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EFFECTS OF SOME ENERGY DRINKS AND ALCOHOL ON ANXIETY-LIKE BEHAVIOURS IN MICE SUBJECTED TO OPEN FIELD AND ELEVATED PLUS MAZE TESTS

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Abstract

Energy drinks (ED) are beverages containing caffeine, taurine, vitamins, herbal supplements, and sugar or sweeteners. Alcohol has a wide spectrum of action within CNS (central nervous system) affecting neurotransmitter systems like adenosine, dopamine, GABA (gamma-amino butyric acid), glutamate and serotonin. Anxiety is an unpleasant state of inner turmoil, often accompanied by nervous behaviour, such as pacing back and forth, somatic complaints and rumination. Health care providers reported seing dehydration, accelerated heart rates, anxiety, seizures, acute mania, and strokes from the consumption of ED. Scientific research has not universally supported the idea that ED can reduce the effects of ethanol in humans or rodents. This study was designed to determine the effect of some ED (red bull and power horse) and alcohol on anxiety-like behaviours in mice using open field and elevated plus maze anxiety neuro-behavioural paradigms. It also evaluates corticosterone hormone of blood of

mice studied. Seventy (70) mice of both sexes were used. For each model, mice were randomly divided into six groups of five mice each. Group 1 (distilled water 10 ml/kg) served as control, Group II and III received ED only (3.75 ml/Kg orally) and (7.5 ml/Kg orally) respectively. Group IV and V received ED plus alcohol (3.75 ml/Kg + 1.0 g/Kg orally) and (7.5 ml/Kg + 2.0 g/Kg orally) respectively. Group VI received alcohol (2.0 g/kg). ED together with alcohol (7.5 ml/Kg + 2.0 g/Kg) were anxiogenic in open field, but not in elevated plus maze. Alcohol (2.0 g/kg) alone has no significant effect on anxiety. ED alone or together with alcohol has no effect on corticosterone level in the blood. The findings of this study suggest that ED together with alcohol may leads to more anxiety instead of counteracting the effects of alcohol. Hence, ED should not be consumed together with alcohol.

Keywords: *Alcohol, anxiety, corticosterone, energy drink*

INTRODUCTION

Energy drinks (ED) are beverages that contain caffeine, taurine, vitamins, herbal supplements, and sugar or sweeteners and are marketed to improve energy, weight loss, stamina, athletic performance, and concentration (Oddy and O'Sullivan, 2009). Half of the ED market is patronized by children (<12 years old), adolescents (12–18 years old), young adults (19–25 years old) (Seifert *et al.,* 2011). By 2006, there were over 500 brandsof ED worldwide (Dombovy-Johnson, 2012). Often, cups of ED are mixed with highly alcoholic drinks like Vodka and Whiskeys as enhancers. There are over 31 ED sold in the open markets in Nigeria (Emmanuel, 2012). In recent times, consumption of ED with alcohol is perceived as a way of ameliorating the toxic effects of alcohol. It has been reported that 20-40% of young people consume ED with alcohol while partying (Ugwuja, 2014).

Caffeine and alcohol are two of the oldest commonly consumed psychoactive compounds, and caffeinated drinks have been mixed with alcohol for many years (Alford *et al.,* 2012). Alcohol has a wide spectrum of action within the CNS (central nervous system) affecting many neurotransmitter systems including major neurotransmitters adenosine, dopamine, GABA (gamma-amino butyric acid), glutamate and serotonin (Nympha *et al.,* 2010).Popular brands of ED contain around 80 mg of caffeine per 250 ml which places them above colas but on a similar level to coffee, although some minority brands can contain higher doses as can other preparations of coffee (Reissig *et al.,* 2009).Taurine, 2-amino-ethanesulfonic acid is one of the most abundant amino acids in mammals. Taurine has been shown to elicit neuronal hyper polarization presumably through its action by opening the chloride channels in the cerebellum and in the hippocampus (Wu and Prentice, 2010).

