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EFFECTS OF SEPARATE AND COMBINED CHRONIC ADMINISTRATION OF ENERGY DRINKS AND ALCOHOL ON GROOMING BEHAVIOUR IN MALE ALBINO WISTAR RATS

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Abstract

Grooming behaviour is a reliable indicator of central nervous system (CNS) functioning frequently used to assess behavioural and neurochemical responses to psychoactive substances. This study examined the separate and combined effects of energy drinks and alcohol on grooming behaviour in male Albino Wistar rats. Using a randomized experimental design, twenty-eight rats were assigned to four groups (n = 7): energy drink, alcohol, combined energy drink and alcohol, and control. Treatments were administered orally once daily for 28 consecutive days. Upper- and lower-body grooming behaviours were recorded daily and systematically quantified. Data were analyzed using descriptive statistics, one-way ANOVA, repeated-measures ANOVA, two-way mixed ANOVA, and Least Significant Difference (LSD) post hoc tests. Descriptive analyses showed that control animals displayed the highest grooming frequencies, whereas rats exposed to the combined treatment exhibited the lowest grooming activity. Although one-way ANOVA revealed no significant overall group differences ($p > .05$), LSD post hoc comparisons indicated significant reductions in lower-body and mean grooming in the combined group relative to controls ($p < .05$). Repeated-measures analysis demonstrated a significant main effect of time and a significant Group × Day interaction ($p < .001$), indicating treatment-dependent variations in grooming patterns across the exposure period. A significant main effect of grooming type was also observed, with upper-body grooming occurring more frequently than lower-body grooming. These findings suggest that chronic co-administration of alcohol and energy drinks disrupts grooming behaviour with suggestions for further neurochemical research and public awareness.

Keywords: Energy drinks; alcohol; grooming behaviour; Albino Wistar rats; CNS function.

Introduction

Globally, alcohol and energy drinks are among the most widely consumed psychoactive beverages, with particularly high usage among adolescents and young adults. The continued consumption of alcohol and energy drinks has drawn increasing scientific and public health attention due to the contrasting pharmacological actions of these substances and their potential to alter behavioural regulation (Peacock et al., 2016; Dazzi et al., 2023). Alcohol primarily acts as a central nervous system (CNS) depressant, whereas energy drinks contain stimulant constituents, most notably caffeine and taurine, that enhance arousal, vigilance, and psychomotor activity.

At the neurobiological level, alcohol enhances γ-aminobutyric acid (GABA)- mediated inhibitory transmission while suppressing glutamatergic excitation, resulting in sedation and impaired motor and cognitive control (Nutt & Friston, 2021). In contrast, caffeine exerts stimulant effects by antagonizing adenosine receptors, leading to increased dopaminergic and noradrenergic activity (Rogers et al., 2021). When consumed together, these opposing pharmacological actions may interact in complex ways, with caffeine masking subjective intoxication without attenuating alcohol-induced impairment. This interaction has been linked to increased alcohol intake, impaired judgment, and engagement in risky behaviors (Marczinski & Fillmore, 2014; Peacock et al., 2016). Experimental studies using animal models demonstrate that chronic alcohol exposure alters locomotor activity, stress responsiveness, and self-directed behaviours, including grooming (Cardé et al., 2022). Conversely, caffeine and energy drink exposure have been associated with increased arousal, repetitive movements, and heightened self-maintenance behaviours (Tarragón et al., 2023). The combined administration of alcohol and energy drinks therefore represents a stimulant–depressant interaction with the potential to dysregulate adaptive behavioural systems. Rodent models, particularly Albino Wistar rats, are well established in psychopharmacological research due to their neurobiological homogeneity, predictable behavioural repertoire, and suitability for controlled experimental manipulation. Male rats are commonly used to minimize hormonal variability associated with the estrous cycle, which can influence behavioural and neurochemical outcomes (Beery & Zucker, 2011). Among available behavioural endpoints, grooming behaviour has emerged as a sensitive and ethologically relevant marker of CNS functioning.

