Effect of Red Bull energy drink on Rats' Submandibular salivary glands (Light and Electron microscopic Study)

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Abstract: Background: Energy drink consumption has continued to gain wide popularity. These drinks are marketed for young people as natural alternatives that improve physical and mental performance such as concentration, attention, and alertness. **Aim:** The purpose of this study was to determine the histological and ultra structural changes in rat submandibular salivary glands induced by Red Bull energy drink. **Material and Methods:** Twenty male albino rats (170 ±10 g) were divided equally into group I (control) and group II (Red Bull). The rats of group II received a daily single dose (3.57 ml/kg b.wt.) of Red Bull energy drink using an oro-pharyngeal metallic curved tube for 8 weeks. At the end of the experimental period, all rats were sacrificed. The submandibular salivary glands were dissected out and prepared for light and transmission electron microscopic examinations. **Results:** Histological examination of submandibular glands of Red Bull group revealed swelling of the secretory portions with numerous intracytoplasmic vacuoles. The connective tissue capsule and septa showed extensive fibrosis and congested blood vessels. Nuclear atypism, pleomorphism, hyperchromatism as well as numerous mitotic figures were detected. The excretory ducts appeared dilated with retained secretion. The granular convoluted ducts appeared dilated with reduced granular eosinophilic content. Electron microscopic examination revealed abnormal divided nuclei and large coalescing electron lucent secretory granules in the secretory cells. Numerous vacuoles and electron lucent granules were detected also in the granular convoluted ducts. There were numerous dilated blood vessels with electron dense erythrocytes.

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Keywords: Energy drinks; Red Bull; salivary glands; histological changes.

1. Introduction:

Energy drinks are non-alcoholic, lightly carbonated beverages that are designed to give the consumer a dose of energy. Energy drinks are more popular than ever and it seems to be getting bigger every year. Energy drinks are used frequently with teenagers, young adults, athletes and physically active subpopulations. These drinks are marketed as natural alternatives that increase fun and improve physical and cognitive performance such as concentration, attention, and alertness [(1)].

There are different types of energy drinks, with names like Boom Boom, Power horse, Burn, Monster, Red Bull and AMP Energy. Energy drinks have sugar-containing and sugar-free versions. Red Bull is the most popular energy drink consumed in Egypt. The company's claim that Red Bul ''gives you wings'' suggests that consumption of their product will provide the consumer with more energy and enhanced performance, both mentally and physically [(2)]. The company also labeled their product with the statement ''it vitalize body and mind''. Carbonated energy drinks such as Red Bull, contain ingredients intended to induce alertness, concentration, and focus [(3)]. The main active ingredient in energy drinks is caffeine, although other substances such as taurine, carbohydrates riboflavin, pyridoxine, nicotinamide, B-complex vitamins, and various herbal derivatives such as ginseng are also present [(4 & 5)]. Ethanol may be also added in certain types of energy drinks [(6)].

There are few studies on the effects of the energy drinks. Some studies reported enhancement of the mood state, as well as physical and psychomotor performance (time of motor reaction, concentration, work memory and subjective sensation of alertness and vigor) after the ingestion of Red Bull [(7 & 8)]. In contrast to these potential beneficial effects, energy drink consumption has been linked to different symptoms of cardiovascular disease. Administration of Red Bull like beverage (with caffeine and taurine) significantly increased the contractility of the left atrium that accounts for the higher left end-diastolic volume leading to an increase in stroke volume [(9)]. Red Bull consumption was also associated with a decrease in heart rate and an increase in the systolic blood pressure and pain tolerance [(2)]. The combined use of energy drinks and alcohol is increasing sharp-

ly, resulting in an increase of the rate of alcohol absorption and dominating the alcohol related injury [10].Several studies suggest that energy drinks may serve as a gateway to other forms of drug dependence [11].

As the popularity of energy drinks continues to rise, it is important to consider their potential adverse effects. Therefore, the aim of the present study was to determine the histological effects of ingestion of Red Bull energy drink on the submandibular salivary glands of male albino rats.

2. Material and Methods:

Twenty healthy adult male albino rats weighing 170 ±10 grams were used in this study. The animals were divided into two main groups (10 rats each) as follows:

Group I (Control group):

The rats were kept on normal diet and water for 8 weeks.