Anxiety is an unpleasant state of inner turmoil, often accompanied by nervous behavior, such as pacing back and forth, somatic complaints and rumination (Seligman *et al.,* 2001*)*. Anxiety occurs in situations only perceived as uncontrollable or unavoidable, but not realistically so (Ohman, 2000). On the physiological level these signals initiate activation of the hypothalamus-pituitary-adrenal (HPA) axis. This leads to increased heart rate and breathing

(Schubert *et al*., 2009).Anxiety disorders are the most common of all mental health problems. It is estimated that they affect approximately 1 in 10 people (Shiri *et al*., 2012). They can exist in isolation but more commonly occur with other anxiety and depressive disorders (Cape *et al*., 2011). Anxiety disorders, including panic disorder, obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), social phobia, specific phobias, and generalized anxiety disorder (GAD), were the most common mental disorders within the EU states in 2010 with 14% prevalence (Wittchen *et al*., 2010).

Health care providers report that they have seen the following effects from the consumption of ED: dehydration, accelerated heart rates, anxiety, seizures, acute mania and strokes (Nicole *et al*., 2010). While ED are often consumed alone, young people have become enamoured with using ED as mixers for alcohol. Recently, this practice has become common and has also come under intense scrutiny (Marczinski *et al*., 2012). Alcohol abuse causes injuries of multiple organs and tissues affecting the brain, lungs, liver, cardiovascular system, immune system (Musa *et al*., 2012). Although the effect of caffeine on cognitive performance, physical endurance, and short and long-term memory has been studied extensively, research on the combination of ingredients contained in functional ED is lacking (Sophia, 2010). Therefore, this study intends to determine the effects of some EDs and alcohol on anxiety-like behaviors in mice within short period of time.The aim of the study is to determine the effect of some EDs alone and together with alcohol on anxiety-like behaviors in mice.

MATERIALS AND METHODS

Energy Drink

Two cans (250ml) each of 'Power horse' and 'red bull' (manufactured by EDs were purchased from a store in Sabon Gari, Zaria. Red bull was used for open field test, while power horse was used for elevated plus maze test.

Alcohol

One litre of ethanol (99% purity) was purchased from Steeve Moore scientific store, Kwangila, Zaria. It was diluted with distilled water to produce 33% alcohol, 3:1 dilution factor (Allam *et al*., 2013).

Animals

Seventy mice (mixed sexes, weight range 18g to 26g and age range of 4-6 weeks), purchased from the animal house of the Faculty of Pharmaceutical Sciences A.B.U. Zaria were used in the study. The animals used were kept under humanly conditions.

Animal husbandry**:** The mice were housed in cages containing dust-free sawdust bedding. They were fed with pellets made from grower's mash and water *ad libitum*.

Animal grouping**:** Two groups (Group A and B) of thirty mice each were randomly created out of the seventy mice. Group A was tagged open field paradigm and Group B, elevated plus

maze paradigm. Each of the two groups was sub-divided into six groups of fives each and were tagged Group I to IV.

Animal treatment**:** Each of the sub-groups were treated orally as follows:

Group I: Distilled water (10 ml/kg only)

Group II: Low dose of energy drink only (3.75 ml/kg)

Group III: High dose of energy drinks only (7.5 ml/kg)

Group IV: Low dose of energy drink (3.75 ml/kg) + alcohol (1.0 g/kg)

Group V: High dose of energy drink (7.5 ml/Kg) + alcohol (2.0 g/Kg) (37; 14).

Group VI: High dose of alcohol (2.0 g/kg)

Neurobehavioral Assessments of Anxiety Using Open Field Test

The open field test was conducted with an open-field apparatus (72 × 72 × 36 cm). The floor of the apparatus was compartmentalized into 4 × 4 = 16 square areas (18 × 18 cm square) and central 4 × 4 = 16 square areas were considered as the central part. Mice were put into the apparatus from the left-hand corner and allowed to move freely for 5 minutes (Wataru *et al.,* 2012). The parameters observed were: number of line crossing, number of centre crossing, number of rearing and level of urination and defecation.

Neurobehavioral Assessments of Anxiety Using Elevated plus maze (EPM)

An elevated plus-maze test was conducted as follows: The elevated plus-maze consists of two open arms (28 × 5 cm) and two enclosed arms (30 x 5cm) with15-cm high walls. The arms and central square were made of wooden plates elevated 51cm above the floor. Arms of the same type are located opposite from each other. Each mouse was placed in the central square of the maze (5 × 5cm), facing one of the closed arms. Mouse behaviour was recorded during a 5 min test period (Komada *et al.,* 2008). The time spent in the open and enclosed arms were recorded and analyzed.

