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# Separate and Combined Effects of Alcohol and Energy Drinks on the Aggressive Behaviour of Male Wistar Albino Rats

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## Abstract

*Despite the growing trend of co-consumption of alcohol and energy drinks, limited knowledge exists regarding their separate and combined effects on aggression, particularly in controlled animal models. This study examined the chronic effects of Alcohol and Energy drinks on the aggressive behaviour of male Wistar albino rats. A total of 28 male Wistar albino rats were randomly assigned to four groups (Control, Energy Drinks, Alcohol, and Alcohol combined with Energy Drinks). Rats were exposed to chronic administration of energy drinks (10 ml/kg) and/or alcohol (0.74 g/kg body weight) daily for 28 consecutive days. Aggressive behaviour was assessed using the validated Resident–Intruder Paradigm. Data were analyzed using One-Way ANOVA. Results revealed no statistically significant effect of Alcohol, Energy Drinks, or their combination on aggressive behaviour, $F(3,23)=1.486$, $p > .05$, $\eta^2 = .162$. Descriptive means were: Control ($M = .706$), Energy Drinks ($M = .604$), Combined ($M = .499$), and Alcohol ($M = .443$). The findings indicate that, under conditions of chronic administration at these specific doses, exposure to alcohol, energy drinks, or their combination did not significantly alter aggressive behaviour relative to the control group baseline.*

**Keywords:** ***Aggression, Alcohol, Energy Drinks, Co-administration, Male Wistar albino rat, Resident-Intruder***

## Introduction

Aggression is a complex behaviour involving actions intended to cause physical or psychological harm (Allen & Anderson, 2017; Bushman & Huesmann, 2010). It arises from the interaction of biological, psychological, and environmental factors, and is often expressed in response to threat, frustration, impulsivity, or impaired self-regulation. Contemporary theoretical models recognise both innate tendencies and learned components. For example, while early psychoanalytic perspectives proposed that aggression is an instinctual drive (Drndarevic, 2021), social learning theory emphasises that aggressive acts are acquired through observation and reinforcement (Bandura, 1973; Huesmann, 2017). The General Aggression Model integrates these approaches by proposing that situational factors (e.g., provocation, substance use) and personal factors (e.g., traits, cognitions) jointly shape aggressive outcomes through their effects on affect, arousal, and appraisal (Anderson & Bushman, 2002).

Aggression carries substantial global health and societal burdens. The WHO reports that violence is a leading cause of morbidity and mortality among young people and contributes significantly to disability-adjusted life years worldwide (WHO, 2024). Aggressive behaviour is also prevalent across psychiatric conditions, including psychotic disorders, bipolar disorder, depression, neurocognitive disorders, and externalising disorders such as conduct disorder and intermittent explosive disorder (Girasek et al., 2022).

Substance use, particularly alcohol, cannabis, stimulants, and opioids, is a well-documented risk factor for aggression. Psychoactive substances impair inhibitory control, increase impulsivity, alter reward and threat processing, and modify neurochemical pathways linked to aggression (Hoaken & Stewart, 2003; Heinz et al., 2011). Alcohol, in particular, is strongly associated with violent offending and interpersonal aggression due to its effects on the GABAergic, dopaminergic, and serotonergic systems (Vengeliene et al., 2008; Mitchell & McCambridge, 2023). Stimulants such as caffeine, amphetamine, and cocaine can heighten agitation and irritability, increasing the likelihood of aggressive outbursts (Smith & Garavan, 2020). Studies in both humans and animals confirm that drug exposure disrupts prefrontal cortical regulation of behaviour, predisposing individuals to impulsive aggression (Kolla et al., 2017).

Alcohol remains one of the most widely consumed psychoactive substances globally and is implicated in a substantial proportion of aggressive incidents. Its depressant effects on the central nervous system reduce behavioural inhibition and impair judgment, increasing the propensity for aggression and violence (Mitchell & McCambridge, 2023). At the neurobiological level, alcohol modulates NMDA, GABA_A, glycine, 5-$HT_3$, and nicotinic acetylcholine receptors, resulting in disinhibition and altered emotional regulation (Oscar-Berman & Marinković, 2007; Vengeliene et al., 2008).