Group II (Red Bull group):

The rats were kept on normal diet and water and received a daily single dose (3.57 ml/kg b.wt.) [5] of Red Bull solution using an oro pharyngeal metallic curved tube for 8 weeks. This dose is equivalent to the minimal human dose [1can (250 ml) /day] and related to the animal's body weight. The drug used was Red Bull energy drink [**Red Bull GmbH, 5330 Fuschl am See, Austria].** Each 100 ml containing: a mixture of water, sucrose, glucose, sodium citrate, carbon dioxide, taurine (0.4%), caffeine (0.03%), gluconolactone (0.24%), inositol, niacin (8 mg), pantothenic acid (2 mg), vitamin B6 (2 mg), B12 (0.002 mg), caramel, riboflavin, natural and artificial flavoring and coloring agents (these are the labeled ingredients of the product company on the cans).

At the end of the experimental period, the rats were sacrificed by cervical dislocation. The submandibular salivary glands were dissected free and cleaned rapidly of any adherent connective tissue, then:

I- The submandibular salivary glands of one side were fixed immediately in 10% calcium formol for 12 hours, washed by tap water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of 6-7M were obtained and mounted on clean glass slides and stained with Haematoxylin and Eosin stain for light microscopic examination.

II- The submandibular glands of the other side were cut into small fragments of about 1×1 mm^3 using a very sharp blade then fixed in a solution prepared by mixing equal volumes of 3% glutaraldehyde and 0.1 phosphate buffer (pH= 7.4) at 4°C for 3-5 hours. Specimens were rinsed in 0.1% phosphate buffer, post fixed with 1% osmium tetroxide and 0.1 phosphate buffer then rinsed with distilled water. Then the specimens were dehydrated in ascending grades of ethanol, infiltrated with resin and embedded into araldite resin capsules. Semithin sections 0.5 um thick were cut then fixed on glass slides and stained by Toulidine Blue for light microscopic examination to select the best areas to be examined by the electron microscope [12]. Ultra thin sections 60–100 A° thick were cut and stained with Uranyl acetate and Lead citrate then, examined by **(Joel 100 S transmission electron microscope)** at different magnifications and photographed using **C.C.D camera** in the electron microscopic unit at the National Institute of Cancer (Cairo university).

3. Results

Light microscopic results:

Group I (Control group):

The Light microscopic examination of the rat submandibular glands of control group showed mixed acini, striated ducts and granular convoluted tubules in between. The mixed acini were lined by secretory cells having uniform rounded basophilic nuclei. The granular convoluted tubules were lined by columnar cells having central rounded nuclei and characterized by presence of numerous eosinophilic granules. The striated ducts were lined by columnar cells having oval nuclei and characteristic basal striations (Fig. 1).

Group II (Red Bull group):

Histological examination of the submandibular glands of Red Bull group revealed thickening of the connective tissue capsule that contained numerous congested blood vessels. The secretory portions appeared swollen with numerous intracellular vacuoles and some of the granular convoluted ducts were degenerated (Fig. 2). There were numerous extravassated red blood cells in between the acini and ducts. The secretory cells showed hyperchromatic nuclei with different size and shape (atypism & pleomorphism) and numerous mitotic figures (Fig. 3). There were numerous dilated blood vessels engorged with red blood cells. Some of the granular convoluted ducts appeared dilated with decreased granular eosinophilic content (Fig. 4).The excretory ducts appeared dilated with degenerated epithelial lining and retained secretion. Thickening and fibrosis of the connective tissue septa were detected. (Fig. 5 & 6). Numerous thick wall blood vessels engorged with red blood cells were also detected in the connective tissue (Fig. 7).

Electron microscopic results:

Group I (Control group):

Electron microscopic examination of the rat submandibular glands of control group revealed the ultrastructure of the secretory portions (acini) and duct system. Each acinus was formed of group of secretory cells. Each secretory cell had central rounded nucleus, numerous cisternae of rough endoplasmic reticulum packed in the vicinity of the nucleus and numerous mitochondria. There was large number of electron lucent secretory granules (Fig. 8). The lining cells of the granular convoluted tubules showed rounded basal nuclei and numerous membrane bounded electron dense secretory granules (Fig. 9).