Serum collection and preparation

Treated and control animals were sacrificed by decapitation under anesthesia (chloroform) and blood taken from each mouse by cardiac puncture, then allowed to clot in EDTA free bottles. Serum samples were extracted by centrifuging the clotted blood at 704 x *g* for 10min in a laboratory centrifuge. The serum samples were stored at -20^0C until utilization for corticosterone hormone assay.

Biochemical analysis of Corticosterone

The Corticosterone (cort) ELISA (Enzyme linked immune sorbent Assay) kit with catalog number EC3001-1, Lot number 11181413 was purchased from Assaypro LLC, U.S.A.

Statistical Analysis

Data were analyzed using SPSS version 20. Results were expressed as Mean ± SEM, analyzed using one-way ANOVA and Dunnett's post-hoc test for multiple comparisons. Values with $P< 0.05$ were considered significant**.**

RESULTS

Assessment of Anxiety using Open Field Test

Line crossing

In the number of line crossing, there was no statistical significant difference between control group (distilled water 10 ml/kg only) (96.70 ± 9.20) and the ED and ethanol treated groups (83.70 ± 18.90, 68.80 ± 8.70, 67.50 ± 9.40, 84.20 ± 8.50 and 68.00 ±14.60) respectively, $F(5, 30) = 0.961$, $P=0.457$ (Table 1)).

Center crossing

In the number of center crossing, there was no statistical significant difference between control group (distilled water 10 ml/kg only) (1.00 ± 0.40) and ED and ethanol treated groups with means (1.50 ± 0.67, 0.50 ± 0.50, 0.83 ± 0.54, 0.50 ± 0.20 and 0.80 ± 0.40) respectively, $F(5, 30) = 0.618$.

Rearing

There was statistical significant difference in the number of rearing between control group (distilled water 10 ml/kg only) (27.80 ± 3.90) and ED + ethanol treated groups with means (23.20 ± 5.90, 13.30 ± 1.90, 15.80 ± 4.30, 34.70 ± 4.00 and 18.70 ± 3.30) at 34.70 ± 4.00^{a} (7.5 ml/kg+2 g/kg), $F(5, 30) = 3.854$ $p=0.037$ (Table 1).

Urination

In the level of urination, there was no statistical significant difference between control group (distilled water 10 ml/kg only) with mean (0.20 ± 0.20) and the ED and ethanol treated groups (2.00 ± 1.30, 0.80 ± 0.50, 1.30 ± 1.00, 1.50± 0.60, 1.20 ± 0.60) $F(5, 30) = 0.629$, $P>0.05$ (Table 1).

Defecation

There was no statistical significant difference between control group (distilled water 10 ml/kg) (1.20 ± 0.50) and the ED and ethanol treated groups with means (0.20 ± 0.20, 0.50 ±0.30, 1.00 ± 0.50, 0.70± 0.30, 2.30 ± 0.70), but ANOVA showed a statistical significant different between the control group and treated groups $F(5, 30) = 2.582$, $P = 0.047$ (Table 1).

Table 1: Effect of acute administration of energy drink (ED) and alcohol on anxiety-like behaviors in mice using open field test, n = 6

Groups	Line crossing	Center crossing	Rearing	Urination	Defecation
Control (distilled water)	96.70 ± 9.20	1.00 ± 0.40	27.80 ± 3.90	0.20 ± 0.20	1.20 ± 0.50
E.D (3.75 ml/kg)	83.70 ±18.90	1.50 ± 0.67	23.20 ± 5.90	2.00 ± 1.30	0.20 ± 0.20
E.D (7.5 ml/kg)	68.80 ± 8.70	0.50 ± 0.50	13.30 ± 1.90	0.80 ± 0.50	0.50 ±0.30
E.D + Ethanol (3.75 ml/kg + 1 g/kg)	67.50 ± 9.40	0.83 ± 0.54	15.80 ± 4.30	1.30 ± 1.00	1.00 ± 0.50
E.D + Ethanol (7.5 ml/kg + 2 g/kg)	84.20 ± 8.50	0.50 ± 0.20	34.70 ± 4.00[a]	1.50 ± 0.60	0.70 ± 0.30
Ethanol (2 g/kg)	68.00 ±14.60	0.80 ± 0.40	18.70 ± 3.30	1.20 ± 0.60	2.30 ± 0.70

