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## Morphological Effects of Long-Term Consumption of Energy Drinks on the Intracranial Auditory Relay Centres of Adult Wistar Rats.

<sup>1</sup>Adjene JO, <sup>1</sup>Emojevwe V, <sup>2</sup>Idiapho DE, <sup>3</sup>Igbigbi PS

<sup>1</sup>Department of Medical Laboratory Science, Achievers University, Owo, Ondo State, Nigeria. E-mail: joadjene@yahoo.com Tel: +2348034084016, +2348053478526.,

<sup>2</sup>Department of Histopathology, Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria. E-mail: davididiapho@gmail.com., Tel: +2348034305403. and <sup>3</sup>Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka. E-mail: pigbigbi@yahoo.com., Tel: +2348036746053, +2348075388657.

**Corresponding Author:** Adjene JO.

E-mail: joadjene@yahoo.com. Tel: +2348034084016, +2348053478526.

### ABSTRACT

Morphological effects of long-term consumption of energy drinks commonly use as flavored beverage drinks on the intracranial auditory relay centres namely inferior colliculus and medial geniculate body of adult wistar rats were carefully studied. The rats of both sexes (N = 20), with average weight of 200g were randomly assigned into treated (n<sub>1</sub>=10) and control (n<sub>2</sub>=10) groups. The rats in the treated group received energy drinks on a daily basis for 10hrs and 14hrs of distilled water liberally in thirty days, while the control group received distilled water daily for thirty days. The growers mash feeds was obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo state, Nigeria and the rats were given feeds liberally. The rats were sacrificed by cervical dislocation on the thirty-first day of the experiment; the inferior colliculus and medial geniculate body were carefully dissected out, weighed and quickly fixed in 10% formal saline for further histological study. The findings indicated that there was an increase in the weight (g) and relative weight (%) of the inferior colliculus and a significant (p < 0.05) decrease in the weight (g) and relative weight (%) of the medial geniculate body of the treated rats as compared to the control group. The section from the treated group with energy drinks showed cellular degenerative changes, sparse cellular population, hypertrophy, microcytic changes and vacuolations in the stroma of the inferior colliculus and medial geniculate body as compared to the control group. Long-term consumption of energy drinks may therefore have an adverse effect on the intracranial auditory sensibilities by affecting the morphology of the inferior colliculus and medial geniculate body of adult wistar rats. It is recommended that further studies aimed at corroborating these findings be carried out.

**Key words:** Morphology effects, Energy drinks, Inferior colliculus, Medial geniculate body, Wistar rats.

### INTRODUCTION

Energy drinks are flavoured beverages containing high amounts of caffeine and typically other additives, such as vitamins, taurine, herbal supplements, creatine, sugar, and guarana, a plant product containing concentrated caffeine. The energy drinks are marketed to improve energy, weight loss, stamina, athletic performance, and concentration<sup>1,2</sup>. These drinks sold in cans and bottles, are readily available in grocery stores, vending machines, convenience stores, bars and other venues where alcohol is sold. Consumption of energy drinks is a rising public health problem because medical and behavioural consequences can result from excessive caffeine intake. A growing body of scientific evidence documents harmful health effects of energy drinks, particularly for children, adolescents, and young adults<sup>3</sup>.

Research has established that among college students, there are associations between energy drink consumption and problematic behaviours such as

marijuana use, sexual risk taking, fighting, smoking, drinking and prescription drug misuse<sup>4,5</sup>. Although healthy people can tolerate caffeine in moderation, heavy caffeine consumption, such as drinking energy drinks, has been associated with serious consequences such as seizures, mania, stroke and sudden death<sup>6,7,8,9,10,11,12</sup>. In children, especially those with cardiovascular, renal or liver disease, seizures, diabetes, mood and behaviour disorders, hyperthyroidism or those who take certain medications may be at higher risk for adverse events from energy drinks consumption<sup>12,13,14,15,16,17,18,19,20,21,22</sup>. However, caffeine has been reported to have detrimental health consequences. Riesenhuber and colleagues found it is caffeine, not taurine in energy drinks that promotes diuresis and natriuresis<sup>23</sup>. Further acute caffeine consumption reduces insulin sensibility<sup>24</sup> and increases mean arterial blood pressure<sup>25</sup>.