Group II (Red Bull group):

Electron microscopic examination of the rat submandibular glands of Red Bull group revealed that, the secretory cells had abnormal divided nucleus, parallel cisternae of rough endoplasmic reticulum and large electron lucent secretory granules (Fig. 10). The lining cells of the granular convoluted tubules showed disfigured basal nucleui, electron dense secretory granules and numerous vacuolization. Some large electron lucent secretory granules were detected (Fig. 11). There were numerous dilated blood vessels engorged with electron dense erythrocytes (Fig. 12).

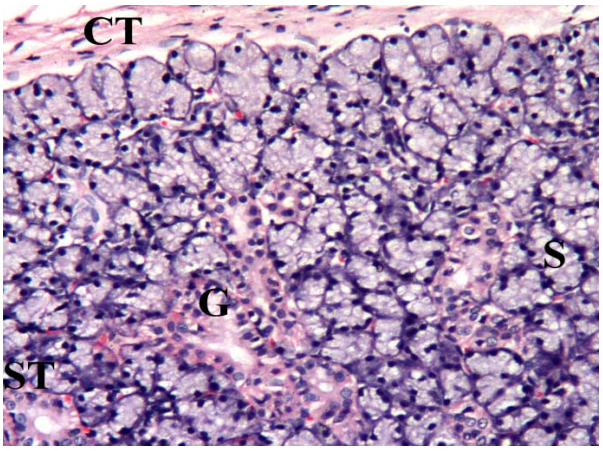

Fig. (1): A photomicrograph of submandibular glands of control group showing mixed secretory portions (S), striated ducts (St) and granular convoluted ducts (G) in between. (H & E Orig.mag. X 200)

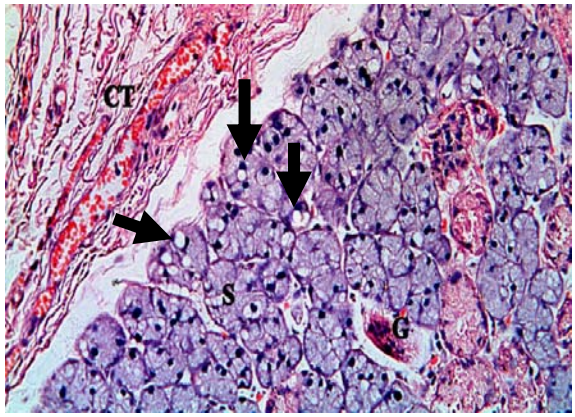

Fig. (2): A photomicrograph of submandibular glands of Red Bull group showing thickening of the connective tissue capsule (CT) with congested blood vessels, swollen secretory portions (s), intracellular vacuoles (arrows), hyperchromatic nuclei with different size and shape (n) and degenerated granular convoluted ducts (G). (H & E Orig.mag. X 200).

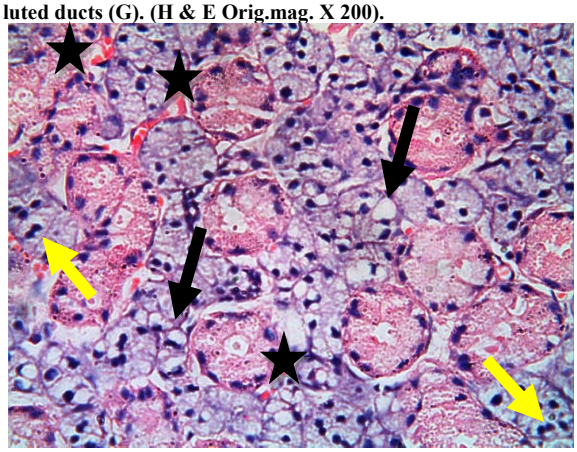

Fig. (3): A photomicrograph of submandibular glands of Red Bull group showing numerous extravassated red blood cells (astrix) , intracytoplasmic vacuoles (black arrows), hyperchromatic nuclei with different shape and size (n) and numerous mitotic figures (yellow arrows) (H & E Orig.mag. X 400).