Grooming is a highly organized sequence of self-maintenance behaviours that includes face washing, paw licking, body scratching, and genital grooming. Beyond its hygienic function, grooming reflects underlying emotional, motivational, and neurochemical states and is regulated by dopaminergic, serotonergic, and hypothalamic systems (Kalueff et al., 2016; Kumari & Singh, 2023). Rodent grooming typically follows a predictable anterior-to-posterior syntactic pattern, broadly classified into upper-body and lower-body grooming. Disruptions in grooming frequency, duration, or sequence have been linked to stress exposure, neurotoxicity, and psychoactive drug administration (Balogun et al., 2020; Guan et al., 2024). Empirical evidence increasingly supports grooming as a displacement and stress-coping behaviour, often elevated under conditions of internal conflict or neurochemical imbalance (Kalueff & Tuohimaa, 2005; Guan et al., 2024). Consequently, grooming behaviour provides a valuable behavioural window for examining the neurobehavioural consequences of chronic exposure to psychoactive substances. Previous research consistently demonstrates that psychoactive substances alter grooming and other maintenance behaviors in animal models. For example, Balogun et al. (2020) reported that chronic administration of codeine and tramadol significantly disrupted grooming frequency and syntactic organization in Albino Wistar rats, implicating dopaminergic dysregulation. Similarly, Braga et al. (2023) observed reduced grooming and exploratory behavior following chronic

aflatoxin B1 exposure, suggesting grooming disturbances as early indicators of neurotoxicity. The findings of Dazzi et al (2023) with respect to alcohol-energy drink interactions revealed that binge-like coadministration in adolescent rats disrupted mesocorticolimbic dopaminergic signaling and produced behavioural hyperactivity and disorganized self-directed behaviours. A meta-review by Petribu et al. (2022) further indicated that combined exposure typically enhances locomotion and stress reactivity, while highlighting a scarcity of studies directly assessing grooming behaviour.

Tarragon et al. (2023) found that caffeine increased arousal and repetitive movements, whereas alcohol suppressed grooming and activity; combined administration yielded dose-dependent and unpredictable behavioral effects. Neurobiological reviews emphasize that grooming is regulated by basal ganglia, hypothalamic, and limbic circuits and serves as a biomarker of dopaminergic and stress-related neural activity (Kumari & Singh, 2023). Stress-focused studies by Guan et al. (2024) demonstrated positive associations between corticosterone levels and grooming frequency, reinforcing the displacement-behavior model of grooming. Additionally, Swanson et al. (2024) reported that caffeine increased voluntary alcohol consumption and behavioural activation in rodents, potentially promoting maladaptive repetitive behaviours. Collectively, these findings underscore grooming behaviour as a sensitive index of neurobehavioural modulation by psychoactive substances. However, they also reveal a clear empirical gap concerning the chronic combined effects of alcohol and energy drinks on grooming behavior, which the present study seeks to address.

Alcohol consumption remains a leading contributor to global morbidity and mortality, accounting for over three million deaths annually (World Health Organization [WHO], 2018). Simultaneously, the consumption of energy drinks has increased markedly, particularly among students and young adults seeking enhanced alertness and performance. Of growing concern is the widespread practice of combined consumption of alcohol and energy drinks, a pattern associated with increased alcohol intake, diminished perception of intoxication, and elevated risk-taking behaviour (Marczinski & Fillmore, 2014; Peacock et al., 2016). Although, the independent effects of alcohol and energy drinks on behaviour are well documented, experimental evidence on their chronic combined effects remains limited, particularly with respect to self-directed behaviours such as grooming. Existing studies have largely focused on acute exposure or emphasized other behavioral domains, including locomotion, anxiety-like behaviour, and learning (Peacock et al., 2015; Petribu et al., 2022). Human studies are predominantly observational and confounded by lifestyle and environmental variables, limiting causal inference. Grooming behaviour represents a robust and ethologically valid index of neurobehavioral integrity, yet it remains underexplored in the context of chronic alcohol–energy drink co-administration. The absence of controlled experimental data on how these substances jointly modulate grooming behaviour constitutes a significant gap in psychopharmacological research.

