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# Toxicological Study of an Alcohol and Non-Alcohol Energy Drink on Wistar Rat



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## ABSTRACT

Energy drinks are widely consumed and they are not without adverse health effects. The aim of this study is to investigate the subacute (28 days) oral toxicity of two energy drinks commonly consumed in Togo, one with alcohol and another one without alcohol. Twenty-four (24) male Wistar rats were set up in four groups, the control group received distilled water, group 2, group 3 and group 4 received respectively 3.6 ml/kg of non-alcohol energy drink, 3.6 ml/kg alcohol energy drink and 10 ml/kg alcohol energy drink. The effect on the stomach and on the accumulation of intra-abdominal fat was assessed. Both drinks have lack of information on their outer packaging. The drinks have increased the relative weight of the testis. Non-alcohol energy drink has significantly increased the relative weight of the liver at a dose of 3.6 ml/kg and alcohol energy drink at a dose of 10 ml/kg ( $p < 0.05$ ). The alcohol energy drink increased significantly ( $p < 0.001$ ) the number of platelets and increased significantly the number of gastric lesions in a dose of 10 ml/kg with the alcohol energy drink.



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## INTRODUCTION

Energy drinks are beverages with stimulating action on the physical or intellectual, and contain a mixture of different compounds usually caffeine, taurine, glucuronolactone, vitamins of B group, the sugars or sweeteners but also sometimes plant extracts such as *Paullinia cupana* (guarana) and *Panax ginseng* (ginseng)<sup>1</sup>.

There are two types of energy drinks namely alcoholic and non-alcohol energy drink. Caffeine, found in coffee and tea or energy drinks, is one of the most commonly consumed worldwide alkaloids<sup>2</sup>. The harmful actions of caffeine on the cardiovascular, hematological and gastrointestinal systems as well as an abnormal stimulation of the nervous system<sup>3, 4</sup>. Indeed, several studies have shown that energy drinks have toxic effects on the heart, kidneys and metabolism (obesity, diabetes type 2)<sup>5-8</sup>.

However, energy drinks are increasingly consumed worldwide especially by teenagers<sup>9</sup>. In the United States, Italy and Argentina, respectively 81%, 57% and 65% of university students have used it<sup>10, 11, 12</sup>. The consumption of energy drinks in Africa is in increase. In Ghana, a study revealed that 62% of students have consumed at least once energy drinks with 80% who consume one to two energy drinks a week and 21%, three to four per week<sup>13</sup>.

In Togo, energy drinks are highly consumed by the majority of teens and have not been studied to assess its toxicity. The objective of this study is to evaluate the (28 days) oral subacute toxicity of non-alcohol energy drink and alcohol energy drink rats.

## MATERIALS AND METHODS

### Framework study

The study was carried out toxicology department of the Faculty of Health Sciences and the Department of Animal Physiology, Faculty of Science, University of Lomé.

### Chemicals substances

The alcohol energy drink (batch number: 5G81BJ17C) and non-alcohol energy drink (batch number: 01-6209) were used. A global market leader non-alcohol energy drink has been chosen for the study<sup>14</sup>. The non-alcohol energy drink, used has a high alcohol content (18%).

Drinks were given to rats as is. Ethical approval was obtained from Institutional Ethical Committee for Teaching and Research under the number (ref no. CNCB- CEER 2801/2019).

### **Animal material**

Male Wistar rats ( $156.63 \pm 4.45$ ) were provided by the department of animal physiology at the University of Lomé (Togo). All these animals were housed in a normal environmental condition and fed standard diets of rodents and water.