a: statistically significant

Assessment of Anxiety using elevated plus maze

Time spent in open arm

In the time spent in open arm, no statistical significant difference between the control group (distilled water 10 ml/kg only) with mean (29.30 ± 9.40) and the ED and ethanol treated group with means (52.20 ± 7.10, 50.20 ± 7.50, 34.30 ± 7.70, 40.80 ± 7.50, 52.70 ± 7.80). F (5, 30) = 1.604, P = 0.189 (Table 2).

Time spent in closed arm

In the time spent in closed arm, no statistical significant difference between the control group (distilled water 10 ml/kg only) with mean (199.20 ± 14.20) and the ED and ethanol treated group with means (183.00 ± 15.80, 215.00 ± 12.00, 225.00 ± 15.10, 214.80 ± 14.10, 186.80 ± 13.50). F (5, 30) = 1.443, P = 0.238 (Table 2).

Table 2: Effect of acute administration of energy drink (ED) and alcohol on anxiety-like behaviors in mice using elevated plus maze, n = 6

Groups	Time spent in open arm (s)	Time spent in closed arm (s)
Control (distilled water)	29.30 ± 9.40	199.20 ± 14.20
E.D (3.75 ml/kg)	52.20 ± 7.10	183.00 ± 15.80
E.D (7.5 ml/kg)	50.20 ± 7.50	215.00 ± 12.00
E.D + Ethanol (3.75 ml/kg +1 g/kg)	34.30 ± 7.70	225.00 ± 15.10
E.D + Ethanol (7.5 ml/kg + 2g/kg)	40.80 ± 7.50	214.80 ± 14.10
Ethanol (2 g/kg)	52.70 ± 7.80	186.80 ± 13.50

The mean difference is not statistically significant when compared to control, p>0.05.SPSS version 20.

Corticosterone Hormone Concentration

Corticosterone Hormone concentration in mice used in Open Field Test

In the open field anxiety paradigm, there was no statistical significant difference between control group (distilled water 10 ml/kg) and ED and ethanol treated groups in the level of corticosterone hormone (ng/ml), P>0.05. The corticosterone concentration for control was 0.25 ± 0.05, while ED and ethanol treated groups were 0.17 ± 0.05, 0.32 ± 0.07, 0.43 ± 0.06, 0.35 ± 0.12, 0.28 ± 0.06, F (5, 22) =1.379, P = 0.270 (Table 3).

Table 3: Effect of acute administration of energy drink (ED) and alcohol on corticosterone concentration in open field test n = 6

Groups	Corticosterone concentration (mg/ml)
Control (distilled water)	0.25 ± 0.05
E.D (3.75 ml/kg)	0.17 ± 0.05
E.D (7.5 ml/kg)	0.32 ± 0.07
E.D + Ethanol (3.75 ml/kg + 1 g/kg)	0.43 ± 0.06
E.D + Ethanol (7.5 ml/kg + 2 g/kg)	0.35 ± 0.12
Ethanol (2 g/kg)	0.28 ± 0.06

The mean difference is not statistically significant when compared to control, p>0.05.SPSS version 20.

Corticosterone hormone concentration in mice used in elevated plus maze

There was no statistically significant difference between control group (distilled water 10 ml/kg) and ED and ethanol treated groups in the concentration of corticosterone hormone (ng/ml), P>0.05. The corticosterone concentration for control was 0.11 ± 0.02, while ED and ethanol treated groups were 0.21 ± 0.07, 0.22 ± 0.05, 0.31 ± 0.08, 0.19 ± 0.07, 0.25 ± 0.07, F (5, 24) = 1.069, P =0.402 (Table 4).