Energy drinks, widely consumed among adolescents and young adults, contain high levels of caffeine, taurine, sugars, and herbal stimulants (Seifert et al., 2011). High caffeine intake enhances dopaminergic activity and increases arousal, which in excessive doses can lead to anxiety, agitation, and aggression-like behaviours (Juliano et al., 2004; Costantino et al., 2023). Emerging research links frequent energy-drink consumption with behavioural dysregulation, risk-taking, and increased aggression in youth (Elvig et al., 2021; Marinoni et al., 2022).

Combined use of alcohol and energy drinks is common among young adults. Caffeine partially masks alcohol-induced sedation, causing individuals to underestimate their level of impairment, drink more, and engage in high-risk behaviours (Brache & Stockwell, 2011; Nawi et al., 2021). This combination is associated with heightened aggression, injuries, and risky decision-making (Elvig et al., 2021; Zeidán-Chuliá et al., 2023). However, despite increased public health concern, the neurobehavioural mechanisms underlying these aggressive outcomes remain insufficiently understood.

The concurrent use of alcohol and energy drinks has become increasingly prevalent, particularly among adolescents and young adults. Energy drinks are often mixed with alcohol to reduce perceived intoxication, resulting in increased consumption, impaired judgment, and heightened behavioural dysregulation. One critical consequence of this pattern of use is increased aggression, which contributes to interpersonal conflict, injuries, and social harm.

Animal models provide controlled and ethical platforms for investigating aggression's biological and neurochemical substrates (Natarajan & Caramaschi, 2010; Figdor, 2022). Male rats, in particular, exhibit stable dominance- and territorial aggression patterns and show

stronger aggression-related behavioural responses to psychoactive substances compared to females due to hormonal and neurochemical differences (Lisciotto et al., 1990; Yu et al., 2025). Thus, male Wistar albino rats represent a valid model for studying drug-induced aggression.

Despite extensive research on alcohol and energy drinks individually, limited experimental evidence exists on their combined effects on aggression. Existing human studies are largely observational and confounded by factors such as personality, impulsivity, and peer influences. Experimental animal studies are therefore essential to isolate the causal effects of chronic alcohol, chronic energy drink exposure, and their combination on aggression. This study, therefore, investigates the separate and combined effects of alcohol and energy drinks on aggressive behaviour in male Wistar albino rats.

Although alcohol and energy drinks have individually been linked to aggressive behaviour, there is limited scientific understanding of their combined effects on aggression. Human studies remain largely correlational, and few controlled animal studies have examined the behavioural outcomes of chronic exposure to these substances, particularly in male Wistar albino rats, an established model for studying aggression-related neurobehavioural mechanisms.

This study addresses these gaps by investigating:

(i) Whether chronic administration of alcohol increases aggressive behaviour in male Wistar albino rats.

(ii) Whether chronic administration of energy drinks increases aggressive behaviour in male Wistar albino rats.

(iii) Whether combined chronic administration of alcohol and energy drinks produces additive or synergistic effects on aggression in male Wistar albino rats.

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

i. There will be a significant effect of Energy Drinks on Aggressive behaviour among Male Wistar albino rats.

ii. There will be a significant effect of Alcohol on Aggressive behaviour among Male Wistar albino rats.

iii. There will be a significant combined effect of Alcohol and Energy Drinks on Aggressive behaviour among Male Wistar albino rats.

## Methodology

### Research Design

The study employed a randomized controlled experimental design with four treatment conditions: Control, Alcohol, Energy Drink, and Alcohol + Energy Drink. The independent variables were chronic oral administration of alcohol, energy drink, or their combination, while the dependent variable was aggressive behaviour, operationalised through the Resident–Intruder Paradigm. Aggression scores were subjected to One-Way Analysis of Variance (ANOVA) to determine treatment effects.

**Subjects**

A total number of twenty-eight (28) male Wistar albino rats were used for this study. The rats were housed under standard laboratory conditions with controlled temperature, a 12:12-hour light–dark cycle, and ad libitum access to food and water. The rats were randomly assigned to the four treatment groups (n = 7 per group). One rat in the alcohol treatment group died before the start of treatment and was not replaced, resulting in:

Alcohol (n = 6)

Energy Drink (n = 7)

Alcohol + Energy Drink (n = 7)

Control (n = 7)

Three additional male rats served exclusively as intruders during the behavioural assessments and were not part of the treatment groups.

**Setting**

All procedures were conducted in the Animal Experimental Laboratory, Department of Psychology, University of Ibadan, using standard experimental housing and testing apparatus.