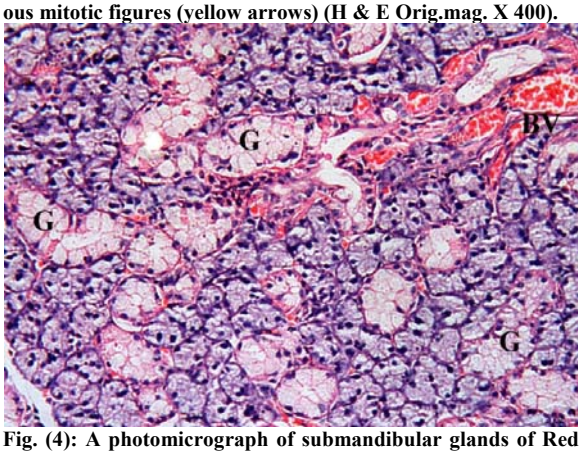

Fig. (4): A photomicrograph of submandibular glands of Red Bull group showing numerous dilated blood vessels engorged with red blood cells (BV) and dilated convoluted ducts with decreased eosinophilic granular content (G). (H & E Orig.mag. X 200).

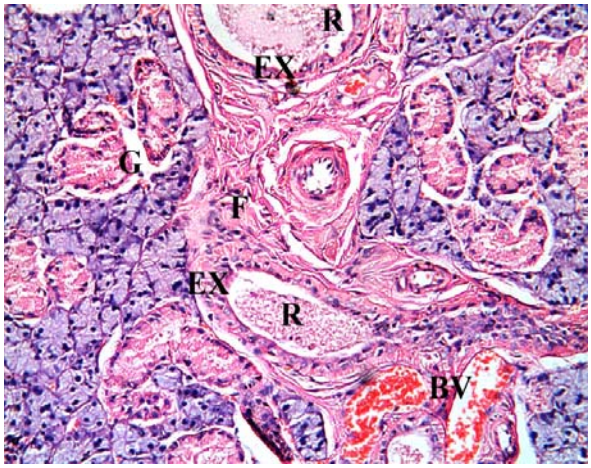

Fig. (5): A photomicrograph of submandibular glands of Red Bull group showing fibrosis (F), numerous dilated excretory ducts (EX) with retained secretion (R) and congested blood vessels (BV) (H & E Orig.mag. X 200).

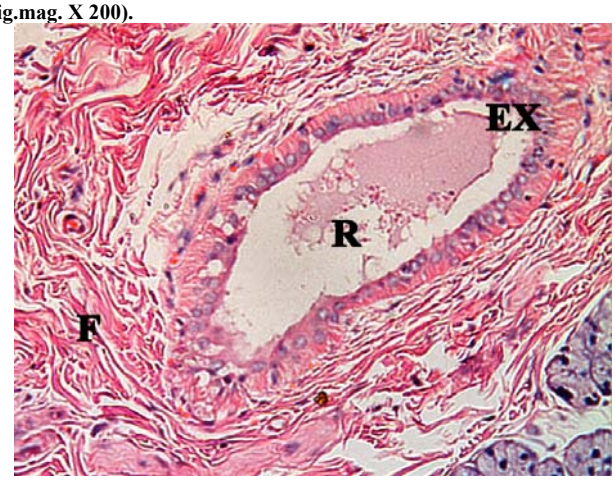

Fig. (6):* A photomicrograph of submandibular glands of Red Bull group showing extensive fibrosis (F) and dilated excretory duct (EX) with degenerated epithelial lining and retained secretion (R). *(H & E Orig.mag. X 400)

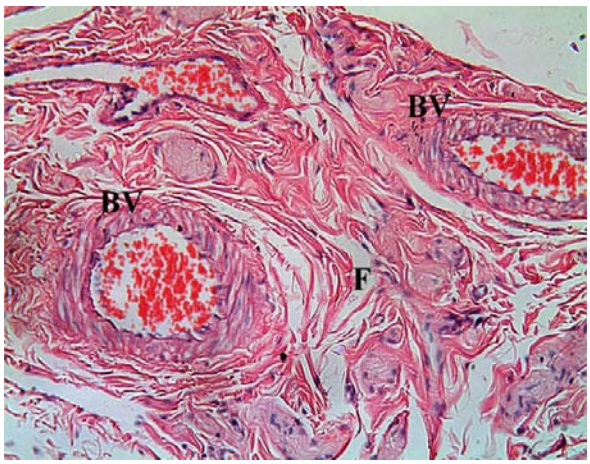

Fig. (7): A photomicrograph of submandibular glands of Red Bull group showing extensive fibrosis (F) and thick wall blood vessels engorged with red blood cells (BV) (H & E Orig.mag. X 200).