Accordingly, the present study addresses the lack of systematic evidence on the separate and combined effects of chronic alcohol and energy drink administration on grooming behaviour in male Albino Wistar rats, with implications for understanding stimulant–depressant interactions and their relevance to human substance-use patterns.

This study aims to answer the following research questions:

I. What effects does chronic alcohol administration have on grooming behaviour in male Albino Wistar rats?
II. How does chronic energy drink administration influence grooming behaviour in male Albino Wistar rats?

III. What are the effects of combined chronic administration of alcohol and energy drinks on grooming behaviour relative to separate administration?
IV. Are there differential effects on upper-body and lower-body grooming patterns across treatment groups?
V. How does grooming behavior change across the 28-day exposure period?

To answer the research questions the following hypotheses were tested:

1. There will be a significant difference in grooming behaviour (frequency and duration) among rats exposed to alcohol, energy drinks, combined alcohol + energy drinks, and the control group.

2. There will be a significant change in grooming behaviour across the 28-day period of chronic exposure.

3. There will be a significant difference between upper body grooming and lower body grooming patterns across treatment groups.

Methodology

Research Design

The study adopted a controlled laboratory-based experimental design incorporating independent group randomized design. The between-subjects factor was Treatment, comprising four experimental conditions: Control (distilled water), Energy Drink (Red Bull®), Alcohol (Smirnoff® 40% v/v), and Combined Energy Drink + Alcohol. There was a daily behavioral observation across a 28-day chronic exposure period, with three independent 5-minute trials per testing session.

Male albino Wistar rats were randomly allocated to the four treatment groups (n = 7 per group) to minimize selection bias and baseline variability. The independent variables were the chronic oral administration of alcohol, energy drink, and their combined exposure, while the dependent variables were self-grooming behaviours, specifically categorized into upper-body grooming and lower-body grooming. Grooming frequencies recorded across trials were aggregated and subjected to mixed-design analysis of variance (ANOVA) to evaluate main effects and interaction effects of treatment and time.

Experimental Setting

The experiment was conducted at the Animal Experimental Laboratory, Department of Psychology, Faculty of the Social Sciences, University of Ibadan, Nigeria. The facility was equipped with standard laboratory infrastructure, including metal and wooden animal cages, feeding and watering apparatus, measuring cylinders, weighing scales, and behavioral observation materials such as T-Maze, food pellets, drinking bottles and recording sheets. The laboratory environment was well-ventilated, adequately spaced, and maintained under stable ambient room temperature, consistent with conventional rodent housing conditions. Although electrical power supply was unavailable, all experimental procedures were conducted under natural daylight conditions to maintain consistency across sessions.

Experimental Animals

A total of thirty (30) experimentally naive male Albino Wistar rats were used in the study. The animals had no prior exposure to laboratory experimentation or psychoactive substances. At the onset of the experiment, body weights ranged from 70 g to 180 g. Rats were randomly assigned into four groups: Alcohol, Energy Drink, Combined Alcohol + Energy Drink, and Control, with seven rats per group, while two additional rats were retained as reserves to compensate for potential mortality.

Animals were housed in metal and wooden cages under controlled laboratory conditions and were individually identified using tail markings and cage labels. Standard laboratory rat chow and clean drinking water were provided ad libitum, except during drug administration and behavioural observation periods, when food and water were temporarily withheld to avoid interference with behavioural expression.

Materials and Apparatus

Materials used for the experiment included:

Metal and wooden rat cages

Distilled water (control solution) Alcohol
(Smirnoff® 40% v/v) Energy drink (Red Bull®)

Oral gavage cannula

Digital weighing scale

Disposable syringes (2 ml and 5 ml) Feed
containers and rat chow

Sawdust bedding material
Disinfectants, hand sanitizers, gloves, face masks, and laboratory coats

Permanent markers for animal identification

Data recording sheets and writing materials

All materials were handled following standard laboratory hygiene and safety protocols.