**Behavioural Assessment: Resident–Intruder Paradigm**

Aggressive behaviour was assessed using the Resident–Intruder (RI) Paradigm, a validated assay for quantifying offensive aggression in rodents (Koolhaas et al., 2013). Each treated rat served as the resident and was paired with a weight-matched unfamiliar intruder introduced into its home cage. Interactions were observed for 5 minutes per trial.

The following offensive behaviours were coded as aggression:

Biting

Biting attempts

Chasing

Offensive posturing

Attack latency (if applicable)

Each animal underwent three RI trials at different time points during the study period, and the mean of the three observations was used as the aggression score.

Drug Administration and Dosing

Doses were calculated according to individual body weight as follows;

Alcohol: Ethanol (37.5% v/v; Smirnoff®) administered via oral gavage at 0.74 g/kg (Varlinskaya et al., 2001; Marley et al., 2023).

Energy Drink: Red Bull® administered at 10 ml/kg (Valle et al., 2017).

Combined Group: Both ethanol and energy drink administered sequentially with a 10-minute interval.

Control Group: Distilled water (10 ml/kg) via oral gavage.

Drug administration occurred once daily for 28 consecutive days.

**Experimental Procedure**

The Albino rats were allowed a two-week acclimatization period before treatment. Body weights ranged from 128–232 g at treatment onset. All rats were handled daily to reduce stress effects.

Following each day's administration, a 30-minute absorption period preceded behavioural testing. RI testing was conducted in the resident's home cage for a 5-minute session. Observers, blinded to treatment groups, recorded aggression using standard ethological scoring procedures.

Each resident rat completed three RI sessions spaced across the treatment period. Aggression scores for each rat were computed as the mean of the three trials. Treatment and behavioural assessment continued for 28 days with a 24-hour inter-session interval. At the end of the study, animals were humanely euthanized in accordance with institutional guidelines.

**Statistical Analysis**

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

**Ethical Considerations**

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

**Results**

This chapter presents the results of the study, including both descriptive and inferential statistics for aggressive behaviour in male Wistar albino rats. Data were analyzed using One-Way Analysis of Variance (ANOVA) to assess differences among groups, and Tukey's HSD post hoc test was conducted to examine pairwise differences. Descriptive statistics, including mean and standard deviation, were calculated for all variables. Results are presented using tables, bar plots, and box plots to illustrate group comparisons clearly.

**Descriptive Statistics**

**Table 1**

*Descriptive Statistics Showing The Average Number Of Aggression Displayed By Groups (Energy Drink, Alcohol, Combined And Control).*

| | | | | | 95% Confidence Interval Mean | | | |
|---|---|---|---|---|---|---|---|---|
| | *n* | *M* | *SD* | Std.Error | Lower | Upper | Min | Max |
| Energy Drink | 7 | .604 | .2347 | .0887 | .387 | .821 | .380 | .950 |
| Alcohol | 6 | .443 | .1633 | .0667 | .272 | .615 | .180 | .670 |
| Combined | 7 | .499 | .0832 | .0314 | .422 | .575 | .390 | .610 |
| Control | 7 | .706 | .3835 | .1449 | .351 | 1.060 | .320 | 1.350 |

*Note. M* and *SD* represent mean and standard deviation, respectively.

The descriptive statistics indicate variations in aggression among the four groups. The Control group showed the highest mean aggression score ($M$ = .706, $SD$ = .384), followed by the Energy Drink group ($M$ = .604, $SD$ = .235). The Combined ($M$ = .499, $SD$ = .083) and Alcohol ($M$ = .443, $SD$ = .163) groups exhibited lower aggression levels. The 95% confidence intervals overlap among groups, suggesting that differences may not be statistically significant. Overall, the treatment groups tended to show lower aggression than the Control group.

**Table 2:** *One-Way ANOVA Results Showing Mean Aggression Scores*

| | Sum of Squares | df | *MS* | *F* | *P* | $\eta^2$ |
|---|---|---|---|---|---|---|
| Between Groups | .269 | 3 | .090 | 1.486 | > .05 | .162 |
| Within Groups | 1.388 | 23 | .060 | | | |
| Total | 1.657 | 26 | | | | |

*Note.* Type III Sum of Squares.