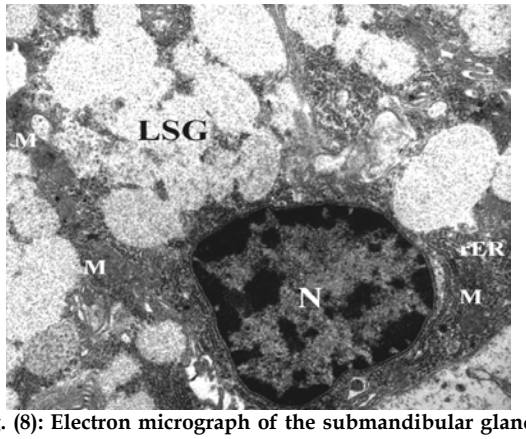

Fig. (8): Electron micrograph of the submandibular glands of control group showing: The ultrastructure of the secretory cells having basal rounded nucleus (N), numerous cisternae of rough endoplasmic reticulum (rER), well formed mitochondria (M) and numerous electron lucent secretory granules (LSG). (Uranyl acetate & Lead citrate x 3000).

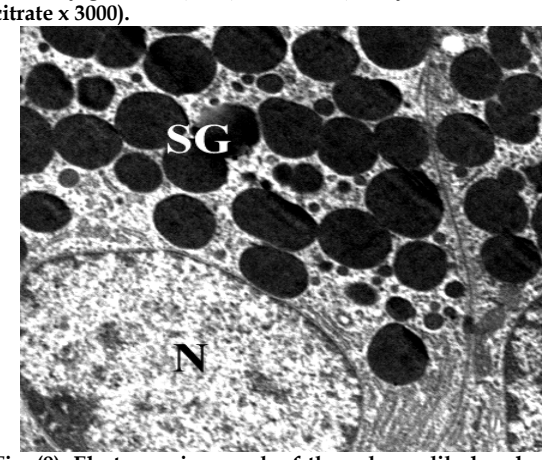

Fig. (9): Electron micrograph of the submandibular glands of control group showing: The ultrastructure of the lining cells of the granular convoluted ducts having basal rounded nucleus (N) and numerous electron dense secretory granules (SG). (Uranyl acetate & Lead citrate x 3000).

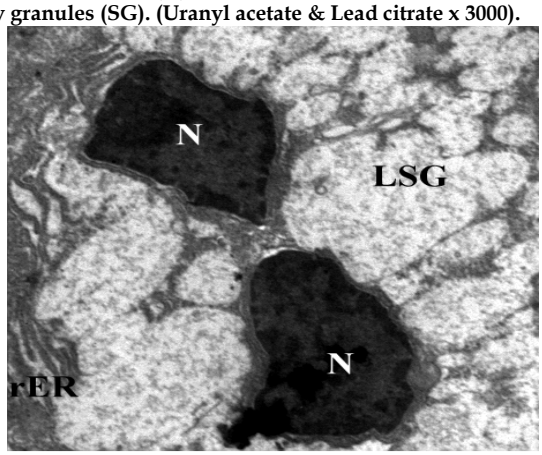

Fig. (10): Electron micrograph of the submandibular glands of Red Bull group showing: The ultrastructure of the secretory cells having abnormal divided nucleus (N), parallel cisternae of rough endoplasmic reticulum (rER) and large electron lucent secretory granules (LSG). (Uranyl acetate & Lead citrate x 3000).