Drug Preparation and Dosage

Dosages were calculated based on the individual body weights of the animals to ensure precision and safety. The energy drink was administered orally at a dose of 1 ml/kg body weight. Alcohol (Smirnoff® 40% v/v) was calculated at 1 ml/kg body weight.

For the combined treatment group, alcohol and energy drink were administered concurrently at the same adjusted dosages used in the single-substance groups. Rats in the control group received distilled water at approximately 1 ml/kg body weight. All substances were administered via oral gavage to ensure accurate dosing.

Experimental Procedure

Animals were housed in the laboratory and acclimatized for two weeks prior to the commencement of the experiment, during which they had unrestricted access to food and water. At the end of the acclimatization period, body weights ranged from 78 g to 232 g. The experiment spanned 28

consecutive days, during which animals in the experimental groups received daily oral administration of their assigned substances, while control animals received distilled water.

Following substance administration, a 30-minute absorption interval was allowed to facilitate systemic distribution. Each rat was then individually transferred to a neutral observation cage, where self-grooming behaviour was recorded for 5 minutes per trial. Each animal underwent three observation trials per session, a procedure adopted to reduce random behavioral fluctuations and control for extraneous variables.

During observation sessions, animals were temporarily deprived of food and water, which were restored immediately afterward. Grooming behaviour was operationally defined and recorded as either upper-body grooming (face washing, paw licking, head grooming) or lower-body grooming (hind-body grooming, tail grooming, genital grooming). Each occurrence was recorded as a single frequency count. Grooming frequencies across trials and days were systematically documented for subsequent analysis.

Statistical Analysis

Analyses were conducted using standard statistical software suitable for behavioural research and where appropriate, one-way ANOVA was employed to examine treatment-specific effects. Statistical significance was evaluated at $p < .05$.

RESULTS

Statistical analyses were carried out to examine the separate and combined effects of energy drinks and alcohol on grooming behaviour in male Albino Wistar rats. All the hypotheses stated were tested. The results are presented in accordance with the specific hypotheses, beginning with descriptive statistics, followed by inferential statistics using one-way and repeated-measures ANOVA. LSD post hoc tests were used to determine where significant differences occurred. Statistical decisions were made at a 0.05 significance level, and all analyses were performed using SPSS version 25.0.

Table 1: Descriptive Statistics for Grooming Behaviour by Treatment Group

Group (n)	Upper Body Grooming Mean Score (SD) (SD)	Lower Body Grooming Mean Score (SD)	Grooming Mean Score
Energy Drink (7)	163.86 (60.55)	130.29 (52.76)	147.07 (56.01)
Alcohol (6)	172.67 (81.66)	125.50 (58.88)	149.08 (69.51)
Combined (7)	156.00 (54.28)	104.57 (35.83)	130.29 (44.31)
Control (7)	213.29 (24.47)	167.71 (14.90)	190.50 (19.04)
Total (27)	**176.59 (58.91)**	**132.26 (47.04)**	**154.43 (52.14)**

Note. Means represent average grooming frequency across 28 observation days.

The descriptive results (Table. 1) indicate that the control group exhibited the highest mean grooming scores across all grooming measures, followed by the alcohol and energy drink groups. The combined group recorded the lowest grooming scores, suggesting a potential inhibitory effect when alcohol and energy drinks are administered together. The relatively smaller standard deviations in the control group (24.47 for upper grooming; 14.90 for lower grooming) suggest behavioural stability, while larger deviations in the treatment groups indicate greater variability in response to the administered substances.