High caffeine consumption has is also associated with chronic daily headaches, particularly among young women and among those with chronic episodic

headaches of recent onset<sup>25</sup>. Central nervous system, cardiovascular, gastrointestinal, and renal dysfunction have been associated with chronic caffeine ingestion<sup>26</sup>. Aspartame is a synthetic sweetener and preservatives, which is of low caloric value added to soft drinks, chewing gum, fruit juices, gelatins and jellies<sup>27, 28</sup>. Aspartame is metabolized in the gastrointestinal tract into aspartic acid, methanol, and phenylalanine<sup>29</sup>. The methanol is later oxidized to formaldehyde and formate in many tissues. Formic acid is the principal metabolite responsible for the deleterious effects of acute intoxication by methanol in humans and animals<sup>30</sup>. In intoxication, it causes blindness and hepatic injury since the liver and retina concentrate the greatest quantity of the metabolic by-products of aspartame<sup>31</sup>.

The inferior colliculus (IC) and medial geniculate body (MGB) constitute the intracranial auditory relay centers. The MGB is the target of ascending projections from the inferior colliculus and descending input from the auditory cortex; this is the obligatory synaptic target in the thalamus for hearing<sup>32</sup>. The cerebral cortex strongly affects the MGB through descending projections. These projections are thought to consist primarily of small areas with slow conduction velocities<sup>33</sup>.

It has been demonstrated that auditory cortex neurons have great physiological plasticity when rats are exposed to specific stimuli coupled with concurrent stimulation of a forebrain subcortical structure in the nucleus basalis. Observed changes have included massive expansion of frequency-specific representation<sup>33</sup>. The inferior colliculus is essential for normal hearing and for the startle reflex. Cortical structures such as the medial and lateral geniculate bodies and the inferior and superior colliculi have higher glucose utilization than other structures<sup>34</sup>. The correlation between functional activity and metabolic rate much as in the visual and auditory systems has been established<sup>34</sup>. There is no document on any toxic effects of energy drinks on the intracranial auditory relay centers. This study aimed at investigating any possible morphological effects of long-term consumption of energy drinks on the inferior colliculus and medial geniculate body of adult Wistar rats. This study is based on the premise that any morphological distortion on the inferior colliculus and medial geniculate body could affect auditory dependent sensibilities.

## MATERIALS AND METHODS

**Experimental Animals:** The ethical committee of the Achievers University, Owo granted the approval before the commencement of the research. Twenty adult wistar rats of both sexes (Animals were caged based on same sex to avoid mating and pregnancy) with average weight of 200g were randomly assigned into two groups: A and B of (n=10) in each group. Animals in group A served as treated group (n<sub>1</sub>=10) while animals in group B (n<sub>2</sub>=10) served as the control. The rats were

obtained and maintained in the Animal Holding of the Department of Medical Laboratory Science, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria. The animals were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given feeds liberally. The energy drinks were obtained from retailer store, Owo, Ondo State, Nigeria.

**Energy Drinks Consumption:** The rats in the treated group received energy drinks and distilled water alternatively on a daily basis for 10hrs and 14hrs liberally in thirty days, while the control group received distilled water liberally for the thirty days in line with an improved method of (Adjene *et al.*, 2010)<sup>35</sup>. The ingredients contained in the energy drinks were as follows: Water, Citric acid, Carbon dioxide, Taurine (0.38%), acidity regulator (Sodium citrate), Sweeteners (Acesulfame and Aspartame), Flavouring, Caffeine (31.5mg), Glucoronolactone (0.01%), Nicotinamide (7.92mg/100ml, 49.5% RDA) Colour ammonia caramel, inositol, Niacin, Pantothenic acid (33% RDA, about 1.98/100ml), vitamin B<sub>6</sub> (143% RDA about 2mg/100ml) vitamin B<sub>12</sub> (80% RDA, 2ug/100ml). The rats were sacrificed by cervical dislocation on the thirty-first day of the experiment and the inferior colliculus and medial geniculate body were extracted, weighed using the Mettler Toledo weighing balance and quickly fixed in 10% formal saline for routine histological techniques.

