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Original Article

Effects of caffeinated energy drinks on cerebellum of Male albino rats

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ABSTRACT

Background: Energy drinks are widely being used by different age groups especially by teenagers in Pakistan. Higher level of caffeine in energy drinks have harmful effects on histology of brain tissue. **Objective:** This study aims to determine the impact of oral administration of red bull on cerebellum of adult male albino rats. **Study Design:** Randomized Control trial. **Place and Duration:** Animal house National institute of Health Islamabad for 8 weeks **Methodology:** Red bull in dosage of 3.75 ml/kg body weight was administered by oral gavage daily for 4 weeks to experimental group B followed by normal diet for next 4 weeks. Experimental group C was given 3.75 ml/kg body weight of red bull for consecutive 8 weeks. The cerebellum was excised in each animal and weighed; fixed in formalin, stained with Hematoxylin and Eosin. Sections were observed for histological cytoarchitecture. **Results:** Results were taken in forms of photomicrographs and analyzed. Observations showed marked detachment of pia mater along with congestion and marked hypertrophy of molecular layer, distortion of purkinje cells and shrinkage of granular layer in experimental group C. Changes were minimal to mild in experimental group B.

Conclusion: Therefore, it is concluded that consumption of caffeinated energy drinks should be restricted to avoid their harmful effects on cerebellum leading to sleep disturbances and motor incoordination.

Keywords: Caffeine, Energy drink, Cerebellum

Introduction

Cerebellum, located in the occipital lobe of skull, is responsible mainly for motor coordination, balance and cognitive functions. Histological structure of cerebellum comprises of three main layers: molecular, purkinje and granular layer. Important cells in these layers are basket and stellate cells in Molecular layer, purkinje cells in Purkinje layer, granular, glomeruli and Golgi cells in Granular layer.[1] Many factors can cause cerebellar damage for example caffeine abuse, alcohol, certain medications, degenerative brain diseases, stroke and tumors. Cerebellar damage can result in short term memory deficits, headache,

motor incoordination, gait ataxia and speech problems[2]

For past few decades there is remarkable increase in use of caffeinated energy drinks. Energy drinks (EDs) were originally invented in UK in 1929, initially used therapeutically in hospitals and appeared in Asia and Europe in 1960,s. Later in 1980 their use was promoted for energy burst. They are now available in > 140 countries.[3] There are varieties of energy drinks available in Pakistan such as Monster, Sting power horse and Red bull, that is the most widely used brand3. Limited data is available regarding daily average intake of caffeinated energy drinks in Pakistan, but studies have shown increased consumption by youth particularly males of age group of 13-35 years.[4,5]

Energy drinks contains combination of sugars, amino acids mainly taurine (800mg/L), herbs and caffeine (150 mg/L).[6] The higher levels of caffeine in energy drinks are associated with behavioural disorders, anxiety, restlessness, irritability, headache and sleep problems. Long term intake of these drinks is also associated with increased risk of myocardial infarction, arrhythmias, hypertension, bone weakness due to increased urinary loss of calcium and spontaneous abortion.[7,8]

The caffeine in energy drinks causes damage by inhibiting adenosine receptors leading to interstitial inflammation, insulin resistance and formation of reactive oxygen species.[9] The oxidative stress caused by reactive oxygen species is responsible for hypoxic injury to brain tissue leading to neuronal cell damage and cell death.[10]

No research has yet been conducted to study the effects of caffeinated energy drinks on histology of cerebellum. So, keeping in view the detrimental effects of caffeinated energy drinks the present study was conducted to observe the effects of these drinks on the histomorphology of cerebellum.

Methodology

This randomized control study was conducted in Animal house National institute of Health Islamabad for 8 weeks. Thirty, healthy adult male albino rats weighing 250±10 grams were used in this experimental study. The animals were kept at animal house Islamabad and were acclimatized for 7 days. The random allocation of groups to the animals was done by lottery method and they were equally divided into three groups.