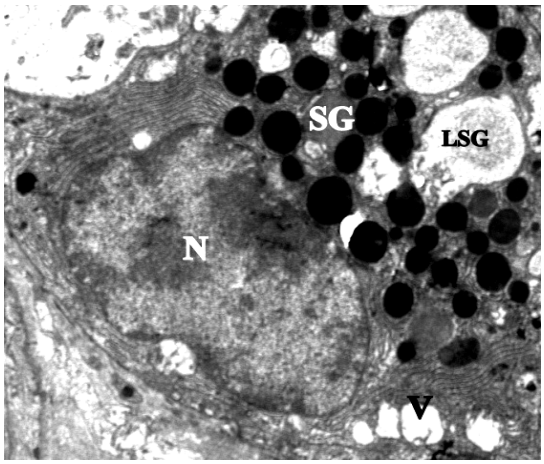

Fig. (11): Electron micrograph of the submandibular glands of Red Bull group showing the ultrastructure of the lining cells of the granular convoluted ducts having disfigured basal nucleus (N), electron dense secretory granules (SG), numerous vacuoles (V) and electronlucent secretory granules (LSG). (Uranyl acetate & Lead citrate x 3000)

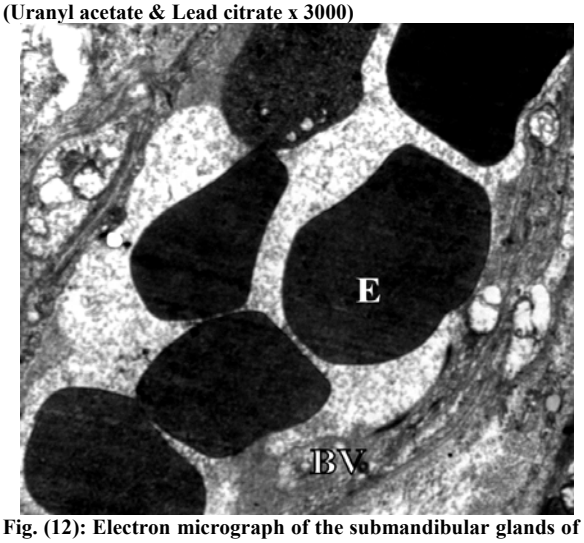

Fig. (12): Electron micrograph of the submandibular glands of Red Bull group showing dilated blood vessel (BV) engorged with electron dense erythrocytes (E) (Uranyl acetate & Lead citrate x 2000).

4. Discussion:

Energy drink consumption has continued to gain popularity all over the world. These drinks are marketed for young people as natural alternatives that increase fun and improve physical and cognitive performance such as concentration, attention, and alertness [(1)]. Adolescents are consuming these types of drinks at an alarming amount and rate. Energy drinks were designed to give the consumer an instant dose of energy provided by combination of different legal stimulants including caffeine, herbal extracts such as guarana, ginseng, B vitamins, amino acids such as taurine, amino acid derivatives such as carnitine, carbohydrates and sugar derivatives, including glucuronalactone and ribose [(13)].

In the present study, ingestion of Red Bull energy drink had adversely affected the histological structure of the rats' submandibular salivary glands. Light microscopic examination revealed swelling of the secretory portions with numerous intracellular vacuolization. This finding might be attributed to degenerative changes within the secretory portions that mostly of fatty nature (fatty degeneration) and aggregation of the degenerative products within the secretory cells leading to their swelling. However, in the routinely processed hematoxylin and eosin sections the lipid droplets were dissolved during fixation and processing of the tissues leaving empty vacuoles.

The nuclei of the secretory cells showed nuclear atypism, pleomorphism and hyperchromatism. Numerous mitotic figures were also detected. All these findings were considered as signs of premalignancy. These premalignant changes might be due to the preservatives added to the energy drinks as **sodium Benzoate.** Sodium benzoate is a type of salt that may occur naturally in some foods but is chemically produced and added as a preservative to energy drinks. There were health concerns about the combination of sodium benzoate and ascorbic acid, another common ingredient in energy drinks. When these two substances were mixed, they could form the chemical **benzene**, which is carcinogenic. Sodium benzoate on its own was not considered a carcinogen, and it would be consumed at a huge amount in order to have toxic levels in the body [(14)]. The premalignant changes might be also attributed to the toxic action of the caffeine content of Red Bull energy drink. Epidemiological studies of caffeine toxicity reported that exposure of women to caffeine during pregnancy is related to the occurrence of congenital malformations, fetal growth retardation, small-for-date babies, miscarriages (spontaneous abortions) and maternal fertility problems. In addition, there were different malformations described in the animal studies at very high doses of caffeine and classified as vascular disruptive types of malformations [(15)].

