

Effects of Separate and Combined Chronic Ingestion of Alcohol and Energy Drinks on Aggressive Behaviour in Female Albino Wistar Rats

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ABSTRACT

Aggressive behaviour is a complex neurobehavioural phenomenon influenced by biological, psychological, and environmental factors, including exposure to psychoactive substances. Although alcohol and energy drinks have each been independently linked to alterations in aggression, limited experimental evidence exists regarding their separate and combined effects, particularly in female animal models. This study investigated the chronic effects of alcohol, energy drinks, and their co-administration on aggressive behaviour in female albino Wistar rats. A total of 28 female albino Wistar rats were randomly assigned to four groups: Control, Energy Drink, Alcohol, and Alcohol combined with Energy Drink. Rats received daily oral administration of alcohol (0.5 ml/kg body weight), energy drink (1.0 ml/kg body weight), or their combination for 28 consecutive days. Aggressive behaviour was assessed using the validated Resident–Intruder Paradigm, with dominant posture, scratching, and biting recorded as indices of aggression. Data were analysed using One-Way ANOVA with Bonferroni post hoc comparisons. Results revealed a statistically significant effect of treatment on aggressive behaviour, $p < .001$, with a large effect size (partial $\eta^2 \approx .87$). Rats exposed to energy drinks exhibited the highest levels of aggression ($M = 76.00$), followed by alcohol-only exposure ($M = 62.43$), while the combined alcohol and energy drink group showed the lowest aggression ($M = 34.57$), even below control levels ($M = 44.86$). The findings indicate that chronic exposure to alcohol and energy drinks significantly modulates aggressive behaviour in female albino Wistar rats, with stimulatory and depressant substances producing distinct and interactive effects.

Keywords: Aggression, Alcohol, Energy Drinks, Co-administration, Female albino Wistar rats, Resident–Intruder

INTRODUCTION

Aggression is a fundamental social behaviour seen in many different animals; intricately linked to evolutionary processes. Aggression initially developed as an adaptive technique to improve survival and reproduction in both human and non-human species. It makes it possible for people to build dominance hierarchies, fight for limited resources, and protect their territory. In these situations, aggressive behaviour serves as a controlled reaction to environmental difficulties and supports social organization and ecological balance. But when aggression becomes excessive, poorly regulated, out of proportion to the demands of the circumstance, or focused on non-threatening stimuli, it no longer qualifies as adaptive; since its costs to people and society outweigh its evolutionary benefits, it is now considered maladaptive (Aleyasin, Flanigan, & Russo, 2018).

Maladaptive aggression is often characterized by Increased irritation, impulsive behaviour, and violent reactions to mild provocation. Such dysregulated behaviour increases the risk of harm, social exclusion, and psychological discomfort by upsetting interpersonal connections and social institutions. According to Aleyasin

et al. (2018), persistent aggressive tendencies are widely acknowledged as prominent symptoms across a variety of neuropsychiatric and neurodegenerative disorders, significantly lowering the quality of life for those who are affected and placing a significant emotional and practical burden on caregivers.

Aggressive behaviour has been extensively documented in a broad spectrum of neuropsychiatric conditions, including major depressive disorder, schizophrenia, PTSD, and substance use disorders. Aggression is now commonly acknowledged as a complex phenomenon that results from the interplay of biological, psychological, social, and environmental factors. Socioeconomic inequalities, governmental policy, exposure to violence, and cultural norms all influence aggressive tendencies within populations. The expression of aggression at the individual level is influenced by a variety of factors, including personality traits, neurodevelopmental processes, endocrine regulation, genetic vulnerability, and situational stressors. Impulsive aggressiveness, a subtype that manifests under elevated emotional arousal or provocation and is closely associated with unique neurobiological mechanisms (Liu, Lewis, & Evans, 2018).

Dietary and chemical factors provide a significant but mostly unexplored pathway among extrinsic influences that may influence aggressive behaviour. Emotional and behavioural regulation has been connected to nutritional factors such as fatty acids, cholesterol, and amino acids like tryptophan. Furthermore, exposure to neurotoxic chemicals has been linked to changes in brain activity that could exacerbate aggressive tendencies. However, little is known about the processes by which these pharmacological and nutritional variables influence aggressive behaviour, especially when coupled with other biological or environmental stresses (Liu et al., 2018). Because of their significant effects on brain chemistry and behaviour, psychoactive drugs in particular have become a prominent subject of concern.