Group A: Rats in this group were given normal diet and water for a period of eight weeks.

Group B: Rats in this group were given 3.75 ml red bulls orally for first four weeks, followed by normal diet and water for next four weeks.[11]

Group C: Rats in this group were given 3.75 ml red bulls orally by gavage for eight weeks.

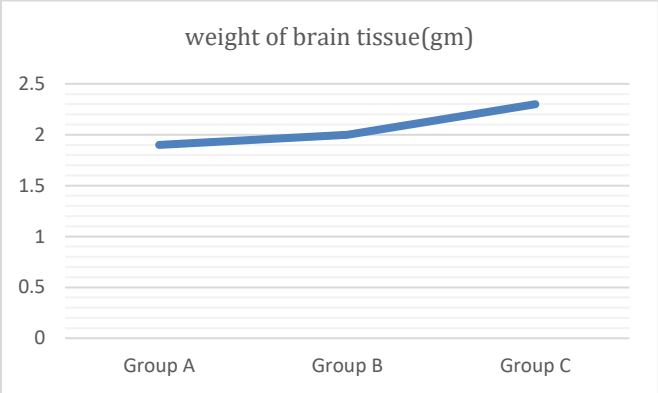
After completion of experimental study all animals were humanly sacrificed by euthanizing with sodium pentobarbital intraperitoneally. Immediately after sacrifice cerebellum was observed for gross parameters that were appearance and weight of the tissue. After washing with saline and fixing in 10% formalin, transverse sections of 5 µm thickness were obtained. Staining with haematoxylin and eosin, sections were done, and slides were examined under light microscope for the detachment of pia mater, haemorrhage and the layers of cerebellar cortex. Data was analysed in SPSS version 22. One-way analysis of variance (ANOVA) was applied for mean comparison of quantitative variables between control group A and experimental group B and C. A p value of equal or less than 0.05 was considered as significant.

Results

On gross examination, the cerebellum appeared normal in control group A. In experimental group

B, no remarkable change was observed macroscopically. However, in experimental group C, there was remarkable increase in weight of cerebellum along with cerebellar swelling (Figure:1:1).

Figure 1: Line chart showing weight of brain tissue by ANOVA in rats of control and experimental groups



On histological examination pia mater was closely adherent to the layers of cerebellar cortex in control group A. In experimental group B, mild detachment of pia mater was observed. However, in experimental group C pia mater was markedly detached along with severe hemorrhage (Figure:2). On observing the molecular layer of cerebellar cortex cytoarchitecture was preserved in control group A and experimental group B. In experimental group C marked hypertrophy of molecular layer was observed (Figure: 3).

In control group A, normal morphology of cells of cerebellar cortex was preserved (Figure: 4a). In experimental group B no significant change was seen in cells of granular layer. However, in purkinje layer mild vacuolization along with slight change in shape of cells were seen (Figure: 4b). In experimental group C there was marked shrinkage of granular layer along with severe vacuolization and distortion of shape of purkinje cells (Figure: 4c).

Figure 2: Cross-section of cerebellum of the adult male albino rats stained with H & E at 100 X showing pia mater closely attached to the layers of cerebellar cortex in control group (Figure: 2a). There is partial detachment of pia mater in experimental group B (Figure: 2b). In experimental group C there is marked detachment along with congestion of pia mater (Figure: 2c).

