Evaluation of Combined Effects of Predator Energy Drink and Caffeine on Motor Skills of Albino Rats

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Abstract

This study evaluates the combined effects of Predator® Energy Drink and Coffee (King Café), *(PEDAC) on Albino Rats for a period of twenty-eight (28) days. The study involved twenty-four (24) adult, and healthy albino rats of both sexes, weighing 90-120 grams and were divided into four different groups of six rats per group. The doses of the PEDAC include 5 mg/kg (low dose) 7.5 mg/kg (medium dose), and 10 mg/kg (high dose), which were administered through oral gavage using nasogastric tube. The objective was to assess the impact of these mixed PEDAC on motor skills, focusing on motor strength and coordination. The Rats underwent grip strength tests and walk beam assessments to evaluate their motor performance. These findings revealed dose-dependent effects, with the Low and Medium Dose groups exhibiting significant improvements in motor strength and coordination compared to baseline and control groups. Additionally, all the treated groups showed reduced completion times in the walk beam task compared to baseline measures and the control group. These findings also disclosed the implication of combined effects of energy drinks mixed with coffee on motor function and emphasize the importance of dosage considerations.*

Keywords: *Predator® Energy Drink, Albino Rats, Walk beam, Grip strength test, Coffee.*

Introduction

The Food and Drug Administration (FDA) defines energy drinks (EDs) as a class of products in liquid form that typically contain caffeine, with or without other added ingredients (NAFDAC, 2014). These beverages often contain significant amounts of caffeine, added sugars, various additives, and legal stimulants like guarana, taurine, and L-carnitine. The levels of these stimulants vary greatly among the different varieties and brands available commercially, of which they may have greater values than are officially allowed (Tanne, 2012). Legal stimulants can heighten alertness, attention, energy, as well as elevate blood pressure, heart rate, and breathing (Costantino *et al*., 2023). Some findings observed that energy drink intake enhances performance in activities such as endurance exercise, muscular strength and endurance, sprinting, jumping, and sport-type activities. Caffeine doses ranged from 40 to 325 mg, with taurine content (dosages 71 to 3105 mg) also influencing performance, indicating a combined effect (Souza *et al*., 2017). Marketed as enhancers of mental activity and physical performance (Seifert *et al*., 2011). The key consumers are mainly adolescents who are drawn to these drinks for rapid energy boosts, improved alertness, and enhanced scholastic (students) or athletic performance (athletes) (Aslam *et al*., 2013). According to findings by Reissig *et al*., (2009), reported that the rate at which energy drinks are intensively consumed is far beyond and more than the estimated values in the

given self-reporting surveys, which can be characterized by the chance of underestimation. The adverse effects and toxicity derived from EDs are of no doubt related to their compositions. However, there is limitation in fully apprehend these relationships between the EDs and the accompanied adverse effects of their intense intake (Seifert *et al*., 2011). Despite their potential beneficial effects, massive consumption of energy drinks results in life-threatening toxicity (Khayyat *et al*., 2013).

Caffeine, which is the most recognized ingredient of these energy drinks, is one of the most globally consumed food substances. In the U.S., approximately 85% of adults, especially young adults, consume caffeine (Fray *et al*., 2005; Fulgoni *et al*., 2015). Caffeine is consumed in various forms, including coffee, energy drinks, chocolate, and sodas. In 2015, Washington Post reported that about 2 billion cups of coffee are consumed daily globally (Fray *et al*., 2005). Comparatively, a cup of coffee contains between 80 to 100 mg of caffeine, and most EDs contained about 70 to 100 mg depending on the coffee roast and mode of preparation (Vaughan 2023). Caffeine enhances alertness in both rested and fatigued individuals with its effects being dosage dependent (Nehlig, 1999). Moderate doses (100–300 mg or approximately 1.5–3.0 mg kg−1) generally prove beneficial, while higher doses (exceeding 400 mg or approximately 5.5 mg kg−1) are more prone to inducing anxiety and, in non-sleep-deprived non-users of caffeine, may impair performance (Smith, 2010). Studies focusing on strength tasks, involving trained or untrained individuals, typically report a commonly consumed caffeine dose ranging from 3 to 6 mg/kg body mass (with the entire range spanning from 2 mg to 11 mg) (Nancy *et al.,* 2021).

Methodology

Experimental site

All experiments were carried out in the Pharmacology laboratory of the Department of Pharmacology and Toxicology Faculty of Pharmacy, University of Maiduguri Borno State.

Energy drinks and Caffeine

The Energy drinks were purchased from the commercial area in the University of Maiduguri Borno State. The Energy drink used for the experiment is Predator® (Gold Strike) a product of Monster Energy with Batch number P2011 23AK3 and expiry date of 20/05/2025. The Caffeine (King Café®) was also purchased from the commercial area in the University of Maiduguri Borno State with expiration date 0f 2025. The King Café® caffeine is a product of an India 100% Instant coffee.