The One-Way ANOVA was conducted to determine whether there is a significant difference in the levels of Aggression among the four experimental groups; Energy Drink, Alcohol, Combined, and Control group.

The result revealed no statistically significant difference in the mean aggression scores among the groups, $F(3, 23) = 1.486$, $p > .05$ $\eta^2 = .162$. The p-value, being greater than the

significance level of .05, indicates that the variations observed in the mean aggression levels across the different treatment conditions are not statistically significant.

The result therefore suggests that the separate and combined administration of alcohol and energy drinks did not have a significant effect on aggressive behaviour in male Wistar albino rats. In other words, although the descriptive statistics showed that the control and energy drinks groups had slightly higher aggression scores compared to the alcohol and combined groups, these differences were not statistically significant.

**Table 3: Tukey Hsd Post Hoc Test Result Showing Pairwise Comparisons Between Groups.**

| | Energy Drink | Alcohol | Combined | Control |
|---|---|---|---|---|
| Energy Drink | — | | | |
| Alcohol | .161 | — | | |
| Combined | .106 | -.055 | — | |
| Control | -.101 | -.262 | -.207 | — |

*Note.* No pairwise comparisons were significant at $p < .05$. Values represent mean differences (*MD*) between groups.

Although the one-way ANOVA result was not statistically significant, a post hoc analysis using Tukey's Honestly Significant Difference (HSD) test was conducted to further examine pairwise mean differences among the groups. The results revealed that none of the comparisons between groups were statistically significant, as all p-values were greater than the .05 level of significance.

Specifically, the energy drink group did not differ significantly from the alcohol group (*MD* = .161, $p > .05$), the combined group (*MD* = .106, $p > .05$), or the control group (*MD* = -.101, $p > .05$ ).

Similarly, the alcohol group showed no significant difference when compared with the combined group (*MD* = -.055, $p > .05$ ) or the control group (*MD* = -.262, $p > .05$ ). The combined group also did not differ significantly from the control group (*MD* = -.207, $p > .05$).

These results indicate that the small mean differences observed were not statistically significant, which confirms that none of the treatments; Energy Drink, Alcohol, or their Combination produced a significantly different effect on aggression when compared with each other or with the control group.

Following the results in Table 3 above of the Post Hoc Test (Tukey HSD), each hypothesis was was interpreted accordingly as follows;

Hypothesis I: The hypothesis stated that *There will be a significant effect of Energy Drinks on Aggressive behaviour among Male Wistar albino rats.* The result shows that the energy drink group had a mean aggression score of .604, which is slightly lower than the control group's mean score of .706, indicating a small reduction in aggression. However, this difference was

not statistically significant ($p$ >.05). Therefore, the hypothesis that there will be a significant effect of Energy Drinks on Aggressive behaviour among Male Wistar albino rats is rejected.

Hypothesis II: The hypothesis stated that *There will be a significant effect of Alcohol on Aggressive behaviour among Male Wistar albino rats,* the result shows that the alcohol group had a mean aggression score of .443, which is lower than the control group's mean score of .706, suggesting a decrease in aggressive behaviour. Nevertheless, this difference was not statistically significant ($p$ >.05). Therefore, the hypothesis that there will be a significant effect of Alcohol on Aggressive behaviour among Male Wistar albino rats is rejected.

Hypothesis III: The hypothesis stated that *There will be a significant combined effect of Alcohol and Energy Drinks on Aggressive behaviour among Male Wistar albino rats.* The result shows that the combined group had a mean aggression score of .499, which is lower than the control group's mean score of .706, implying reduced aggression. However, this difference was not statistically significant ($p$ >.05). Hence, the hypothesis that there will be a significant combined effect of Alcohol and Energy Drinks on Aggressive behaviour among Male Wistar albino rats is rejected.

*Bar Plot showing the mean aggression scores with 95% confidence intervals for the four groups (Energy Drink, Alcohol, Combined and Control).*