### **Subacute toxicity**

The oral toxicity of repeated dose study was conducted in accordance with Directive 407 OECD (1998)<sup>15</sup>. Four groups of 6 male rats were formed. The Control (received distilled water for 28 days), the group D1 (received energy drink at 3.6 ml/kg for 28 days), the group D2 (received alcohol energy drink at 3.6 ml/kg for 28 days) and the group D3 (received alcohol energy drink at 10 ml/kg for 28 days). The dose of 3.6 ml/kg is taken base on the fact an adult subject weighing 70 kg drink 250 ml per day and the dose of 10 ml/kg is the maximum dose of energy drink that rats can take without modification of their homeostasis. During the period of the experiment, the rats were observed macroscopically for potential adverse effects. Thus mortality, behavioral and physiological parameters such as aggression, agitation, feed intake, diarrhea, mobility and weight were measured daily. At day 29, after an overnight fast, the rats were anesthesia and a blood sample was taken from the retro-orbital sinus according to the experimental protocol used by Weiss *et al.*,<sup>16</sup>. The collected blood was collected with capillary tubes in tubes containing EDTA and dry tubes. The blood was used for biochemical and haematological examinations. For biochemical tests, urea, creatinine, transaminases (AST, ALT), gamma GT, alkaline phosphatase (ALP), triglycerides, total cholesterol, glucose and electrolytes (Na + and K +) have assayed. Regarding hematological parameters, blood counts were performed. Hemoglobin (HBG), the number of red blood cells (RBC), white blood cells (WBC), platelets (PLT), the hematocrit (HCT), mean corpuscular volume (MCV), the mean corpuscular concentration hemoglobin (MCHC), the mean corpuscular hemoglobin (MCH) was determined. The rats were anesthetized again with ether and then sacrificed. The heart, liver, spleen, kidneys and testes were removed, weighed and the relative weight of each isolated organ was calculated using the formula:  $PR = (\text{body/weight rat weight}) \times 100$ .

### Effects on the stomach

After the sacrifice of the rats, the stomachs were opened along the greater curvature. They were then washed with distilled water and observed using a magnifying glass. The lesions observed were counted.

### Effects on the accumulation of intra-abdominal fat

Intra-abdominal fat rats was also removed and weighed. Intra-abdominal ratio: fat/body weight was calculated.

### Histological sections

After fixation by formalin 10%, organs such as the heart, kidneys, spleen, testes and liver were dehydrated by successive baths of increasing concentrations of alcohol. These organs were then cleared in toluene and packed in paraffin. They were then cut into thin (5 mm) pieces using a microtome. The sections were stained with hematoxylin-eosin (HE) and examined microscopically. Photos of histological sections were taken.

### Statistical analysis

The software Graph Pad Prism 6.02 (Graph Pad Software, Inc. USA) was used to analyze our results. These were expressed as mean value accompanied by the standard error of the mean ( $m \pm SEM$ ). The analysis of variance (ANOVA) was used to compare different groups each other. The difference between two groups was determined using the Tukey test. The level of significance is set at  $p < 0.05$ .

## RESULTS

The evaluation of the behavior of the animals showed aggressiveness and a major agitation in rats which took alcohol energy drink and non-alcohol energy drink (Table No. 1). No mortality was recorded during the experimental period.

Drinks administration resulted in no significant change in rats body weight. No significant changes were observed in rat kidney, spleen and heart when compared with control. But there was a significant increase in testis relative weight with alcohol energy drink. Non-alcohol energy drink increased significantly ( $p < 0.01$ ) the relative weight of the liver at a dose of 3.6 ml/kg and alcohol energy drink increased it at a dose of 10 ml/kg ( $p < 0.05$ ) (Table No. 2).

In Table No. 3, alcohol energy drink increased significantly ( $p < 0.001$ ) the number of platelets but has no significant effect on other haematological parameters studied.

The analysis of biochemical parameters and the relative weight of intra-abdominal fat reported no significant change. About the evaluation of effects on the stomach of rats, there was a significant ( $p < 0.01$ ) increase of the number of gastric lesions in a dose of 10 ml/kg with the alcohol energy drink (Figure No. 1).

Energy drinks induced no abnormality in the histological section of the heart, kidneys, spleen, testis and liver.

## DISCUSSION

In our study, we have evaluated the sub-chronic study of alcohol energy drink and energy drinks non-alcohol energy drink. Energy drinks resulted in behavioral modification marked by an aggressive and significant agitation of the rats. These results could be explained by the high content of caffeine in our drinks (320 mg/l in non-alcohol energy drink). According to Smith *et al.*, In 2002<sup>17</sup> and Childs and Wit., in 2008<sup>18</sup>, caffeine induces psychological and behavioral disturbances including nervousness, irritability, anxiety or panic attacks or psychotic phenomena (including hallucinations). By its structural analogy with adenosine, caffeine binds to membrane receptors A1 adenosine in the brain and induces the release of catecholamines: noradrenaline and adrenaline<sup>19</sup> especially dopamine. The latter increases aggression, sexual activity and initiative. Our results are important from made these do not even respect BE the legislation of the European Union (EU) which imposes a reference to "high caffeine content" on beverages containing more than 150 mg of caffeine per liter<sup>20</sup> and that would prevent their uncontrolled consumption<sup>21</sup>. But, Eduardo S. *et al.*, 2017<sup>22</sup> have reported that during the 120 days of treatment, animals treated with the energy drink showed no behavioral changes.