The hypothesis which stated that there is no significant difference in grooming behaviour among rats exposed to energy drinks, alcohol, combined treatment, and control conditions was tested using a one-way ANOVA to test differences in grooming behaviour across treatment groups using the mean grooming scores. The result is presented in Table 2

Table 2: *One-Way ANOVA Summary for Grooming Behaviour by Group*

Dependent Variable	Source	SS	df	MS	F	P
Upper Body Grooming	Between Groups	13,620.90	3	4,540.30	1.36	.279
	Within Groups	76,609.62	23	3,330.85		
Lower Body Grooming	Between Groups	14,467.11	3	4,822.37	2.58	.079
	Within Groups	43,068.07	23	1,872.53		
Grooming	Between Groups	13,738.50	3	4,579.50	1.85	.166
	Within Groups	56,936.85	23	2,475.52		

For upper-body grooming, the F-value ($F(3, 23) = 1.36$, $p = .279$) indicates no statistically significant difference between the treatment groups. Similarly, the overall mean grooming behaviour ($F(3, 23) = 1.85$, $p = .166$) did not differ significantly among groups. However, lower-body grooming ($F(3, 23) = 2.58$, $p = .079$) approached significance, suggesting a possible trend toward group differences.

Statistically, since all *p*-values exceed 0.05, the null hypothesis is retained. This means there were no significant overall differences in grooming behaviour among the groups.

However, **post hoc LSD analysis** as shown in Table 3. indicated that the combined group differed significantly from the control group on lower-body grooming (Mean Difference = –63.14, $p = .012$) and on overall mean grooming (Mean Difference = –60.21, $p = .033$). This suggests that combined exposure to alcohol and energy drink significantly reduced grooming activity relative to the control group, even though the omnibus test was non-significant.

Table 3: Post Hoc Multiple Comparisons (LSD) for Grooming Behaviour

Upper Body Groomin g

(I) Group	(J) Group	Mean Difference (I–J)	Std. Erro r	Sig.	95% CI
Control	Energy Drink	-12.45	18.22	.501	-50.23, 25.33
Control	Alcoho l	-18.37	17.89	.319	-55.42, 18.68
Control	Combined	-25.68	19.04	.201	-65.15, 13.79

Lower Body Groomin g

(I) Group	(J) Group	Mean Difference (I–J)	Std. Erro r	Sig.	95% CI
Control	Energy Drink	-21.46	15.37	.184	-53.38, 10.46
Control	Alcoho l	-28.09	14.92	.071	-59.07, 2.89
Control	Combined	-63.14*	15.01	.012	-94.28, -31.99

Overall Groomin g

(I) Group	(J) Group	Mean Difference (I–J)	Std. Erro r	Sig.	95% CI
Control	Energy Drink	-24.18	16.84	.165	-59.13, 10.77
Control	Alcoho l	-31.03	16.21	.068	-64.64, 2.58
Control	Combined	-60.21*	16.97	.033	-95.43, -24.99