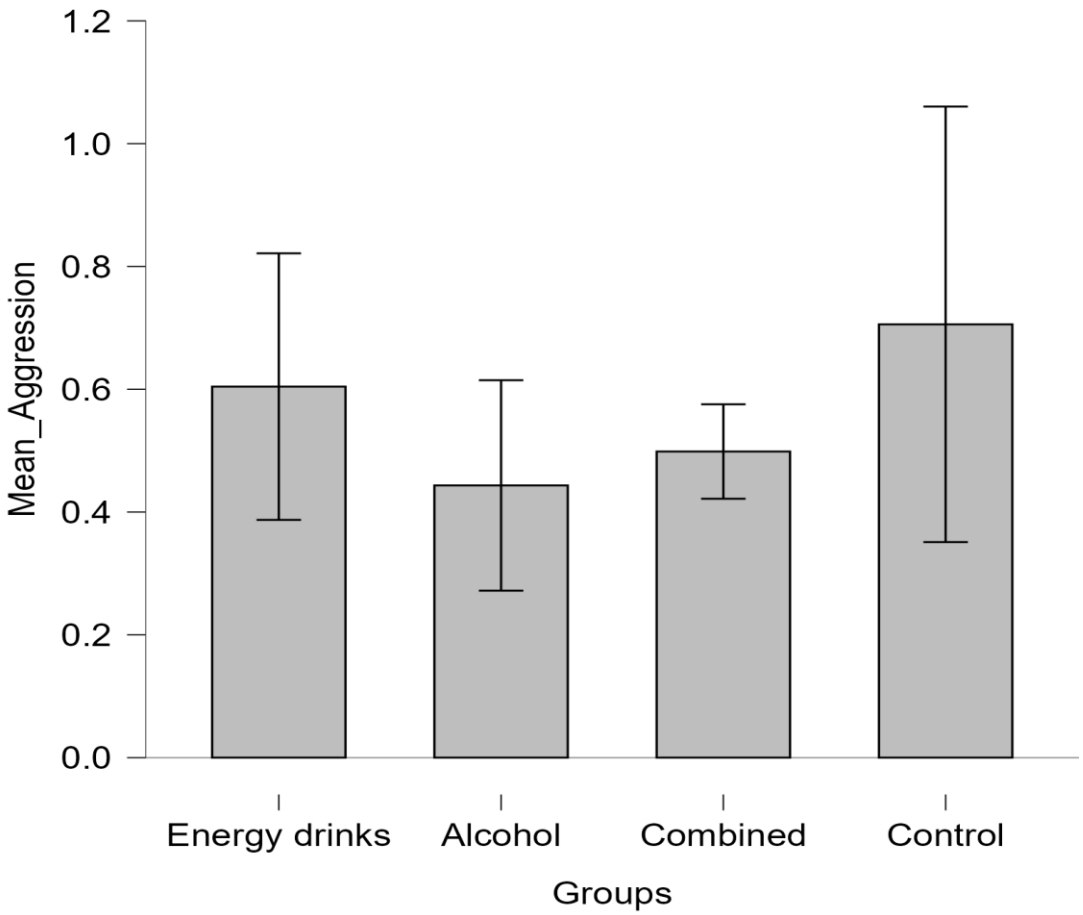


**Figure 1**

The bar plot illustrates the mean levels of aggression displayed by each group. From the illustration above, it can be observed that the control group recorded the highest mean level

of aggression, followed closely by the energy drink group. The combined group and alcohol group showed comparatively lower mean aggression scores with the alcohol group being slightly lower than the combined group .

The error bars represent the 95% confidence intervals, which overlap considerably across the groups indicating that the differences observed among the means are not statistically significant. This visual overlap supports the ANOVA and post hoc results, which showed no significant differences in aggression levels between the groups.

*Box plot showing the distribution of aggression scores across the four groups (Energy Drink, Alcohol, Combined and Control).*