Both learning processes and intrinsic predispositions have been incorporated into theoretical explanations of aggressive behaviour. Aggression was viewed as an innate human urge in early psychoanalytic theories (Drndarevic, 2021). Social learning theory, on the other hand, emphasizes the importance of imitation, reinforcement, and observation. It suggests that people adopt aggressive behaviours by watching others and experiencing the results of their acts (Bandura, 1973; Huesmann, 2017). This theory posits that violence is acquired through social settings rather than being innate.

The General Aggression Model (GAM) has offered an all-encompassing framework that integrates social and biological viewpoints. According to the GAM, personal elements like beliefs, personality traits, and cognitive schemas combine with situational factors like provocation, stress, and substance abuse to produce aggression. According to Anderson and Bushman (2002), these elements have an impact on internal states of affect, physiological arousal, and cognition, which in turn shape appraisal and decision-making processes that drive behavioural outcomes. Because psychoactive substances directly arouse, emotional processing, and inhibitory control, this model is especially helpful for comprehending substance-induced aggressiveness.

Aggression is a major public health concern. According to the World Health Organization, violence is a substantial contributor to disability-adjusted life years and a leading cause of illness and mortality globally, particularly among teenagers and young adults (WHO, 2024). Aggressive behaviour not only causes physical harm but also long-term psychological anguish, social disturbance, and financial expenses. Additionally, psychotic disorders, bipolar disorder, depression, neurocognitive disorders, and externalizing disorders including conduct disorder and intermittent explosive disorder are also associated with a high prevalence of aggression (Girasek et al., 2022).

Substance use has been repeatedly found to be a significant risk factor for aggressive behaviour. Psychoactive chemicals undermine prefrontal cortical regulation of behaviour, alter reward and threat processing, increase impulsivity, and impair executive functioning. According to Hoaken and Stewart (2003) and Heinz et al. (2011), these neuropsychological impacts make people more prone to impulsive and reactive aggressiveness. Research

on humans and animals also shows that long-term substance use impairs emotional control, which raises the risk of aggressive behaviour (Kolla et al., 2017).

Alcohol is still one of the most often used psychoactive substances in the world and is closely linked to violent and aggressive behaviour. Alcohol facilitates aggression by lowering behavioural inhibition, impairing judgment, and increasing emotional reactivity since it depresses the central nervous system (Mitchell & McCambridge, 2023). Many neurotransmitter systems, such as the GABAergic, glutamatergic, dopaminergic, and serotonergic pathways, are affected by alcohol at the neurobiological level, which results in disinhibition and poor emotional regulation (Oscar-Berman & Marinković, 2007; Vengeliene et al., 2008).

Unlike alcohol, energy drinks are stimulants that activate the neurological system. High amounts of caffeine, taurine, sugar, and herbal stimulants are present in these drinks, which are particularly well-liked by adolescents and young adults (Seifert et al., 2011). Excessive caffeine use raises dopaminergic activity and physiological arousal, which may cause anxiety, irritation, agitation, and aggressive behaviour even though it is meant to improve alertness and performance (Juliano et al., 2004; Costantino et al., 2023). Frequent consumption of energy drinks has been associated in recent research with increased aggression, risk-taking, and behavioural dysregulation (Elvig et al., 2021; Marinoni et al., 2022).

Consumption of energy drinks and alcohol combined is particularly alarming. Alcohol-induced drowsiness can be masked up by caffeine, which causes people to underestimate their level of intoxication, consume larger amount of alcohol, and act dangerously (Brache & Stockwell, 2011; Nawi et al., 2021). Co-consumption of alcohol and energy drinks has been linked in studies to increased aggression, injuries, and poor decision-making (Elvig et al., 2021; Zeidán-Chuliá et al., 2023). The neurobehavioural mechanisms behind these consequences are still not fully understood, despite growing public health concern.

An ethically and methodologically robust framework for investigating the biological causes of aggressive behaviour is provided by animal models. Causal inference is made easier by the exact control that rodent models provide over dosage, environmental factors, and behavioural evaluation (Natarajan & Caramaschi, 2010; Figdor, 2022). Despite the fact that male rats have historically been employed in studies on aggression, mounting data emphasizes the need of examining females, whose aggressive behaviour is impacted by unique hormonal and neurological mechanisms. For studying sex-specific patterns of substance-induced aggression, female albino Wistar rats offer a useful model.