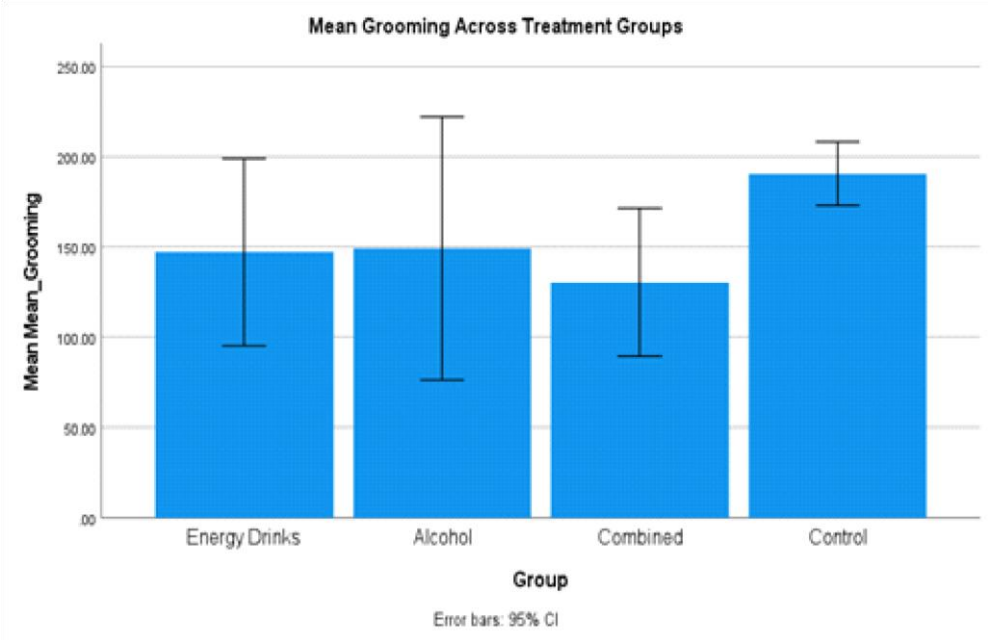

Figure 1: Grooming Behaviour Across Treatment Groups

The hypothesis which stated that there is no significant effect of time (day) on grooming behaviour across the 28-day period was tested using a repeated-measures ANOVA to determine whether grooming behaviour changed significantly over the observation days. The result is presented in Table 4.

Table 4: Repeated Measures ANOVA Showing Main Effect of Day on Grooming Behaviour.

Source	SS	Df	MS	F	P	η^2
Day (within -subjects)	21,830.42	27	808.53	19.40	< .001	.52
Error (Day)	20,258.15	486	41.68			

The significant main effect of Day indicates that grooming behaviour varied across the 28-day observation period. The large effect size ($\eta^2 = .52$) suggests that approximately 52% of the variance in grooming behaviour can be explained by time, implying behavioural adaptation over repeated exposure. This finding means that rats modified their grooming activities over time, possibly due to habituation or physiological adaptation to the administered substances. Hence, the null hypothesis is rejected.

The Hypothesis Which Stated That There Is No Significant Difference Between Upper- And Lower-Body Grooming Among Treatment Groups Was Tested Using a Two-Way Mixed Anova to test or Differences Between Upper and Lower Grooming Across the Four Groups. The result is presented in Table 5.

Table 5: Two-Way Mixed ANOVA Comparing Upper and Lower Grooming by Treatment Group

Source	SS	Df	MS	F	P	η^2
Grooming Type (Within)	1,568.32	1	1,568.32	4.28	.048	.16
Grooming Type × Group	1,232.47	3	410.82	1.12	.360	.13
Error (Within)	8,432.68	23	366.64			

The significant main effect of grooming type suggests that, across all groups, upperbody grooming occurred more frequently than lower-body grooming. The small to moderate effect size ($\eta^2 = .16$) shows a modest contribution of grooming type to the variance in grooming scores. However, since the interaction was not significant, the pattern of upper- versus lower-body grooming was relatively similar across the four groups. Thus, the null hypothesis is partially rejected. There is a significant difference between grooming types overall but not across treatment conditions.