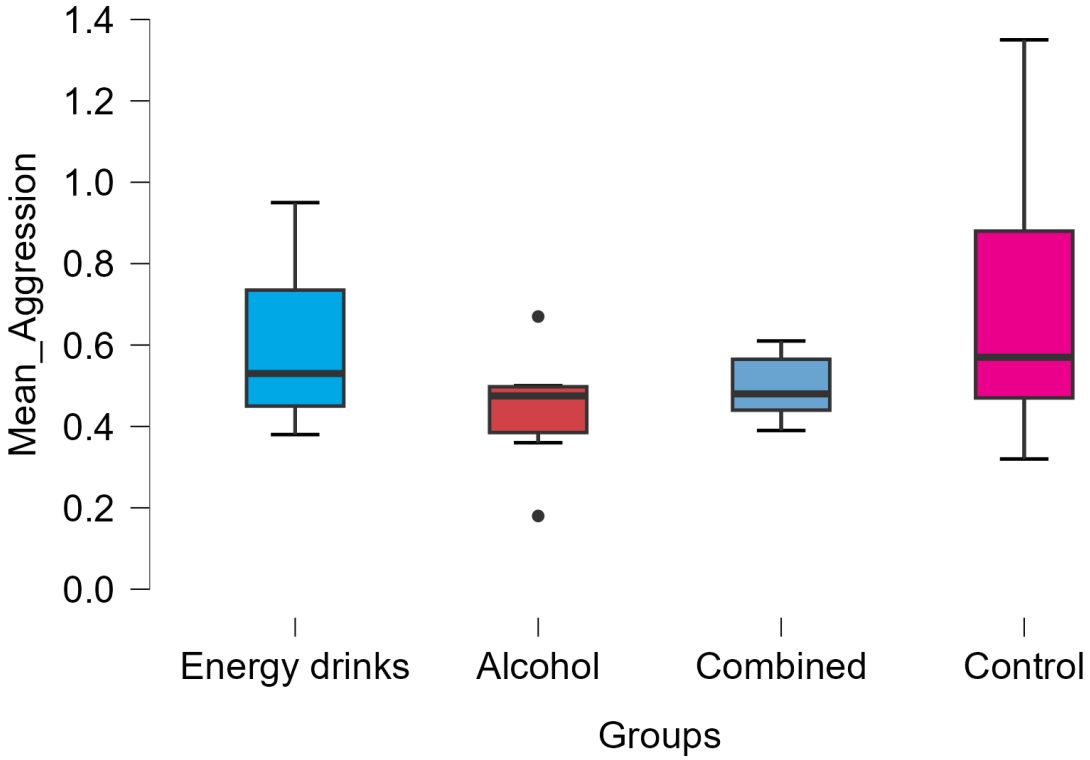


**Figure 2**

The box plot illustrates the spread, median, and variability of aggression scores for each group. The control group shows the widest range, indicating greater variability in aggression among the rats in that condition. The energy drink group also exhibits moderate variability, while the combined and alcohol groups display narrower boxes, suggesting more consistent or lower aggression level.

Two outliers are visible in the alcohol group, represented by points outside the whiskers, indicating that a few rats in that group displayed aggression levels that differed notably from the rest. The median aggression levels across all groups are relatively close, and the interquartile ranges overlap considerably.

The box plot shows that while the control and energy drinks groups recorded slightly higher median aggression than the alcohol and combined groups, the overlap and presence of outliers suggest that the differences among the groups are not significant. This aligns with the

ANOVA and post hoc results, confirming that there were no statistically significant differences in aggression levels across the groups.

**Summary of findings**

Descriptive statistics indicated variations in mean aggression scores among the four experimental groups. The control group showed the highest aggression ($M$ = .706, $SD$ = .384), followed by the energy drink group ($M$ = .604, $SD$ = .235). The alcohol ($M$ = .443, $SD$ = .163) and combined alcohol-energy drink groups ($M$ = .499, $SD$ = .083) showed lower scores. One-way ANOVA revealed no significant differences among groups, $F(3, 23) = 1.486$, $p > .05$, $\eta^2 = .162$. Post hoc Tukey comparisons confirmed that none of the pairwise differences were significant. Visual illustration of bar and box plots supported these results. Overall, all three hypotheses were rejected: alcohol, energy drinks, and their combination did not significantly affect aggressive behaviour in male Wistar albino rats.

**Discussion**

This study investigated the separate and combined chronic effects of alcohol and energy drinks on aggressive behaviour in male Wistar albino rats. Contrary to expectations, no statistically significant differences were observed among the treatment groups and the control group. These findings suggest that long-term administration of alcohol, energy drinks, or their combination did not reliably potentiate overt aggressive behaviour under the experimental conditions.

*Effects of Energy Drinks on Aggression*

The absence of significant aggression in the energy drink group aligns with recent animal and human studies showing that caffeine—energy drinks' primary psychoactive component—does not consistently increase aggression, especially under chronic exposure (Temple et al., 2019; Richards & Smith, 2022). Caffeine primarily acts as an adenosine A1/A2A receptor antagonist, increasing arousal and psychomotor activation without consistently eliciting offensive aggression (Nehlig, 2018; Ribeiro & Sebastião, 2019).