Table 4: Effect of acute administration of energy drinks (ED) and alcohol on corticosterone concentration in elevated plus maze. n = 6

Groups	Corticosterone concentration (mg/ml)
Control (distilled water)	0.11 ± 0.02
E.D (3.75 ml/kg)	0.21 ± 0.07
E.D (7.5 ml/kg)	0.22 ± 0.05
E.D + Ethanol (3.75 ml/kg + 1 g/kg)	0.31 ± 0.08
E.D + Ethanol (7.5 ml/kg + 2 g/kg)	0.19 ± 0.07
Ethanol (2 g/kg)	0.25 ± 0.07

The mean difference is not statistically significant when compared to control, p>0.05.SPSS version 20.

DISCUSSION

The anxiogenic activity observed by decrease in the number of line crossing and center crossing in OFT means that ED may be acting through caffeine (which is anxiogenic) by antagonizing adenosine receptors. Caffeine's stimulatory effects are primarily associated with its capacity to block adenosinereceptors(Lee and Chern, 2014). This finding is in accordance with the findings of Ferreira *et al.,* (2004). In that study done in mice, oral administration of ED did not significantly alter the effects of ethanol, but was able to reduce the suppressant effects of a higher dose of ethanol. However, combining ED and ethanol led to increase anxiety in OFT instead of counteracting each other as speculated. Here, the ethanol may be acting through other pathways such as GABArgic and not by stimulating adenosine receptors.

In the number of rearing, the statistical significance observed between control and high doses of ED and ethanol may indicates that the combination of the two leads to increased anxiety. This is shown by increased number of rearing, which is a measure of anxiety in rodents. This result is contrary to that of Marczinski *et al.,* (2011) which found no significant difference between ED alone or ED and alcohol. The anxiolytic activity shown by ethanol alone indicates that it may be acting through neurotransmitter GABA, which acts as a brake to excitatory neurotransmitters that mediate anxiety. The slight anxiogenic effect shown by ethanol may be due to its ataxic effect. The increased anxiety urination in OFT add more weight to the use of urination as a measure of anxiety in OFT, while the decreased and increased defecation means that its validity is still in question. This is in accordance with Ekong *et al.,* (2008), they state defecation and urination are often used as measures of anxiety, but their validity has been questioned (Ekong *et al.,* 2008). Also, Lister, (1990) state that defecation and urination are often used as anxiety measures, but the validity of defecation has been questioned. It is also in accordance with Bindra and Thompson, (1958) who argue that there is no significant relationship between fearfulness and urination and defecation as measured in OFT, nevertheless they agree that defecation and urination in a novel environment are signs of emotionality, which is not to be equated with fearfulness or timidity. However, the result contradicts Hall (1934) who describe defecation as anxiety index in rodents.

In elevated plus maze (EPM), slight anxiolytic property was shown by ED and ethanol alone. While ethanol is known to be anxiolytic, combination of the two indicate anxiogenic activity, so, they counteract each other. This is contrary to Aarthi and Parsad, (2011) who state that in general, high doses of caffeine may increase anxiety. The increased time spent in closed arm might be due to the natural exploratory behavior of the mice which make them to quickly escape to enclosed arm. Methylxanthines such as caffeine and theophylline have been demonstrated to increase anxiety in humans and rodents in different anxiety paradigms. This agrees with finding of Blatt and Takahashi, (1998) who identified that, mice in the EPM escaped from the open arm to the enclosed arm because of fear and anxiety. In addition, the increase in the concentration of corticosterone hormone (not significant) may indicate increase anxiety because it is a stress hormone. This is in corroboration with studies ofEscrig *et al.,* (2012) and Patz *et al.,* (2005) who states that in regard to ethanol and caffeine, moderate acute

doses of ethanol or caffeine have been shown to increase the plasma corticosterone levels in rodents and cortisol in humans. This increase was not observed after ethanol or caffeine was administered alone. The insignificance may also be due to the fact that physiological indicators of stress in rodents, such as serum corticosterone levels at the time of behavior testing, do not always predict the effect of environment stimulus on anxiety-like behavior.

CONCLUSION

Energy drinks together with alcohol increased anxiety at doses of7.5 ml/kg + 2 g/kg (high) in open field, but not in elevated plus maze paradigms for anxiety. Energy drinks and alcohol have no effect on corticosterone level in mice subjected to these tests, ($p>0.05$). It is recommended that ED and alcohol should not be consumed at the same time or mixed before consumption.

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