Moreover, no significant change in body weight of the treated rats was observed. The same results were reported in Saudi Arabia by Raeesa AM *et al.*, 2018<sup>23</sup> and Brazil Eduardo S. *et al.*, 2017<sup>22</sup>. Hassan *et al.*, 2012<sup>24</sup> obtained a significant decrease ( $p < 0.01$ ) in body weight of rats. This difference could be explained by the dose, duration of study and rat species used. Indeed, Raeesa AM *et al.*, (2018) and Eduardo S. *et al.*, (2017) have also used male Wistar rats. Hassan *et al.*, (2012) used Sprague-Dawley rats, which were more sensitive than Wistar rats<sup>25, 26</sup>, at a higher dose and over a long period (6 weeks against 4 in our study).

The assessment of the weight of organs such as the liver, kidney, spleen, testes, heart, pancreas, brain and language is very important in toxicological studies. The relative weights of organs provide information about possible hypertrophy, atrophy or swelling of these bodies <sup>27</sup>. Thus, the heart, kidneys and spleen have shown no significant differences regarding their relative weight. These results confirm those obtained by Eduardo S. *et al.*, 2017 <sup>22</sup>. Our results have shown a significant increase ( $p < 0.01$ ) in testicular weight with both energy drinks, reflecting testicular hypertrophy which could be attributed by the presence of taurine in energy drinks. In 1997, Xiao *et al.* <sup>28</sup> reported the promoting effect of taurine in the development of the testes. This increase in relative testis weight, with the non-alcohol energy drink, was not confirmed by histological. Nadia *et al.*, 2019 <sup>29</sup> observed a discrete muscle fiber necrosis rabbit's heart. While the analysis of biochemical parameters and histological section of liver reported no change or significant morphological changes, however, there is a significant increase ( $p < 0.05$  and  $0.01$ ) of the relative liver weight at 10 ml/kg with alcohol energy drink and 3.6 ml/kg with the non-alcohol energy drink. Hassan *et al.*, 2012 also reported a significant increase in relative liver weight at different doses used <sup>24</sup>.

Alcohol energy drink increased significantly ( $p < 0.0001$ ) the platelet number. Indeed, achieving blood counts is very important in toxicological studies. The hematopoietic system is one of main targets of toxic substances. This observed thrombocytosis can be attributed to increased production and secretion of thrombopoietin, the major regulator of platelet production <sup>30</sup>. Platelets elevated in the blood increase the risk of thrombosis (blood clot) because of platelet aggregates that form in arteries and veins. Thus, consumption of alcohol energy drink could be a risk factor for venous thrombosis and / or arterial. In addition, the dose of 10 ml/kg of alcohol energy drink led to a significant increase ( $p < 0.05$ ) of the number of gastric lesions. These lesions of the stomach, observed in this study, could be attributed to the caffeine<sup>31</sup> and taurine<sup>32</sup> content in alcohol energy drink and its high alcohol content (18%). Caffeine stimulates the secretion of hydrochloric acid<sup>33</sup>. It stimulates also the release of gastrin and hydrochloric acid and extends the relaxation of the stomach might slow gastric emptying <sup>34</sup>. The slowing of gastric emptying may prolong the contact time of energy drinks with the lining of the stomach. Moreover, Nawrot *et al.* in 2003 <sup>35</sup> reported that the inhibitory effect of caffeine on gastric mucus secretion may be one of the important factors of gastric mucosal lesion.

## CONCLUSION

Our study has shown that the alcohol energy drink and non-alcohol energy drink, in our country, do not respect European regulations on energy drinks. Energy drinks has increased significantly the relative weight of the liver and increased the number of gastric lesions.