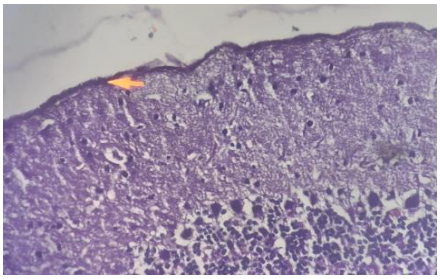


Fig 2a

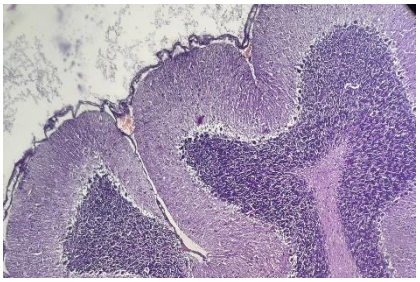


Fig 2b

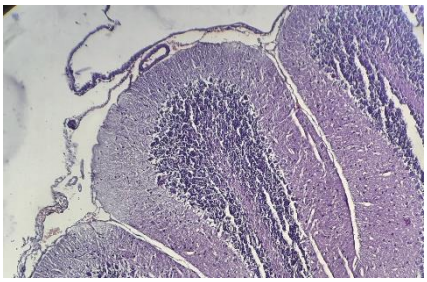


Fig 2c

Figure: 3 Cross-section of cerebellum of the adult male albino rats stained with H & E at 100 X showing preserved cytoarchitecture in control group A and experimental group B (Figures: 3a, 3b). Marked hypertrophy of molecular layer seen in experimental group C (Figure: 3c).

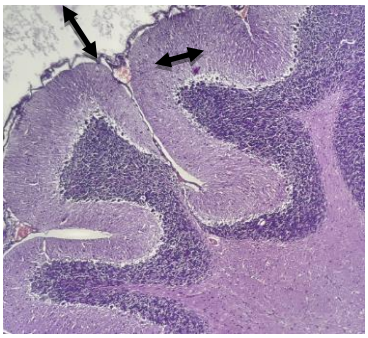


Fig 3a

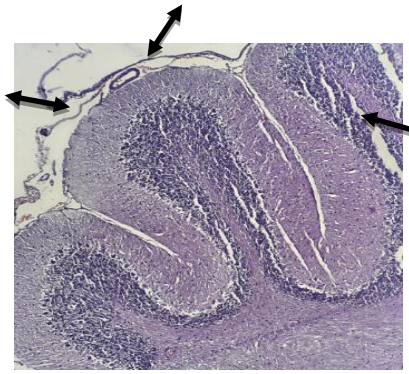


Fig 3b

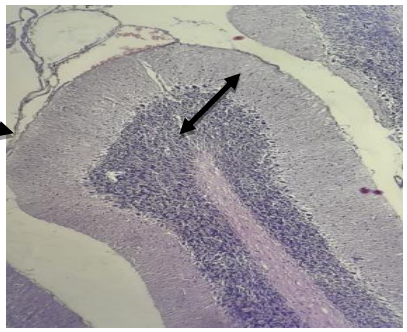


Fig 3c

Figure: 4 Cross-section of cerebellum of the adult male albino rats stained with H & E at 400 X showing normal morphology of cells of cerebellar cortex in control group A (Figure: 4a). In experimental group B mild vacuolization along with slight change in shape of purkinje cells seen (Figure: 4b). Marked shrinkage of granular layer along with severe vacuolization and distortion of purkinje cells seen in experimental group C (Figure: 4c).

