (arrow). Dilated congested blood vessel (double arrows) can be observed. **(c): A photomicrograph of section in the cerebral cortex of adult male albino rat of PH+ ω3 group (H&E X 400),** showing moderate improvement. The pyramidal cells (P) have normal histological structure. Some pyramidal cells (arrow) appear shrunken and surrounded with empty space.

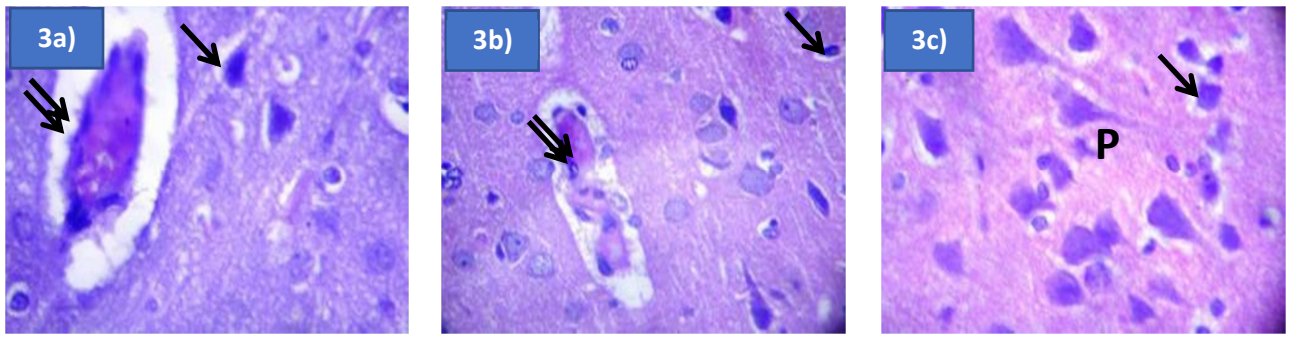

Fig. (3): (a)A photomicrograph of section in the cerebral cortex of adult male albino rat of RB group (H&E X 400), showing many pyramidal cells (arrow) having small darkly stained nuclei and surrounded with empty space. Dilated congested blood vessel (double arrows) can be observed. **(b): A photomicrograph of section in the cerebral cortex of adult male albino rat of RB+ recovery period group (H&E X 400)**, showing no improvement and many pyramidal cells (arrow) having small darkly stained nuclei and surrounded with empty space. Dilated congested blood vessel (double arrows) can be observed. **(c): A photomicrograph of section in the cerebral cortex of adult male albino rat of RB+ω3 group (H&E X 400),** showing moderate improvement. The pyramidal cells (P) have normal histological structure. Some pyramidal cells (arrow) appear shrunken and surrounded with empty space.

DISCUSSION

Beverages marketed as "energy drinks" contain stimulants, mainly caffeine and are marketed with the claims that they are mental and physical stimulants [25]. Previous studies demonstrated histopathological lesions in different organs following administration of energy drinks. These organs include liver, kidney, brain, testis, pancreas and fundus of stomach. These lesions could be attributed to imbalance of oxidant/antioxidant environment in these tissues with increased oxidant stress as a result of reactive oxygen species production. Reactive oxygen species (ROS) are very harmful causing direct damage to vital cell components as lipids, proteins and deoxyribonucleic acid (DNA) [26].

In the current study, oral administration of the two different energy drinks; power horse (PH) and red bull (RB) to the adult male albino rats for 4 weeks induced a state of oxidative stress represented by a significant elevation of malondialdehyde level (MDA) as lipid peroxidation marker in the serum with significant elevation of serum antioxidant superoxide dismutase (SOD) and reduction of catalase (CAT) activities.

These results were in agreement with **Reis et al.** [27] and **Mansy et al.** [28]. **Seifert et al.** [2] found that these effects may be caused by the mixture of various ingredients in the ED due to synergistic interaction and augmented oxidative effect of them. Also, **Zeid´an-Chuli´a et al.** [29] found that ED ingredients caused major alteration in cellular CAT and SOD activities. While **Alsunni** [1] suggested that the caffeine is the most component responsible for major toxicity**. Ekaluo et al.** [30] found that the possible mechanisms involved in ED induced-oxidative stress are the decrease in tissue sensitivity to insulin, impaired glucose metabolism, and increase in stress hormone secretion. In turn this will result in elevated blood glucose level, lipolysis, and gluconeogenesis. This hyperglycemic environment results in subsequent glycation of membrane phospholipids of the cell membrane or even the intracellular organelles leading to lipid peroxidation (oxidative stress) and DNA damage in organs.

Also, in the present study, power horse produced more alteration in antioxidant activities than red bull this could be explained by **Khayyat et al.** [31] who attributed the different action of the energy drinks to their different ingredients**.**

The current study revealed that recovery period produced improvement in the changes of MDA and the studied antioxidant markers indicating that the effect of EDs are reversible.