Chronic caffeine exposure induces neuroadaptive changes that can reduce excitability over time, including downregulation of adenosine receptors and modulation of dopaminergic and glutamatergic signaling (Batel et al., 2020). Recent neurobehavioral evidence suggests that taurine—abundant in energy drinks—may exert anxiolytic and inhibitory effects by modulating GABAergic transmission, which could counteract stimulant-induced agitation (Jong et al., 2023). These mechanistic insights support the non-aggressive behavioural outcomes observed in this study.

*Effects of Alcohol on Aggression*

Although acute alcohol intake is widely associated with increased impulsivity and aggression, chronic exposure often produces the opposite outcome—behavioural suppression—due to cumulative central nervous system (CNS) depressant effects (Miczek et al., 2018; Heinz et al., 2020). Long-term alcohol administration disrupts serotonergic, GABAergic, and glutamatergic pathways, frequently leading to reduced motor activity, sedation, and diminished responsiveness (Abrahao et al., 2017; Koob et al., 2023).

Recent studies have shown that prolonged ethanol exposure increases oxidative stress and neuroinflammation, particularly in the hippocampus and prefrontal cortex—changes that typically manifest as cognitive slowing and decreased behavioural reactivity rather than

aggression (Almeida-Silva et al., 2021; Matthews et al., 2022). This aligns with the lethargy and behavioural dampening observed in the alcohol-treated rats.

*Combined Effects of Alcohol and Energy Drinks*

The combined administration of alcohol and energy drinks did not produce increased aggression relative to controls. Contemporary research shows that caffeine can partially mask the sedative effects of alcohol, creating a behavioural profile often termed the "wide-awake drunk" phenomenon (Marczinski & Fillmore, 2014; Peacock et al., 2018). However, masking does not reverse alcohol-induced neurochemical suppression or neurotoxicity.

Recent rodent studies indicate that chronic co-exposure alters the balance of dopaminergic, noradrenergic, GABAergic, and glutamatergic transmission while elevating oxidative stress markers (Petribu et al., 2023; Cameron et al., 2021). These changes frequently result in behavioural blunting, reduced locomotion, and neuroadaptive downregulation of excitatory pathways, consistent with the mild reductions in aggression observed in this study.

Taken together, the findings suggest that chronic exposure to alcohol, energy drinks, or their combination exerts modulatory—not amplifying—effects on aggression, likely mediated by complex, dose-dependent neurochemical interactions.

## Limitations

Several limitations should be acknowledged:

Sex-Specific Restriction: Only male rats were used; findings may not generalize to females, whose hormonal cycles influence behavioural and neurochemical responses to psychoactive substances.

Single-Dose Design: The study used fixed doses of alcohol and energy drinks, limiting insights into dose–response relationships.

Behavioural-Only Assessment: Absence of biochemical or histological analyses restricted mechanistic interpretation of neurotransmitter changes or brain-region-specific effects.

Chronic Exposure Focus: Acute behavioural responses, which often differ markedly from chronic effects, were not assessed.

## Conclusion

This study found no significant effects of chronic alcohol, energy drink, or combined administration on aggressive behaviour in male Wistar albino rats. Qualitative behavioural observations, however, indicated that energy drink exposure produced hyperactivity and heightened arousal, whereas alcohol and combined treatments yielded signs of CNS depression. These patterns reflect established neuropharmacological profiles, suggesting that chronic stimulant or depressant exposure influences general arousal states but may not sufficiently modulate aggression under controlled laboratory conditions.

The findings contribute to the growing evidence that chronic co-use of alcohol and energy drinks does not reliably increase aggression, while nonetheless posing potential neurobiological risks due to oxidative stress, neuroinflammation, and neurotransmitter dysregulation.

## Recommendations

(i) Regulatory oversight should be strengthened regarding alcohol–energy drink co-marketing, given documented risks related to impaired judgment and risk-taking.

(ii) Health education programs, especially targeting young adults, should highlight the dangers of regular consumption of highly caffeinated beverages and their combination with alcohol.

(iii) Clear labeling requirements for caffeine and stimulant content should be enforced to limit excessive intake.

(iv) Studies should incorporate dose–response paradigms to examine varying concentrations of alcohol and caffeine/taurine.

(v) Future investigations should examine acute, sub-chronic, and chronic exposure patterns for better translational validity.

(vi) Studies should include female animals to explore sex-based differences in aggression, neurotoxicity, and substance response.

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