**LINKS OF INTEREST** The authors said they had no interest link.

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**Table No. 1: Effect of non-alcohol energy drink and alcohol energy drink on the rat's behavior**

Parameters	Control	Group 1 (3.6 ml/kg)	Group 2 (3.6 ml/kg)	Group 3 (10 ml/kg)
Death	-	-	-	-
Diarrhea	-	-	-	-
Vomiting	-	-	-	-
Aggressiveness	-	++	+	++
agitation	-	++	+	++
Mobility	+	++	+	++
Food intake	+	++	+	++

+ (Observed effect)    ++ (intense observed effect)    - (unobserved effect)

Group 1 = non-alcohol energy drink at 3.6 ml/kg; Group 2 = alcohol energy drink at 3.6 ml/kg;  
Group 3 = alcohol energy drink at 10 ml/kg.

**Table No. 2: Effect of non-alcohol energy drink and alcohol energy drink on the relative weight of rat's organs**

Parameters	Control	Group 1 (3.6 ml/kg)	Group 2 (3.6 ml/kg)	Group 3 (10 ml/kg)
Heart	0.44 ± 0.04	0.46 ± 0.05	0.44 ± 0.06	0.38 ± 0.02
Kidney	0.63 ± 0.04	0.64 ± 0.06	0.63 ± 0.06	0.56 ± 0.02
Rate	0.23 ± 0.03	0.22 ± 0.03	0.20 ± 0.03	0.22 ± 0.04
Testis	0.82 ± 0.16	<b>1.26 ± 0.14*</b>	<b>1.32 ± 0.14**</b>	<b>1.33 ± 0.02**</b>
Liver	2.85 ± 0.06	<b>3.45 ± 0.28**</b>	2.83 ± 0.13	<b>3.25 ± 0.08*</b>

Each value represents the mean ± ESM, (n = 6) is the number of animals per group. \* P < 0.05 significant difference as compared to the control, \*\* P < 0.01 significant difference as compared to the control.

Group 1 = non-alcohol energy drink at 3.6 ml/kg; Group 2 = alcohol energy drink at 3.6 ml/kg;  
Group 3 = alcohol energy drink at 10 ml/kg.

**Table No. 3: Effect of non-alcohol energy drink and alcohol energy drink on biochemical parameters in rats**

Parameters	Control	Group 1 (3.6 ml/kg)	Group 2 (3.6 ml/kg)	Group 3 (10 ml/kg)
WBC ( $10^3$ /UL)	9.22 $\pm$ 0.78	9.52 $\pm$ 0.61	9.65 $\pm$ 0.84	10.10 $\pm$ 0.39
HBG (g/dL)	12.83 $\pm$ 0.49	13.33 $\pm$ 0.17	13.20 $\pm$ 0.15	13.30 $\pm$ 0.12
RBC ( $10^6$ /UL)	7.95 $\pm$ 0.29	8.06 $\pm$ 0.07	8.01 $\pm$ 0.13	8.11 $\pm$ 0.11
HCT (%)	39.90 $\pm$ 1.21	41.20 $\pm$ 0.68	40.50 $\pm$ 0.36	40.68 $\pm$ 0.36
MCV (fL)	50.32 $\pm$ 0.70	51.18 $\pm$ 0.56	50.63 $\pm$ 0.48	50.25 $\pm$ 0.51
MCHC (Pg)	16.10 $\pm$ 0.17	16.48 $\pm$ 0.14	16.42 $\pm$ 0.22	16.37 $\pm$ 0.21
MCH (g/dL)	32.07 $\pm$ 0.34	32.32 $\pm$ 0.22	32.55 $\pm$ 0.24	32.62 $\pm$ 0.17
Platelet ( $10^3$ /UL)	693.50 $\pm$ 68.80	728.80 $\pm$ 22.23	<b>819.50<math>\pm</math> 63.60****</b>	<b>776.75 <math>\pm</math> 26.82***</b>

Each value represents the mean  $\pm$  ESM, (n = 6) is the number of animals per group. \*\*\*\* P <0.0001 significant difference as compared to control, \*\*\* P <0.001 significant difference as compared to control.