Despite extensive research on alcohol and energy drinks independently, limited experimental studies have examined their separate and combined effects on aggression in female rodents. Previous studies demonstrate that combined psychoactive substances can produce behavioural outcomes distinct from single-drug exposure (Balogun, 2006; Balogun, Osuh, & Olorode, 2020). However, aggression-related outcomes under alcohol, energy drink, and combined conditions in female Wistar rats remain underexplored. In order to provide empirical evidence, this study adopts a controlled experimental design to investigate the separate and combined effects of alcohol and energy drinks on aggressive behaviour in female albino Wistar rats, contributing empirical evidence to behavioural neuroscience and substance-use research.

Statement of the Problem

A significant global clinical, social, and public health concern is aggressive behaviour. Excessive and poorly controlled aggression is linked to criminal activity, psychological trauma, interpersonal violence, and a lower quality of life. Additionally, it is a common symptom of many neurological and psychiatric conditions, where it exacerbates clinical outcomes, complicates treatment, and increases caregiver burden (Aleyasin et al., 2018). Despite decades of research, effective prevention and management of maladaptive aggression remain limited, partly due to insufficient understanding of its underlying biological and environmental determinants.

It has been repeatedly shown that substance use is a significant risk factor for aggression. Alcohol, stimulants, and other psychoactive substances influence neurochemical systems involved in behavioural regulation, increase emotional reactivity, and impair inhibitory control (Hoaken & Stewart, 2003; Heinz et al., 2011). Due to its disinhibitory effects and influence on emotional processing, alcohol in particular is linked to a significant percentage of aggressive occurrences globally (Mitchell & McCambridge, 2023). Similar to this, energy drinks, which are popular due to their stimulating effects, have been linked to higher levels of agitation, irritability, and aggression, particularly when taken in excess (Juliano et al., 2004; Costantino et al., 2023).

The combined use of alcohol and energy drinks presents an emerging concern. Caffeine's capacity to mask alcohol-induced sedation leads to increased alcohol consumption, impaired judgment, and elevated risk-taking behaviours (Brache & Stockwell, 2011; Nawi et al., 2021). Empirical studies associate this pattern of use with heightened aggression and injury (Elvig et al., 2021; Zeidán-Chuliá et al., 2023). However, much of the existing evidence is derived from human observational studies that are confounded by personality traits, social context, and environmental influences, limiting causal inference.

A vital technique for separating the neurobehavioural effects of psychoactive drugs is animal research. Few research have looked at the combined effects of alcohol and caffeine under controlled experimental conditions, despite the fact that many have looked at the effects of either substance alone. Furthermore, despite evidence that females display unique patterns of aggression affected by hormonal cycles and neurobiological variations, the majority of aggression research has typically concentrated on male individuals.

Previous studies using albino rats have demonstrated that combined psychoactive substances often produce behavioural outcomes that differ qualitatively and quantitatively from those produced by single substances (Balogun, 2006; Balogun et al., 2020). However, there remains a notable gap in research specifically examining how alcohol, energy drinks, and their combination influence aggressive behaviour in female albino Wistar rats. This gap limits understanding of sex-specific mechanisms of substance-induced aggression and constrains the generalisability of existing findings.

Without empirical data from controlled studies on female models, current knowledge remains incomplete, hindering the development of comprehensive theories of aggression and effective intervention strategies. Addressing this gap is essential for advancing behavioural neuroscience and informing public health approaches to substance-related aggression. Thus, the problem which this study attempts to solve is the paucity of experimental data about the independent and combined effects of energy drinks and alcohol on aggressive behaviour in female albino Wistar rats. The current study aims to shed light on the behavioural impacts of exposure to both single and combination substances and advance a more sophisticated understanding of aggression by methodically examining these effects.

This study seeks to fill this critical gap by answering these research questions:

1. What effect does exposure to alcohol and energy drinks have on the aggressive behaviour of female albino Wistar rats?
2. Do female albino Wistar rats exposed to alcohol exhibit significantly higher aggressive behaviour than those exposed to energy drinks?