**Histological Study:** The inferior colliculus and medial geniculate body were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffused sections were stained routinely with haematoxyline and eosin<sup>36</sup>. Photomicrographs of the desired results were obtained using the olympus research photographic microscope in the Department of Medical Laboratory Science, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria.

## RESULTS

The findings indicated that there was an increase in the weight (g) and relative weight (%) of the treated inferior colliculus and a significant (p < 0.05) decrease in the weight (g) and relative weight (%) of the treated medial geniculate body as compared to the corresponding control group (Table 1).

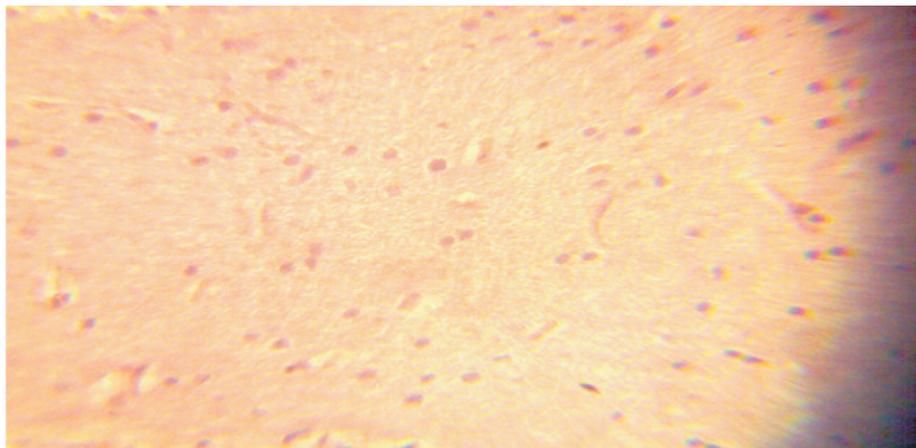
The sections of the inferior colliculus and medial geniculate body from the control animals showed normal histological features with the neurons appearing distinct and of various sizes. The neuron and glial cells appeared normal and showed no signs of vacuolations in the stroma of the inferior colliculus and medial geniculate body. The sections from the treated group with energy drinks showed some cellular degenerative changes, sparse cellular population, hypertrophy, some

microcystic changes and vacuolation in the stroma of the inferior colliculus and medial geniculate body as compared to the control group (figures 1, 2, 3 & 4).

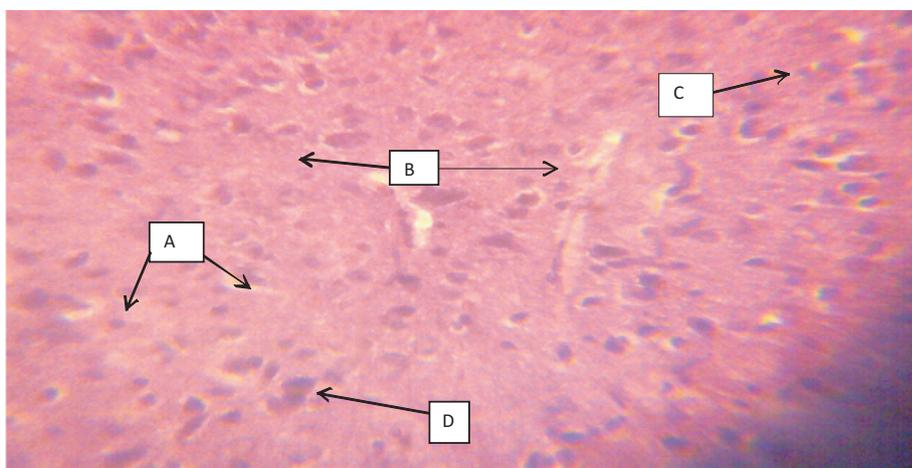
**Table 1:** The Mean SEM Weight (g) and Relative Weight (%) of the Inferior Colliculus: (IC) and Medial Geniculate Body (MGB) of the Animals.