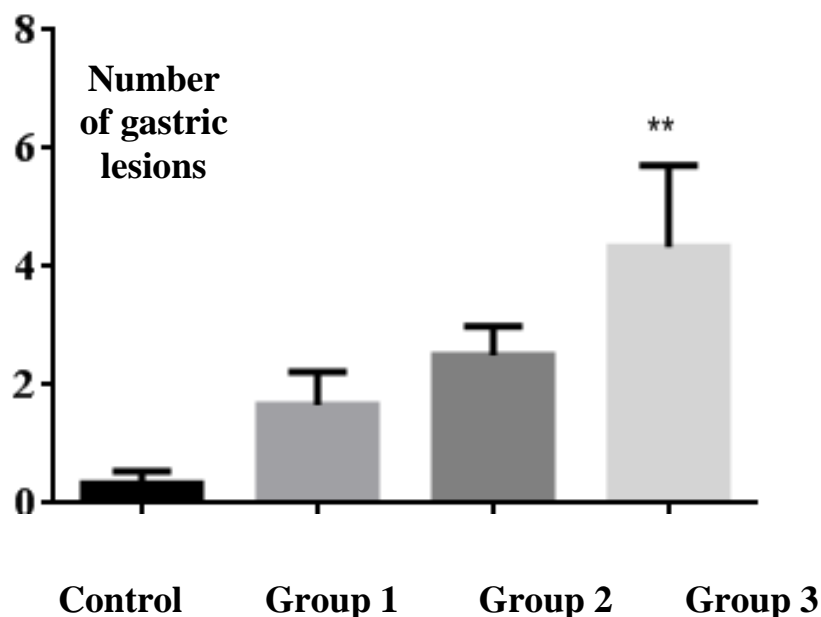
Group 1 = non-alcohol energy drink at 3.6 ml/kg; Group 2 = alcohol energy drink at 3.6 ml/kg; Group 3 = alcohol energy drink at 10 ml/kg.

**Table No. 4: Effect of non-alcohol energy drink and alcohol energy drink on biochemical parameters in rats**

Parameters	Control	Group 1 (3.6 ml/kg)	Group 2 (3.6 ml/kg)	Group 3 (10 ml/kg)
Urea (g/L)	0.43 ± 0.05	0.50 ± 0.09	0.49 ± 0.03	0.50 ± 0.08
Creatinine (mg/L)	8.50 ± 0.62	8.00 ± 0.52	8.00 ± 0.52	8.50 ± 0.56
ASAT (UI/L)	149.20 ± 25.01	165.67 ± 31.05	153.67 ± 20.66	173.83 ± 27.34
ALAT (UI/L)	46.20 ± 7.81	50.67 ± 6.61	49.50 ± 4.33	62.33 ± 9.37
γGT (UI/L)	3.00 ± 0.45	2.50 ± 0.22	3.33 ± 0.21	3.33 ± 0.49
ALP (UI/L)	301.33 ± 48.76	291.00 ± 61.22	351.33 ± 66.10	291.60 ± 59.05
Tryglycerides (g/L)	2.99 ± 0.26	3.08 ± 0.21	2.63 ± 0.28	3.56 ± 0.45
Total cholesterol (g/L)	4.67 ± 0.66	4.14 ± 0.44	4.57 ± 0.29	4.81 ± 0.34
Glucose (mg/dL)	5.84 ± 0.49	4.15 ± 0.43	5.03 ± 0.20	4.12 ± 0.47
Na <sup>+</sup> (mmol/L)	154.26 ± 4.99	141.74 ± 1.73	145.78 ± 4.52	145.57 ± 6.37
K <sup>+</sup> (mmol/L)	476.32 ± 100.19	510.82 ± 52.17	441.52 ± 60.32	414.62 ± 71.75

Each value represents the mean ± SEM, n (the number of animals per group) = 6.

Group 1 = non alcohol energy drink at 3.6 ml/kg; Group 2 = alcohol energy drink at 3.6 ml/kg;  
Group 3 = alcohol energy drink at 10 ml/kg.



**Figure No. 1: Effect of non-alcohol energy drink and alcohol energy drink on the stomachs of rats**

The stomachs were opened along the great curvature. They were then washed with distilled water and observed with a magnifying glass. The lesions observed were counted. Each value represents the mean  $\pm$  ESM. (n = 6 is the number of animals per group). \*\* p < 0.01 significant difference as compared to control.

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