To answer these research questions, the following hypotheses were tested:

Hypothesis

1. Exposure to alcohol and energy drinks will significantly affect the aggressive behaviour of female albino wister rats exposed to the drugs
2. Female albino wister rats exposed to alcohol will display more aggressive behaviour than female albino wister rats exposed to energy drinks

METHODOLOGY

Research design

The design used in this study is an independent group randomized design with four treatment conditions. Alcohol was given to the first group, energy drinks were given to the second, both drugs and alcohol were given to the third, and the control group was assigned to the fourth. The chronic oral administration of alcohol, energy drinks, or both to female albino rats are the independent variables. The aggressive behaviours that the female albino rats display is the dependent variable.

Setting

The experiment took place at the Department of Psychology, Animal Laboratory, University of Ibadan, Oyo State, Nigeria, using standard experimental housing and testing apparatus.

Animal Population

The animals used for this study were female Albino rats. A total of 30 female Albino rats weighing between 120 -150g and 4 – 6 weeks old were used. They were divided into five (5) groups with seven (7) female rats in each group and 2 female rats in a group to serve as the intruder

The groups were alcohol group, energy drink group, combined alcohol and energy drink group, control group.

The rats were randomly assigned to the different groups.

Drugs

The drugs used for this study were Smirnoff vodka (containing 40% alcohol) and Red bull energy drinks (containing 80mg of caffeine). Alcohol and energy drinks were administered orally with the use of an oral cannula. The rats were given 0.5ml/kg bodyweight of alcohol, while energy drinks was administered at a dose of 1.0ml/kg body weight every 24 hours.

Materials/Instruments

The following materials and instruments were used for this study;

1. 6 experimental rat cages.
2. Recording sheets
3. Distilled water/saline
4. Laboratory coat
5. Oral cannula for administration of drugs
6. Hand Gloves.
6. Face/Nose Mask
7. Colored markers for easy identification of female albinorats from, the control and experimental rat.
8. Measuring cylinders used in diluting and measuring the solution.
9. Weighing balance for the daily weighing of rats.
10. Stopwatch for time keeping and recording
11. Disposable syringes
12. Alcohol
13. Energy drinks
14. Distilled water

Procedure

The rats were brought into the lab and given two weeks to acclimate before the studies commenced. The rats were not deprived in any way during this time; they were given water and food. The rats were subsequently divided into four groups at random: the alcohol group, the energy drink group, the combined group, and the control group.

The medications were administered orally to the rats in the experimental group for the course of the 28-day trial. The drug category was prominently displayed on each cage. Additionally, each cage's rats were labeled 1 to 4 for identification. The resident-intruder paradigm was applied. This method involves introducing an intruder rat to the resident rat in order to study or observe aggressive behaviour.

Each day at the commencement of the experiment, all the rats were weighed, and the weights of each rat were recorded in a chart. This is done in order to decide how much drugs each rat should receive each day. To allow for the onset of drug action, the rats were given 30 minutes after the drug was administered before data collection started. All of the cages had their food and water removed during the first half hour after the drug was administered. One rat from the experimental group was put in a free experimental cage and an intruder rat was added after the medication was administered for 30 minutes. The resident rat's behavior was watched, and data was gathered.

During the five minutes of each experimental session, each rat's aggressive behaviour was observed. Dominant posture, scratching, and biting were the signs of aggression that were noted. A recording sheet was used to document these behaviours, noting how frequently the experimental rat displayed each behavior. The intruder rat and the experimental rat were taken out of the experimental cage and put back in their own cages after five minutes. All of the remaining rats in the alcohol, energy drink, and combined alcohol and energy drink experimental groups underwent the same procedure, followed by the control group.

Over the course of the 28-day experiment, each rat underwent three attempts of the same observation process each day. The following, along with regular feeding, was done every day of the experiment:

- Weighing of each rat
- Oral administration of drugs
- Observation of the dominant postures, scratching, and biting.

All of the rats were disposed of at the conclusion of the experiment in accordance with the guidelines provided by the Cruelty to Animal Act for the disposal of animals used in research.

Statistical Analysis

Data were analysed using One-Way ANOVA to assess group differences in aggression. Where applicable, post-doc comparisons were conducted to dominate pairwise differences. Statistical significance was set at $p < .05$.

Ethical Consideration

All procedures adhered to internationally accepted guidelines for the care and use of laboratory animals. Ethical approval was obtained from appropriate institutional review board prior to the commencement of the study.

RESULTS

This chapter presents the results and discussion of the study. The sequence of the presentation of the results is in accordance with the hypotheses proposed in chapter one. The hypotheses were analyzed using Randomize block ANOVA.