PARAMETERS	GROUP OF ANIMALS	
	CONTROL (n <sub>1</sub> = 10)	TREATED (n <sub>2</sub> = 10)
Brain weight (g)	*1.649 ± 0.027	*1.790 ± 0.035
Inferior colliculus weight (g)	0.118 ± 0.011	0.304 ± 0.104
Relative Inferior colliculus weight (%)	7.095 ± 0.601	16.615 ± 5.599
Medial geniculate body weight (g)	*0.204 ± 0.019	*0.131 ± 0.019
Relative Medial geniculate body weight (%)	*12.444 ± 1.226	*7.600 ± 1.365

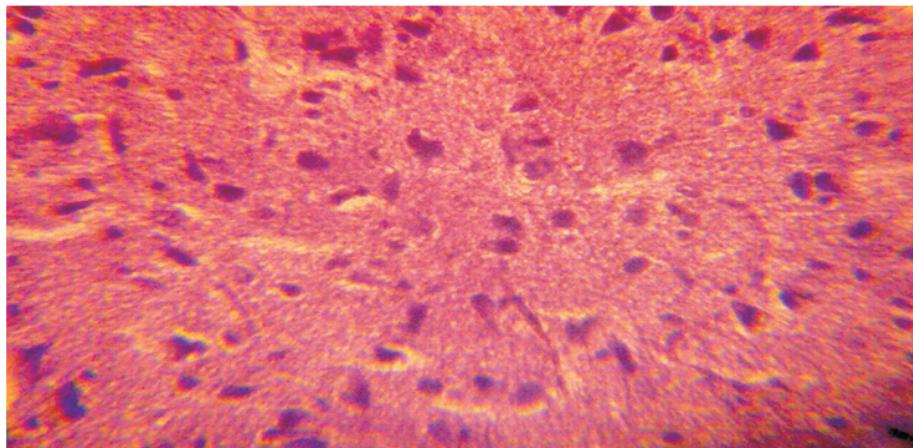
\*Significant (P< 0.05)



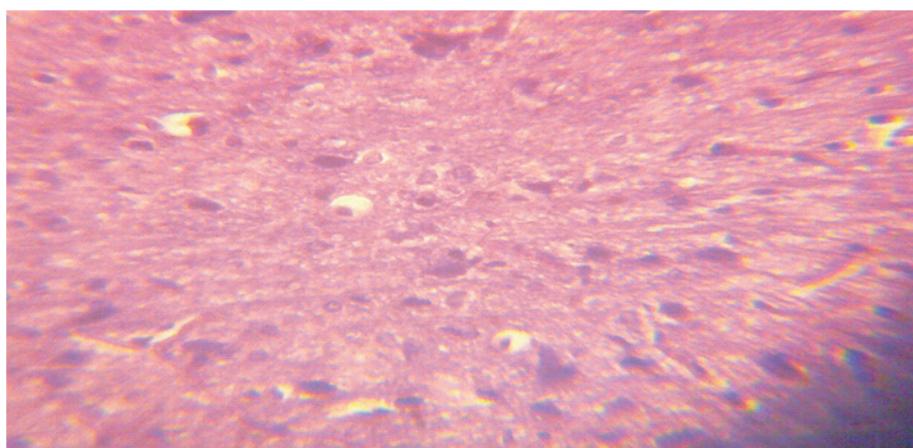
**Figure 1:** Control section of the Inferior colliculus. (H & E x400)



**Figure 2:** Treated section of the Inferior colliculus showing: Vacuolation (A), Degenerative cell (B), Microcystic Vesicle (C) and Hypertrophy (D). (H & E x400)



**Figure 3:** Control section of the Medial Geniculate Body (MGB). (H & E x400)



**Figure 4:** Treated section of the Medial Geniculate Body (MGB) showing: Vacuolation (A), Degenerative cell (B) and Microcystic Vesicle (C). (H & E x400)

## DISCUSSION

The findings revealed that there was an increase in the weight (g) and relative weight (%) of the treated inferior colliculus and a significant ( $p < 0.05$ ) decrease in the weight (g) and relative weight (%) of the treated medial geniculate body as compared to the control group. The treated sections of the energy drinks showed some cellular degenerative changes, sparse cellular population, hypertrophy, some microcytic changes and vacuolation in the stroma of the inferior colliculus and medial geniculate body as compared to the control group of the adult wistar rats. The results indicated that prolonged consumption of energy drinks resulted in increased toxic effects on the intracranial auditory relay centres of adult wistar rats.