Energy drink ingestion adversely affected the duct system of the submandibular salivary glands. The granular convoluted ducts appeared dilated and their apical granular eosinophilic content was reduced. This finding was detected previously in the submandibular glands of advancing aged mice. It was consistent with functional decline [(16)]. The excretory ducts showed dilatation with retaining of secretion in their lumen. This finding might be attributed to accumulation of the salivary secretion and failure of exocytosis due glandular injury and dysfunction.

Thickening of the connective tissue capsule and extensive fibrosis of the connective tissue septa were detected. Excessive fibrosis might be due to toxic

effect of caffeine as the effect of caffeine on wound healing of rat gingiva revealed increased depositions of fibrin on the underlying connective tissue [17]. In addition, investigation of the effect of caffeine on the growth of mandible and long bone revealed that, caffeine consumption had increased collagen synthesis of the mandible [18]. Generally, fatty degeneration of the secretory portions and fibrosis of the connective tissue stroma were considered as characteristic findings of aging [19]. Light and electron microscopic results revealed numerous thick wall blood vessels engorged with red blood cells. In addition, extravassated red blood cells were detected in between the acini and ducts as the thick wall vessels were fragile and easy to produce hemorrhage within the glands. The dilatation and congestion of the blood vessels might be also attributed to microcirculatory disturbances that developed due to the caffeine content of the energy drinks that play an important role in glandular degeneration. Energy drink consumption had been linked to cardiovascular disease [9].

Taurine is one of the active ingredients of energy drinks. Taurine (2-aminoethanesulfonic acid) is a non protein amino acid containing a sulfonic acid group. It is found in many tissues of man and animals. Taurine conjugates with bile acids, and aids digestion of lipids. Besides this well-known function, taurine has several important regulatory actions. These include detoxification, membrane stabilization, osmoregulation and modulation of cellular calcium levels [20]. Taurine has protective properties when administered therapeutically. For example, supplementation studies have documented antioxidative [21], hepatoprotective [22], antihypertensive [23] and antidiabetic [24] properties of taurine. In addition, antioxidant medications including taurine have been reported to possess antifibrotic efficacy in experimental liver fibrosis [25]. In spite of the reported benefits of taurine, Red Bull energy drinks produced degeneration, fibrosis, thickening and congestion of blood vessels and premalignant changes in the secretory cells of the submandibular salivary glands. This might be due to different reaction of taurine associated with other active ingredients of the energy drinks as caffeine. An otherwise healthy 28-year-old man had a cardiac arrest after a day of motocross racing. He had consumed excessive amounts of a caffeinated "energy drink" throughout the day. It was postulate that a combination of excessive ingestion of caffeine- and taurine-containing energy drinks and strenuous physical activity can produce myocardial ischaemia by inducing coronary vasospasm [26].

It was reported that, the amounts of guarana, taurine, and ginseng found in popular energy drinks were far below the amounts expected to deliver either therapeutic benefits or adverse events. However, caffeine and sugar are present in amounts known to cause a variety of adverse health effects [27].

In conclusion, Red Bull ingestion has revealed different histological changes in the rat submandibular salivary glands. It produced fibrosis, degeneration and dysfunction of the salivary glands. Premalignant changes in some secretory cells and abnormal thickening of the blood vessels were detected. Granular convoluted tubules as well as excretory ducts were adversely affected. As Red Bull energy drink produced all these unexpected histological changes in the salivary glands and ingestion of energy drinks continued to gain popularity, we recommended more researches on different types of energy drinks and their possible adverse histological effects on different tissues.