Equipment

Beakers (Pyrex, New York), syringes 1 ml, and 2 ml (Agary, 2022, China) NG-Tube ((Agary, 2022, China) weighing balance (Mettler Toledo, 2011, Grmany), hand Gloves (Uniglov es, 2022, Malaysia), cotton wool (Agary, 2022, China), stopwatch (Fisher Scientific, 2011, USA), Barnes maze (Stoelting Co. 2009. USA), paw grip apparatus (Stoelting Co. 2009. USA), and elevated beam (Stoelting Co. 2009. USA).

Animals and Adaptation Period

Swiss albino rats were procured from Bayero University Kano from the Faculty of Pharmaceutical Sciences weighing 90-120 grams. The rats were housed in conventional housing conditions in the Faculty of Pharmacy Animal House University of Maiduguri. The animals were fed on rat's pellets feeds (TOPFEEDS®), manufactured by Premier Feed mills Co. Ltd and water *ad libidum*. All the rats were maintained under standard laboratory conditions of temperature and optimum ventilation. Good hygiene was maintained by

constant cleaning of droppings and spilled feds. The Rats were exposed to the study environment for 10 days before the test period.

Grouping of Experimental Rats

Twenty-four rats were grouped into six rats per group of four. Group one as the control, group two as the low dose, group three as medium dose and group four as the high dose for the combined Caffeine and Energy drink.

Calculation of dose and administration of EDs and Coffee

The volumes administered of the Predator® ED were equivalent to one can (5 ml/kg, Low Dose), two cans (7.5 mL/kg, Medium Dose) and three cans (10 mL/kg, High Dose). The doses and the calculation of the energy drink and the King Café® caffeine were adjusted on the basis of (Ferreira *et al.,* 2013). A single instant coffee/serving for an average man (3g) was used which was dissolved in Predator® energy drink (350 ml) to make mixture of 9 mg/ml. And finally administered base on the Energy drink dose for each group. While the control group not exposed to any treatments received distilled water.

Treatments were based on sub-chronic toxicity test guidelines by the Organization for Economic Cooperation and Development (OECD 407, 2008). Treatment was administered orally for 28 days by nasogastric tube.

Fore Paw Grip Time Test

Each rat was made to grip the paw grip apparatus with the fore paw by slightly holding the tip of the tail. The time it takes for each rat to grip and fall off the apparatus (fore paw grip time) was then recorded for alternate days and last 4 days following a daily continuous administration of the mixture of caffeine crystals and EDs starting from the last day of training (Day 7) up to 28 days (Smith *et al*., 1995).

Elevated Beam Test

Each rat was placed on an elevated beam and allowed to walk from the starting point and reach the endpoint. The time taken for each rat to reach the endpoint was recorded and the number of stripping on the beam while walking was recorded after the 28th day of continuous administration of the mixture of energy drink and Caffeine (Paul *et al.,* 2011; Carter *et al*., 2001).

Data Collection and Analysis

All data were collected as a measure of time and recorded in Excel. Results from the beam walk and grip strength test were analysed with a repeated measure ANOVA and comparison between groups using Bonferroni's post-hoc at a significant level of $p<0.05$. Values were represented as mean±SEM. All statistics were performed using SPSS version 20 software (Cormack *et al*., 2008).

Results

Table 1: represents the mean time taken (MTT) for rats to release a horizontal rod in a grip paw test under different conditions. In the Control group, the (MTT) for rats to release the rod varies between 9.4 and 11.2 seconds across the 28 days. There are fluctuations, but overall, it remains relatively stable. However, Rats in the High dose with mean time ranging between 10.0 and 12.4 seconds and Low dose groups with mean time between 9.8 and 12.0 seconds, generally take longer to release the rod compared to the control group. Interestingly, rats in the medium dose group also show increased release times compared to the control group, with mean times ranging from 8.8 to 13.0 seconds.

Table 2: Showing estimated mean time(s) (EMTs) taken to release the horizontal rod for 28 days (grip paw). The Control group has the lowest (EMTs) for releasing the horizontal rod (10.013 seconds), followed by the high dose group (10.8 seconds). However, the low dose and medium dose groups both have higher estimated mean times compared to the control and high dose groups.

Table 3: Showing (MTT) for the animals to walk on the walk beam from starting point to the end following administration of the PEDAC. In the high dose group, the rats generally show longer times compared to the control group initially, but the trend reverses around day 9, indicating a potential adaptation or tolerance effect. However, in both medium and low dose groups the doses show a similar trend to the overall pattern, with decreasing mean times compared to the control group as the days progress.