The increase in the weight of the treated inferior colliculus and the significant ( $p < 0.05$ ) decrease in the weight of the treated medial geniculate body may be due to the neurotoxic effects of the energy drinks on the cells of the intracranial auditory relay centres of the adult wistar rats. Under such conditions, there is a net shift of water from the extracellular space to the interior of the brain cells<sup>37</sup>. Ischemic or pharmacologic

disruption of cellular transporters can cause swelling of the brain parenchyma. Cytotoxic edema usually involves intracellular swelling of glial, endothelia and neurons<sup>37</sup>. Regulation of brain water content and therefore of the volume is critical for maintaining the intracranial pressure within tolerable limits<sup>37</sup>. In this study, energy drinks could have acted as toxins to the cell of the inferior colliculus and medial geniculate body thus affecting their cellular integrity and causing a defect in membrane permeability and cell volume homeostasis. As brain tissue swells or shrinks as seen in this study, the activity of the cellular transporters is approximately modified by the up or down regulations as reported in the case of hyponatremia or hypernatremia<sup>37</sup>. The disruption of the inferior colliculus and medial geniculate body caused by energy drinks is a cardinal feature of this experiment.

The significant difference in weight observed in this experiment could be due to the high rate of catabolism caused by the effects of high availability of insulin induced by the sweetening constituents of the energy drinks thereby causing increase in the rate of lipid storage<sup>38</sup>. This supports an existing hypothesis that an

increase in sweeteners consumption can be associated with increased risk of weight gain because of decreased satiety and incomplete compensatory reduction in energy intake<sup>39,40,41</sup>.

Brain cell swellings or shrinkages can lead to severe cytotoxic edema and may lead to marked reduction<sup>37</sup>. The sparse cellular population observed in this study may have caused by the toxic effect of energy drinks thus resulting in cell death. Neuronal degeneration due to toxic agents could be progressive and may include shrinkage of the neurons<sup>42</sup>. Energy drinks may have acted as toxin to the cells of the inferior colliculus and medial geniculate body thus affecting their cellular integrity. The sparse cellular population observed in this study may have been due to cell death caused by the toxic effects of energy drinks. Beside aspartame being an excitoneurotoxic agent and caffeine are contents of energy drinks that can cause the excretion of water from the body thus leading to high concentrations of sugar entering the blood stream<sup>43,22</sup>. It could be inferred from this study that prolonged consumption of energy drinks resulted in an adverse effects on the inferior colliculus and medial geniculate body of adult wistar rats.

The vacuolations and microcystic changes observed in the stroma of the treated inferior colliculus and medial geniculate body in this experiment may be due to energy drinks interference, since some of its constituents has an excitoneurotoxic and excitotoxin effects that could help in the breaking down of the blood brain barrier<sup>45</sup>. It is probable that the results obtain in this experiment may have been due to the neurotoxic effects of energy drinks on the neuronal cells of the inferior colliculus and medial geniculate body of adult wistar rats. This report presents evidence of the possibility damage to the intracranial auditory relay centres of adult wistar rat with long- term consumption of energy drinks.

### Limitations

The energy drinks used in this experiment contain aspartame and caffeine, but we acknowledged that consumption of energy drinks is not as liberally provided to humans as done to the wistar rats in this experiment. However, our report provides an indication of toxic potential of energy drinks to intracranial auditory relay centres in mammals. To our knowledge, this is the first report on the morphological effects of energy drinks on the intracranial auditory relay centres using an adult wistar rats (mammals).

### CONCLUSION

The study revealed that long-term consumption of energy drinks resulted in morphological changes in the stroma of the inferior colliculus and medial geniculate body of adult wistar rats. These changes may affect the auditory sensibility of the inferior colliculus and medial geniculate body in the adult wistar rats. We

recommend further studies aimed at corroborating these observations.

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