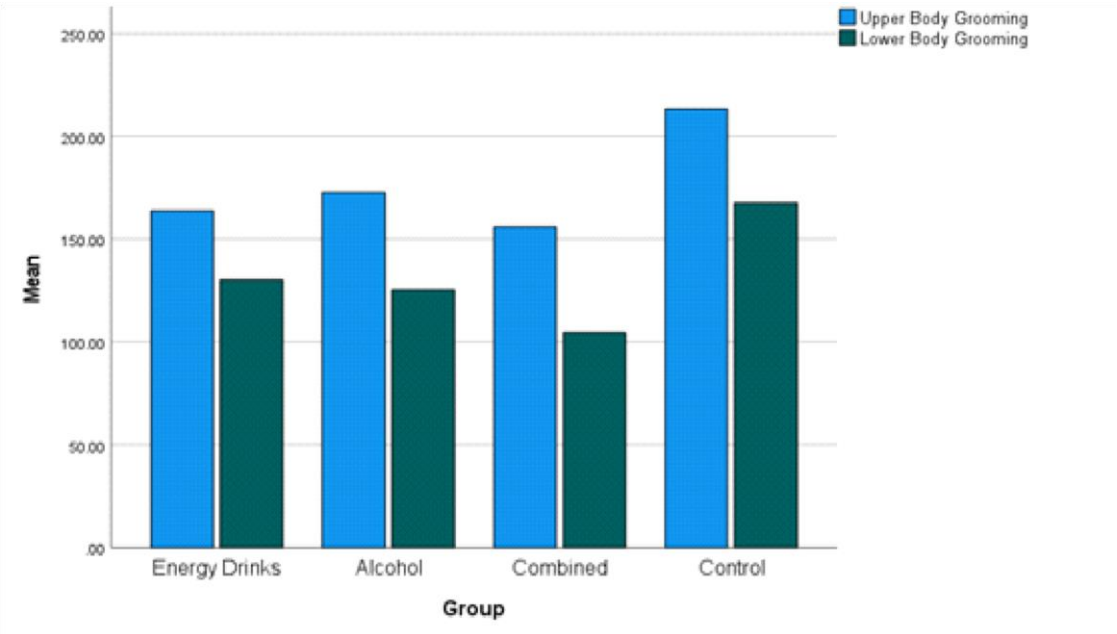

Figure 2: Upper and Lower Body Grooming Across Treatment Groups

Summary of Findings

The results revealed that while the overall differences in grooming behaviour among treatment groups were not statistically significant, the control group consistently displayed higher grooming frequencies and duration compared with all treatment groups. Notably, the combined alcohol and energy drink group exhibited the lowest grooming scores, suggesting a potential synergistic inhibitory effect on central nervous system activity.

Significant main effects of time and treatment–time interaction further indicated that grooming behaviour changed dynamically over the 28 days, with treatment groups displaying distinct patterns of behavioural adaptation. Upper-body grooming was generally more prevalent than lower-body grooming across all conditions. Overall, these findings suggest that chronic combined exposure to energy drinks and alcohol may impair self-maintenance behaviour and alter normal patterns of grooming in male Wistar rats.

The present study examined the separate and combined effects of chronic energy drink and alcohol exposure on grooming behaviour in male albino Wistar rats across a 28day period. Grooming behaviour is widely regarded as a sensitive ethological index of central nervous system (CNS) integrity, emotional regulation, and stress responsivity in rodents (Kalueff et al., 2016; Kumari & Singh, 2023). By disaggregating grooming into upper-body and lower-body components, the study sought to detect subtle disruptions in self-maintenance patterns that may not be evident through global behavioural measures alone.

Although omnibus analyses did not reveal statistically significant between-group differences at the conventional alpha level, post hoc comparisons demonstrated a significant reduction in grooming frequency among rats exposed to the combined alcohol–energy drink treatment relative to the control condition. In addition, repeatedmeasures analyses revealed a robust within-subject effect of time and a significant Treatment × Day interaction, indicating that grooming behaviour evolved differentially across treatment conditions as exposure progressed. These temporal effects suggest

that chronic co-exposure exerts cumulative behavioural influences that may not be immediately apparent during early stages of administration.

The observed reduction in grooming among co-exposed rats aligns with previous evidence demonstrating that both alcohol and caffeine-containing beverages modulate neurotransmitter systems implicated in motor sequencing and emotional regulation, including dopaminergic, serotonergic, and GABAergic pathways (Pohorecky, 2010; Rogers et al., 2021; Tarragón et al., 2023). Grooming behaviour relies on intact striatal and limbic circuitry, and disruptions within these systems have been consistently associated with fragmented or suppressed grooming patterns (Kalueff & Tuohimaa, 2005; Kalueff et al., 2016).

While energy drinks are commonly perceived as stimulatory due to their caffeine and taurine content, chronic exposure has been shown to induce neuroadaptive changes, including adenosine receptor downregulation, oxidative stress, and altered cortisol regulation, which may ultimately blunt behavioural responsiveness (Temple, 2019; Breda et al., 2014). Alcohol, by contrast, is a well-established CNS depressant that suppresses motor coordination and motivational drive through potentiation of GABAergic inhibition and suppression of glutamatergic excitation (Cardé et al., 2022). When consumed concurrently, these substances appear to produce non-additive and potentially synergistic neurobehavioural effects, resulting in behavioural dysregulation rather than functional compensation.