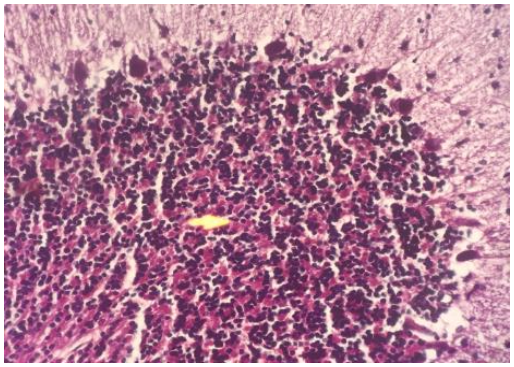


Figure: 4a

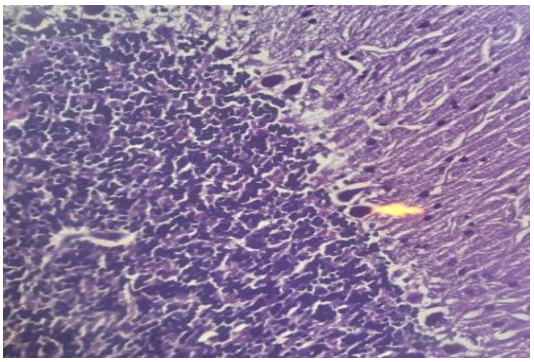


Figure: 4b

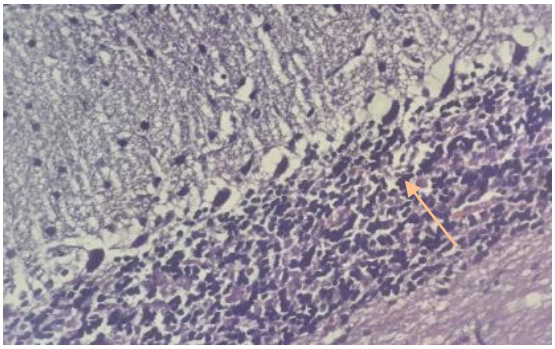


Fig 4c

Discussion

Intake of caffeinated energy drinks results in formation of reactive oxygen species and oxidative damage, which leads to hypoxic injury to brain tissue, neuronal cell damage and cell death. The oxidative stress is also responsible for morphological and histological changes in cerebellum.

Present study revealed remarkable increase in weight of cerebellum along with cerebellar swelling in experimental group C. Adjene studied the effect of long term intake of energy drinks and observed increase in weight of brain tissue of adult rats This was explained by an increase in insulin levels brought about by the sweetening constituents of Energy Drinks which resulted in lipid storage and weight gain of brain.[12] The increase in weight of brain tissue could be due to caffeine content of energy drink (Howard and Marczinski, 2010). Reis observed no significant difference in weight of brain tissue after 14 days' intake of energy drinks.[13]

On histological examination there was marked detachment of pia mater along with congestion in experimental group C. Our results are consistent with study conducted by Salih et al who observed congestion along with neuronal degeneration in cerebral cortex after consumption of energy drinks for 30 days.[14] Abdel Wahab also reported congestion of cerebral cortex after intake of energy drinks for period of 4 weeks. Observed effects were due to oxidative stress caused by energy drinks leading to haemorrhage and congestion.[15]

On observing the layers of cerebellar cortex, marked hypertrophy of molecular layer was observed in experimental group C. However, in purkinje layer vacuolization along with change in shape of purkinje cell was observed in experimental groups B and C. There was marked shrinkage of granular layer in experimental group C. Eluwa et al observed hyperplasia of molecular and granular layers in cerebellum of female albino rats, along with degeneration of purkinje cells after intake of soft drinks for 21days.[1] El sayed et al observed vacuolization and shrinkage of pyramidal cells in cerebral cortex after consumption of energy drinks for 4 weeks.[15] Owolabi et al concluded that higher doses of caffeine had deleterious effects on cerebellar histology with marked distortion of purkinje cells. As purkinje cells are only efferent neuron of cerebellum, their distortion may lead to inadequate signals to higher centre leading to motor incoordination. Russell et al observed neurodegenerative changes in hippocampus of adult male albino rats after intake of caffeinated energy drinks for period of 8 weeks. Observed effects were due to high caffeine content in energy drinks leading to oxidative damage and inflammatory response in brain tissue.

Conclusion

The findings of this study suggested that caffeinated energy drinks may have deleterious effects on histology of cerebellum of adult male albino rats that can lead to motor incoordination. Therefore, their consumption should be minimized.

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Conflict of Interest

The authors declare no conflict of interest.

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Contributions of the Authors

SSB conceptulized the project, did literature research and data collection

FS did literature research and data collection

SS did statistical analysis

SB did drafting and revision

AH wrote the manuscript