The first hypothesis stated that there will be a significant difference in the behaviour among female albino wister rats exposed to alcohol and energy drinks was tested using the Randomized Factorial ANOVA and the result presented in Table 4.1.

Table 4.1: Summary Factorial ANOVA table showing the influence exposure to alcohol and energy drink affecting the aggressive behaviour among female albino wister rats

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Block	7106.679	3	2368.893	4.548	.012	.362
Treatment	83058.036	1	83058.036	159.468	.000	.869
Error	12500.286	24	520.845			
Corrected Total	19606.964	27				

The result from Table 4.1 shows that exposure to alcohol and energy drinks significantly affect aggressive behaviour among Wister rats $F(4.548) = 159.47, p < 0.001, \eta^2 = .87$. Further analysis was carried out to determine the mean difference among the treatment groups using Bonferroni mean comparison Test and the result is presented in Table 4.2.

Table 4.2: Summary Bonferonni mean comparison analysis showing the mean difference between rats exposed to energy drinks and alcohol intake and those exposed to abnormal aggressive behaviour

	Mean	S.E.M	1	2	3	4
Energy drinks	76.00	2.645	-	13.57	41.43*	31.14
Alcohol	62.43	2.645		-	27.86	17.57
Combined	34.57	2.645			--	-10.29
Control	44.86	2.645				-

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

From the analysis, mean differences showed that rats exposed to different type of drinks; combination of (Energy drinks and Alcohol) ($\chi = 34.57$), Energy drink($\chi = 76.00$), and Alcohol only($\chi = 62.43$) significantly exhibited aggressive behaviour compared to animal rats in the control group ($\chi = 44.86$). The mean differences were significant ($p < .001$). The result demonstrated that aggressive behaviour decreased by 87% with exposure to energy drinks and alcohol compared to the control group. Based on this, hypothesis states that rat treated with exposure to energy drinks and alcohol will significantly have aggressive behaviour than rats in control group is accepted. The result demonstrated that aggressive behaviour increased for the control group while it decline significantly for exposed rats by 21% as the exposure increase more than seven days.

The second hypothesis slated that female albino Wister rats exposed to alcohol will display more aggressive behaviour than female albino Wister rats exposed to energy drinks

Table 4.3: Summary Factorial ANOVA table showing female albino Wister rats exposed to alcohol display more aggressive behaviour than female albino Wister rats exposed to energy drinks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Block	3645.214	6	607.536	0.799	0.581	.186
Treatment	83058.036	1	83058.036	109.275	.000	.839
Error	15961.750	21	760.083			
Corrected Total	19606.964	27				

The result from Table 4.3 shows that exposure to alcohol display more aggressive behaviour than female albino Wister rats exposed to energy drinks $F(0.799) = 109.275, p < 0.001, \eta^2 = .84$. Further analysis was carried out to determine the mean difference among the treatment groups using Bonferroni mean comparison Test and the result is presented in Table 4.4.

Table 4.4: Summary Bonferonni mean comparison analysis showing the mean difference between female albino Wister rats exposed to alcohol display more aggressive behaviour than female albino Wister rats exposed to energy drinks

	Mean	S.E.M	1	2	3	4
Energy drinks	76.00	2.185	-	13.57	41.43*	31.14
Alcohol	62.43	2.185		-	27.86	17.57
Combined	34.57	2.185			--	-10.29
Control	44.86	2.185				-

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

From the analysis, mean differences showed that rats exposed to Alcohol only ($\chi = 62.43$) display aggressive behaviour more than rats exposed to Energy drink ($\chi = 76.00$), and significantly exhibited aggressive behaviour compared to animal rats in the control group ($\chi = 44.86$). The mean differences were significant ($p < .001$). The result demonstrated that aggressive behaviour decreased by 84% with exposure to alcohol compared to energy drinks. Based on this, hypothesis states that rat treated with exposure to alcohol will significantly have aggressive behaviour than rats exposed to energy drinks.

DISCUSSION

This study examined the separate and combined effects of alcohol and energy drink administration on aggressive behaviour in female albino Wistar rats, using a controlled experimental design. Aggression was operationalised through observable behaviours including dominant posture, scratching, and biting within a resident intruder paradigm. The findings provide empirical evidence that chronic exposure to alcohol, energy drinks, and their combination significantly alters aggressive behaviour, with distinct behavioural patterns observed across treatment conditions.