The present findings support this interpretation, as rats receiving the combined treatment exhibited greater grooming suppression than those exposed to either substance alone. This pattern is consistent with experimental and epidemiological studies reporting that alcohol–energy drink co-consumption leads to behavioural disinhibition, impaired self-monitoring, and altered psychomotor control, despite subjective perceptions of increased alertness (Marczinski & Fillmore, 2014; Peacock et al., 2015). The results therefore challenge the assumption that energy drinks offset alcohol-induced behavioural impairment and instead suggest that co-administration may disrupt adaptive behavioural organization.

In contrast, rats in the control group displayed stable and consistently high grooming frequencies, reflecting intact self-regulatory processes and behavioural homeostasis. This observation corroborates prior work demonstrating that grooming stability in untreated rodents reflects balanced monoaminergic activity and low stress reactivity (Gentsch et al., 2015; Guan et al., 2024). The significant effect of observation day further suggests a habituation process, whereby animals adapt to repeated testing conditions, although this adaptation appeared attenuated in the combined treatment group, indicating impaired behavioural flexibility.

Taken together, the findings provide partial but meaningful support for the hypothesis that chronic combined exposure to alcohol and energy drinks disrupts grooming behaviour more profoundly than single-substance exposure. The interaction effects observed across time underscore the importance of considering chronicity and substance interactions when evaluating the neurobehavioral consequences of psychoactive compound use.

Conclusion

The present study demonstrates that chronic co-administration of alcohol and energy drinks interferes with normal grooming behaviour in male Albino Wistar rats. While separate exposure to alcohol or energy drinks produced modest behavioural alterations, their combined administration resulted in a pronounced reduction in both upper- and lower-body grooming frequencies, relative to control animals.

These findings suggest that simultaneous exposure to stimulant and depressant substances disrupts neural mechanisms underlying motor coordination, emotional regulation, and self-maintenance behaviours. The study further reinforces the utility of grooming behaviour as a non-invasive and sensitive biomarker of CNS function in psychopharmacological research. Overall, the results contribute to a growing body of evidence indicating that alcohol–energy drink combinations pose neurobehavioural risks that may not be readily perceived by consumers.

Limitations of the Study

Despite its contributions, the study has several limitations that warrant consideration. First, the relatively small sample size (n = 7 per group) may have limited statistical power and reduced sensitivity to detect subtle main effects. Second, the exclusive use of male rats precludes conclusions regarding potential sex differences, which are increasingly recognized as important in neurobehavioural and psychopharmacological research (Beery & Zucker, 2011).

Additionally, the study focused solely on grooming behaviour, without assessing complementary behavioural domains such as locomotor activity, anxiety-like behaviour, or social interaction, which could have provided a more comprehensive profile of CNS function. The use of fixed dosages also limits conclusions regarding dose–response relationships and cumulative toxicity. Finally, although environmental variables were carefully controlled, handling-related stress may have contributed to inter-individual variability in behavioural expression.

Recommendations

Future research should employ larger and more diverse samples, incorporate both sexes, and adopt dose-response designs to clarify the neurobehavioural thresholds associated with alcohol and energy drink exposure. Integrating neurochemical and neurohistological analyses, particularly of dopamine, serotonin, GABA, and oxidative stress markers, would further elucidate the biological mechanisms underlying observed behavioural changes.

From a public health perspective, the findings underscore the need for enhanced awareness regarding the risks of mixing alcohol with energy drinks, particularly among adolescents and young adults. Educational campaigns and policy initiatives should emphasize that perceived stimulation does not equate to reduced neurobehavioural impairment. Regulatory agencies may also consider clearer labeling and consumption guidelines for energy drink products.

Finally, the study supports the continued use of grooming behaviour as a reliable ethological tool in neuropharmacological and toxicological investigations, particularly for evaluating the behavioral consequences of chronic substance exposure and drug interactions.

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