Also, results of the current study showed that **ω3** showed more improvement in the changes of MDA and the studied antioxidant markers than the recovery period**.** This result was in agreement with **Hussein et al.** [32]**.**

The anti-inflammatory and/ or antioxidant effects of omega-3 fatty acids (ω3- FAs) through suppression of lipid peroxidation and scavenging of free radicals have been reported by **Ernest and Magdalena** [33]. It has been suggested that the use of ω3 FAs may have ameliorating effect on this oxidative stress by two possible ways: 1- Increase catalase level within the cytoplasm by ω3- FAs resulting in enhancement of defense against free oxygen radicals. 2- Supplemented Omega-3 FAs may be substituted with Polyunsaturated fatty acid components of the membranes that had been launched by oxygen free radicals such as hydrogen peroxide [34]**.**

Acetylcholinesterase enzyme has very vital role in decreasing the neurotransmitter acetylcholine level and converting it to acetic acid and choline **[35]**. This enzyme is widely distributed throughout the body in both neuronal and non-neuronal tissues. Acetylcholinesterase inhibition increases the concentration of ACh at both muscarinic and nicotinic receptors, producing a diversity of CNS, sympathetic, parasympathetic, and skeletal muscle signs and symptoms [36].

In the current study activity of acetylcholinesterase was measured in the brain tissue of the experimental rats. The results revealed that acetylcholinesterase activity was significantly decreased in **PH** and **RB** groups as compared to control group with more decrease in **PH** group. This result was in agreement with **Ebuehi et al.** [37].

Also, the current study revealed that both of recovery period and **ω3** treatment produced improvement in AchE activity with more improvement in **ω3** groups. These results were in accordance with **Attia and Nasr [38]**. According to **Simioni et al.,** [39] high sensitivity of the brain to oxidative stress is due to its weak antioxidant defense and high metabolic activity.

The results of the present study showed that **PH and RB** induced histopathological alteration in the brain tissue as there were many apoptotic cells having small darkly stained nuclei and surrounded by empty space. Also, dilated congested blood vessel could be observed. This result agreed with that of **Ayuob and ElBeshbeishy** [16] and **Salih et al.** [40]**.**

This result could be explained by **Kassab and Tawfik** [41] who stated that these changes were manifestations of cell damage and were caused by lipid peroxidation (oxidative stress) and DNA damage as a sign of toxicity and were attributed to the preservatives or caffeine gradient that are present in the energy drinks. Also**, Attia and Nasr** [38] found that the suppression of AChE resulted in the cumulation of acetylcholine (ACh), that may cause lymphocytes stimulation and elevated cellular cyclic guanosine monophosphate concentration,

increased lymphocyte motility and cytotoxicity. **Mansy et al.** [28] explained the congestion of blood vessels by microcirculatory disturbance that caused by energy drink intake. Also, **Kassab and Tawfik,** [41] mentioned that ED had been associated with many cardiovascular disorders as well as increased platelet aggregation and impaired endothelial function. These findings were due to different reaction of taurine with caffeine present in the energy drink**.** While **Ebuehi et al.** [37] and **Reis et al.** [27] found no changes in brain tissue although presence of biochemical alterations. They explained this by the short time of exposure to produce brain damage.

The current results revealed that **(ω3)** resulted in moderate improvement. The pyramidal cells have normal histological structure. Some pyramidal cells appear shrunken and surrounded with empty space. This result was in accordance with **Avramovic et al.** [42] **and Ayuob and ElBeshbeishy** [16] who observed that omega-3 fatty acids had valuable effects on brain tissue. Several studies reported that ω-3 fatty acids can decrease the damage caused by oxidative stress through docosanoids derived from Docosahexaenoic acid (DHA) which act as neuroprotectants [43]. According to **Chen et al.** [44] supplementation of ω-3 FAs decrease the inflammatory response and microglial activation.

Concerning to DNA and RNA contents in the brain of the current study, there were significant reduction in DNA and RNA contents in brain of rats treated by **PH and RB** as compared to control group with more reduction in PH group. These current results were in harmony with **Al-Zahrani et al. [45]** and supported by **George and George [46]** who found that caffeine in soft drinks causes diseases in different organs through changing of DNA repair system. These findings could be attributed to high levels of ROS that resulted in cellular lipids modifications, proteins and DNA degradation and finally cell death. ROS mainly affected proteins by oxidation **[39].**

The current result revealed high significant increase in total content of DNA and RNA in brain tissue after the recovery period and coadministration of ω3 as compared to their toxic groups with more improvement in ω3 groups. These results were in harmony with **Ghorbanihaghjo et al.** [47] and were supported by **Sakai et al.,** [48] who concluded that ω3 FAs decrease oxidative DNA damage and cell death through upregulation of messenger RNA (mRNA) expression of antioxidant molecules via Nuclear factor like 2 (NRF2) mediated antioxidant response.