Effect of Alcohol and Energy Drinks on Aggressive Behaviour

The first hypothesis proposed that exposure to alcohol and energy drinks would significantly affect aggressive behaviour among female albino Wistar rats. The Randomized Factorial ANOVA revealed a statistically significant main effect of treatment on aggression, with a very large effect size (partial $\eta^2 = .869$), indicating that substance exposure accounted for a substantial proportion of variance in aggressive behaviour. This finding strongly supports the hypothesis and confirms that psychoactive substances exert powerful modulatory effects on aggression.

The significant treatment effect aligns with the General Aggression Model (Anderson & Bushman, 2002), which posits that situational factors such as substance exposure influence internal states of arousal, affect, and cognition, thereby shaping aggressive outcomes. Alcohol and energy drinks, through their distinct neuropharmacological actions, likely altered arousal and inhibitory control systems, leading to measurable changes in aggressive responding during the resident–intruder encounters.

Bonferroni post-hoc comparisons further clarified group differences. Rats exposed to energy drinks exhibited the highest mean aggression scores, followed by rats exposed to alcohol alone, while rats exposed to the

combined alcohol–energy drink condition showed the lowest aggression scores among the treatment groups. All treatment groups differed significantly from the control group, indicating that chronic substance exposure altered baseline aggression patterns.

The elevated aggression observed in the energy drink group is consistent with existing literature linking stimulant consumption particularly caffeine to increased arousal, irritability, and aggressive behaviours (Juliano et al., 2004; Costantino et al., 2023). Energy drinks contain high levels of caffeine and other stimulants that enhance dopaminergic activity and sympathetic nervous system activation, which may heighten reactivity to social provocation. In the context of the resident–intruder paradigm, this heightened arousal likely increased aggressive responses such as biting and dominant posturing.

Similarly, rats exposed to alcohol alone demonstrated significantly higher aggression than the control group. This finding is consistent with extensive evidence that alcohol reduces behavioural inhibition and impairs emotional regulation, thereby facilitating impulsive and reactive aggression (Oscar-Berman & Marinković, 2007; Mitchell & McCambridge, 2023). Although alcohol is a central nervous system depressant, its disinhibitory effects at moderate doses often increase aggressive behaviour by weakening prefrontal control mechanisms.

Interestingly, rats exposed to the combined alcohol and energy drink condition exhibited lower aggression scores compared to both the alcohol-only and energy drink-only groups. This finding suggests that the interaction between alcohol and energy drink constituents may produce behavioural effects distinct from those of either substance alone. Similar interaction effects have been reported in earlier animal studies, where drug combinations yielded non-additive or paradoxical behavioural outcomes (Balogun, 2006; Balogun, Osuh, & Olorode, 2020).

One possible explanation is that chronic co-administration of alcohol and caffeine produced competing neuropharmacological effects. While caffeine increases arousal and vigilance, alcohol induces sedation and motor impairment. Over repeated exposure, these opposing actions may have attenuated overt aggressive responses, possibly due to fatigue, behavioural suppression, or neuroadaptation. This interpretation supports the assertion that combined psychoactive substances can modulate aggression differently from single-substance exposure.

Comparison Between Alcohol and Energy Drink Exposure

The second hypothesis proposed that female albino Wistar rats exposed to alcohol would display more aggressive behaviour than those exposed to energy drinks. The Factorial ANOVA indicated a significant treatment effect with a large effect size (partial $\eta^2 = .839$), confirming meaningful differences between the two exposure conditions.

However, mean comparisons revealed that rats exposed to energy drinks exhibited higher aggression scores than those exposed to alcohol alone. Although both groups showed significantly greater aggression than the control group, the stimulant effects of energy drinks appeared to provoke stronger aggressive responses than alcohol under the experimental conditions used in this study.

This finding contrasts with some human-based literature that often identifies alcohol as the stronger predictor of aggression (Mitchell & McCambridge, 2023). However, it aligns with experimental evidence showing that stimulants can elicit heightened agitation, irritability, and reactivity in animal models, particularly during social challenge tasks (Juliano et al., 2004; Marinoni et al., 2022).