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References:

1- Kaminer Y. Problematic use of energy drinks by adolescents. Child Adolesc Psychiatr Clin N Am., 2010; 19(3):643-50.
2- Frances RR, Tyler DG, Narjes B, Nicole H, April M, *et al.* Effect of Red Bull energy drink on cardiovascular and renal function. Amino Acids. 2010; 38:1193–1200
3- Babu KM, Church RJ, Lewander WL. Energy drinks: the new eye-opener for adolescents. Clin Ped Emerg Med., 2008; 9:35– 42.
4- Aranda M, Morlock G. Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry. J Chromatogr A., 2006; 1131:253–260.
5- Sionaldo EF, Isabel MH, A´ gatha A, Shirley T, Renata GK, *et al.*, Can energy drinks reduce the depressor effect of ethanol? An experimental study in mice Physiology & Behavior., 2004; 82: 841–847
6- Lutmer B, Zurfluh C, Long C. Potential effect of alcohol content in energy drinks on breath alcohol testing. J Anal Toxicol., 2009; 33:167–169.
7- Seidl R, Peyrl A, Nicham R, Hauser E. A taurine and caffeine containing drink stimulates cognitive performance and well-being. Amino Acids. 2000; 19 (3–4): 635– 42.

8- Alford C, Cox H, Wescott R. The effects of red bull energy drink on human performance and mood. Amino Acids, 2001; 21(2):139– 50.
9- Baum M, Weiss M. The influence of taurine containing drink on cardiac parameters before and after exercise measured by echocardiography. Amino Acids. . 2001; 20:75–82.
10- Roberts C, Robinson SP. Alcohol concentration and carbonation of drinks: the effect on blood alcohol levels. J Forensic Leg Med., 2007; 14:398–405.
11- Chad JR, Eric CS, Roland RG. Caffeinated Energy Drinks – A Growing Problem. Drug Alcohol Depend., 2009; 99(1-3): 1–10.
12-Hayat MA. Principles and techniques of electron microscopy.Van Nastrand Reinhold, Princeton London, 1970; V: 1&2.
13- Boyle M, Castillo VD. Monster on the loose. Fortune, 2006; 154:116-122.
14- Plant M. Should You Be Drinking Energy Drinks? November 17th, 2008. http://plantmiracle.com.
15- Christian MS, Brent RL. Teratogen update: evaluation of the reproductive and developmental risks of caffeine. Teratology, 2001; 64(1): 51-78.
16- Gresik EW. Changes with senescence in the fine structure of the granular convoluted tubule of submandibular gland of the mouse. Am J Anat., 1989; 184(2): 147-156.
17- Takesue M. Effect of caffeine or NaCl on wound healing of the rat gingiva. Light and electron microscopic studies. Aichi Gakuin Daigaku Shigakkai Shi., 1989; 27(1):277-316.
18- Nakamoto T, Shaye. Effects of caffeine on the growth of mandible and long bone in protein-energy malnourished newborn rats. Proc Soc Exp Biol Med., 1984; 177(1):55-61.
19- Scott J. Quantitative age changes in histological structure of human submabdibular salivary glands. Arch Oral Biol., 1977b; 22: 221-227.
20- Huxtable RJ. Physiological actions of taurine. Physiol Rev., 1992; 72:101–163.
21- Green TR, Fellman JH, Eicher AL, Pratt KL. Antioxidant role and sub cellular location of hypotaurine and taurine in human neutrophils. Biochim Biophys Acta, 1995; 1073: 91–97.
22- Dogru-Abbasoglu S, Kanbagli O, Balkan J, Cevikbas U, Aykac-Toker G, Uysal M: The protective effect of taurin against thioacetamide hepatotoxicity of rats. Hum Exp Toxicol., 2001; 20: 23–27.
23- Dawson R , Liu S, Jung B, Messina S, Eppler B. Effects of high salt diets and taurine on the development of hypertension in the stroke-prone spontaneously hypertensive rats. Amino Acids, 2000; 19: 643–655.
24- Trachtman H, Futterweit S, Maesaka J, Ma C, Valderrama E *et al.* Taurine ameliorates chronic streptozotocin-induced diabetic neuropathy in rats. Am J Physiol., 1995; 269: 429–438.
25- Ilker T, Nuket M, Mehmet M, Murvet T, Bilgin C. Ultrastructural changes in hepatocytes after taurine treatment in CCl4 induced liver injury. World J Gastroenterol., 2008; 14(31): 4897-4902.
26- Berger AJ, Alford K. Cardiac arrest in a young man following excess consumption of caffeinated "energy drinks". Med J Aust., 2009; 190(1):41-3.
27- Clauson KA, Shields KM, McQueen CE, Persad N. Safety issues associated with commercially available energy drinks. J Am Pharm Assoc., 2008; 48(3):55-63.

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