As regards to chromosomal aberrations (CAs) in bone marrow cells in the present study showed significant increase in frequency of numerical and structural aberrations with reduced mitotic index in rats treated with PH and RB EDs as compared to the control group. **Boehm et al.** [49] mentioned that the increase in the chromosomal aberrations in bone marrow cells observed in the current study may be attributed to induced oxidative stress that resulted in DNA strand breaks, fragmentation and loss of chromosomal integrity. According to **Karaismailoglu** [50] decline in mitotic index may reflect cytotoxicity of energy drinks through the inhibition of cell cycle from the synthetic (S) phase to the mitosis (M) phase, or by preventing cell mitosis and DNA synthesis.

The results of the present study showed that recovery period and ω3 decreased the frequency of chromosomal aberration and increased mitotic index as compared to EDs groups. This result was similar to **Ali et al.** [51]. **Liu et al.** [52] suggested that ω3 FAs can help in prevention of cancer through suppression of transactivation of transcription activator protein 1 (AP-1), which is responsible for cell multiplicity genes. **Gogus and Smith,** [53] reported that Omega-3 fatty acid not only effective, as an antitumor agent but also having a role in prolonging life span, inhibiting autoimmune diseases, suppressing tumorigenesis and tumor necrosis factor which is markedly elevated in various types of tumor. Omega-3 FAs also have a very important rule as a complementary medicine and can increase the effectiveness of cancer chemotherapeutics [54]**.**

CONCLUSION

Results of the present study can be concluded into; the different energy drinks (power horse and red bull) induced biochemical, histological and cytogenetic changes in the brain of adult male albino rats which were partially ameliorated either by their withdrawal or co-administration of omega 3 fatty acids. On account of these toxicological findings, strict control of EDs consumption is urgently needed. Further researches and regulations on marketed energy drinks are needed.

Meeting presentation
The material has not been presented previously.

Financial support
There is no any financial support

Conflict of interest declaration
There is no any conflict of interest
.

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الملخص العربي

دراسة الآثار البيوكيميائية و النسيجية و الخلوية لنوعين مختلفين من مشروبات الطاقة (باور هورس وريدبول) على أدمغة ذكور الفئران البيضاء وتحديد الدور الوقائي المحتمل لأوميغا 3 على الآثار الضارة الناتجة عن هذه المشروبات

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ملخص البحث:

الخلفية: لقد أصبحت مشروبات الطاقة ظاهرة عالمية وشعبية خاصة بين البالغين والشباب ويعتبر المخ من أكثر الأعضاء تعرضًا للسمية.

الدف : دراسة التأثيردون المزمن لتناول نوعين مختلفين من مشروبات الطاقة (باور هورس وريدبول) على أدمغة ذكور الفئران البيضاء البالغين من خلال التغيرات الكيميائية الحيوية والنسيجية والجينوخلوية. ولتحديد الدور الوقائي المحتمل لأوميغا 3 على الآثار الضارة الناتجة عن هذه المشروبات .

الطرق: تم تقسيم ثمانية وأربعين من ذكور الفئران البيضاء البالغين إلى 8 مجموعات ؛ المجموعة الضابطة ، مجموعة أوميغا 3 ، ريدبول ، باورهورس ، كل من هذه المشروبات جنبا إلى جنب مع أوميغا 3 وايضا تركها لمدة اسبوعين اخرين بدون اعطاء المشروب. تم الحصول على عينات من الدم والدماغ والفخذ من الفئران لتقييم التغيرات الكيميائية الحيوية والنسيجية والجينوخلوية.

النتائج: تبين ان هذه المشروبات التى تم دراستها تسببت فى زيادة مستوى كل من عامل التأكسد ثنائي المالون الدهيد ، والخلل الصبغي الهيكلي والعددي. في حين أنها قللت من علامات مضادات الأكسدة (الكاتالاز وسوبروكساید ديسميوتاز) و نشاط أستيل كولين استراز و المحتوى الكلي للحمض النووي والحمض النووي الريبوزى في أنسجة المخ ومؤشر الانقسام الفتيلي مع اضطراب في الشكل الطبيعي لأنسجة المخ. وقد لوحظ تحسن جزئي في هذه التغييرات بعد استخدام أوميغا 3 وأيضا بعد ايقاف هذه المشروباتوكان التأثير أفضل مع أوميغا 3.

الاستنتاجات: مشروبات الطاقة لها تأثير سام على المخ قد يتحسن جزئيا بعد توقف هذه المشروبات او بتناول الأوميغا3. لذلك هنا حاجة ماسة الى رقابة صارمة على استهلا مثل هذه المشروبات لما لها من اثار ضارة.

الكلمات المفتاحية : مشروبات الطاقة - باور هورس (حصان الطاقة) -ريد بول (الثور الأحمر) ، سمية الدماغ

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