In female rats, hormonal and neurobiological factors may further amplify stimulant-induced aggression. Unlike males, female aggression is often more context-dependent and sensitive to arousal states. Elevated physiological activation induced by caffeine may therefore produce stronger aggressive responses during provocation. This supports the argument that sex-specific mechanisms play a critical role in substance-induced aggression and

underscores the importance of studying female models, which have historically been underrepresented in aggression research.

The finding that alcohol-exposed rats showed lower aggression than energy-drink-exposed rats may also reflect alcohol's sedative and motor-impairing properties during chronic exposure. While alcohol reduces inhibition, prolonged exposure can also diminish locomotor activity and responsiveness, potentially limiting the expression of overt aggression during short observation periods.

Implications for Theories of Substance-Induced Aggression

The findings of this study provide empirical support for integrative models of aggression, particularly the General Aggression Model. Both alcohol and energy drinks altered aggressive behaviour by modifying internal states of arousal and control, but their effects differed depending on pharmacological properties and exposure patterns.

The results also reinforce social learning and neurobiological perspectives that view aggression as an emergent behaviour shaped by interactions between biological predispositions and situational influences. Substance exposure represents a powerful situational factor capable of altering aggression even in the absence of learning or prior aggressive conditioning.

Furthermore, the distinct behavioural outcomes observed in the combined exposure condition support prior work by Balogun (2006) and Balogun et al. (2020), demonstrating that psychoactive drug combinations can yield unique behavioural profiles that cannot be predicted from single-substance effects alone.

Public Health and Research Implications

Although this study employed an animal model, the findings have broader implications for understanding substance-related aggression in humans. The heightened aggression observed following energy drink exposure raises concern given the widespread consumption of these beverages, particularly among adolescents and young adults. The interaction effects observed under combined exposure conditions further highlight the complexity of alcohol–energy drink co-use and its potential behavioural consequences.

Importantly, the study contributes to the limited body of research examining aggression in female subjects. By demonstrating that female albino Wistar rats exhibit significant and substance-specific changes in aggression, the findings challenge the male-centric bias of much prior aggression research and underscore the need for sex-inclusive experimental designs.

CONCLUSION

Based on the statistical analyses and behavioural observations, this study concludes that alcohol, energy drinks, and their combination have distinct and significant effects on aggression levels in female albino Wistar rats. Energy drink consumption produced the highest level of aggression, followed by alcohol consumption. Interestingly, the combination of alcohol and energy drinks produced the lowest aggressive response, even lower than the control group.

These findings demonstrate that:

1. Psychoactive substances significantly modulate aggressive behaviour.
2. The interaction between depressants and stimulants can produce outcomes different from their individual effects.
3. Female Wistar rats respond sensitively to neurochemical alterations associated with ethanol and caffeine-
taurine mixtures.

Thus, the hypothesis stating that exposure to alcohol and energy drinks significantly affects aggressive behaviour is supported.

Limitations

Although the study produced significant and interpretable findings, several limitations should be noted:

1. **Limited Sample Size:** Only 28 rats were used. A larger sample would improve the generalizability and statistical power of the findings.
2. **Single Species and Gender:** Only female albino Wistar rats were used. Male rats may respond differently due to testosterone-related behavioural dynamics.
3. **Single Dosage Level:** Only one concentration of alcohol and energy drink was administered. Dose-response analysis would provide a clearer understanding of behavioural sensitivity.
4. **Behavioural Measure Constraint:** Aggression was measured solely through attack frequency. Additional measures such as latency to attack, duration of aggression, and defensive responses would enrich behavioural interpretation.
5. **Lack of Biochemical Analysis:** No neurochemical or hormonal assays were conducted. Such biomarkers would help clarify mechanisms underlying the observed behavioural patterns.
6. **Possible Observer Bias:** Although behavioural scoring followed standardized procedures, there is always potential for observer subjectivity unless full blinding is ensured.

RECOMMENDATIONS

For Public Health: Adolescents and young adults should be educated about the behavioural risks associated with excessive consumption of energy drinks and alcohol.

For Policy Makers: Regulation of simultaneous marketing of energy drinks and alcoholic beverages is important because combined effects may not follow predictable patterns.

For Schools and Universities: Awareness programs should address the rising trend of mixing alcohol with stimulant beverages.

For Psychologists and Health Professionals: The interaction between stimulant and depressant substances should be considered when treating behavioural and emotional dysregulation.

For Researchers: Future animal studies should integrate behavioural, neurochemical, and histological analyses to develop a comprehensive understanding of how these substances influence aggression.

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