Table 4: Showing (EMTs) taken to release the horizontal rod for 28 days (walk beam). The control group, with a mean time of 4.613 seconds, is slower in releasing the rod compared to all other treatment groups. The high dose group has a mean time of 3.813 seconds, low dose group 3.575 seconds, and medium dose group 3.375 seconds. All these groups show a decrease in time compared to the control, suggesting an improvement in motor coordination or faster reaction times due to the treatments.

Table 1: Showing the mean time taken for the rat to release the horizontal rod (paw grip) following administration of the PEDAC.

	Control	**High Dose**	**Low Dose**	**Medium Dose**
Day 1	9.6±1.3	10.0±1.6	9.8±1.9	8.8±3.1
Day 3	9.8±1.0	10.0±1.2	10.6±2.1	9.8±1.9
Day 5	9.4±1.1	10.4±0.9	10.4±1.5	10.0±1.0
Day 7	10.0±1.5	10.2±0.5	10.8±0.8	10.8±1.6
Day 9	9.4±0.8	10.2±0.8	10.0±0.7	11.0±1.6
Day 11	10.0±1.0	10.6±1.3	11.0±0.7	11.0±0.7
Day 13	10.0±0.7	10.2±0.8	10.4±1.8	11.4±1.1
Day 15	9.6±0.9	10.6±0.6	10.6±1.1	11.0±1.0
Day 17	9.8±0.8	10.8±0.8	11.4±1.1	11.4±1.1
Day 19	10.2±1.3	10.6±0.6	11.2±1.9	11.8±1.3
Day 21	9.8±0.8	11.0±1.0	11.4±1.3	11.6±0.6
Day 23	10.2±1.3	11.0±0.7	11.4±1.1	11.8±1.3
Day 25	9.8±0.4	11.4±1.3	11.2±1.5	12.4±1.5
Day 26	11.0±0.7	11.8±1.1	12.0±0.71	12.8±0.8
Day 27	10.4±0.5	11.6±0.9	12.0±1.0	12.4±1.7
Day 28	11.2±0.8	12.4±0.6	12.0±0.7	13.0±1.4

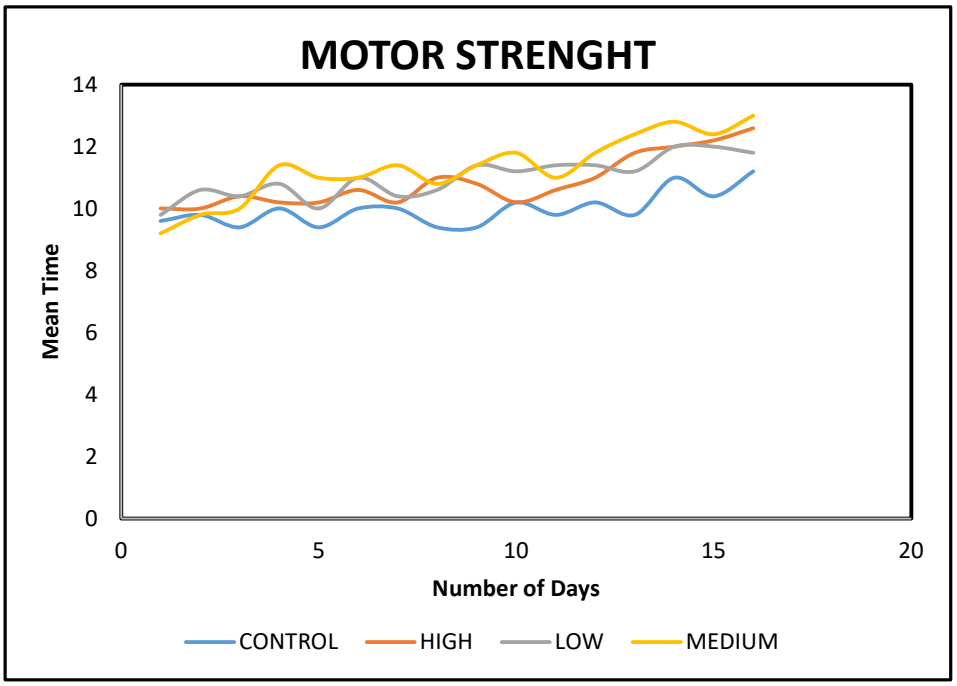

Figure 1: Mean Time taken for the animals to release the horizontal rod following administration for 28 days.

Table 2: Showing estimated mean time(s) taken to release the horizontal rod for 28 days

Group	**Mean**	**SEM**	**95% Confidence Interval**	
			Lower Bound	Upper Bound
Control	10.013	0.2	9.567	10.458
H/Dose	10.8	0.2	10.355	11.245
L/Dose	11.013	0.3	10.567	11.458
M/Dose	11.313	0.3	10.867	11.758

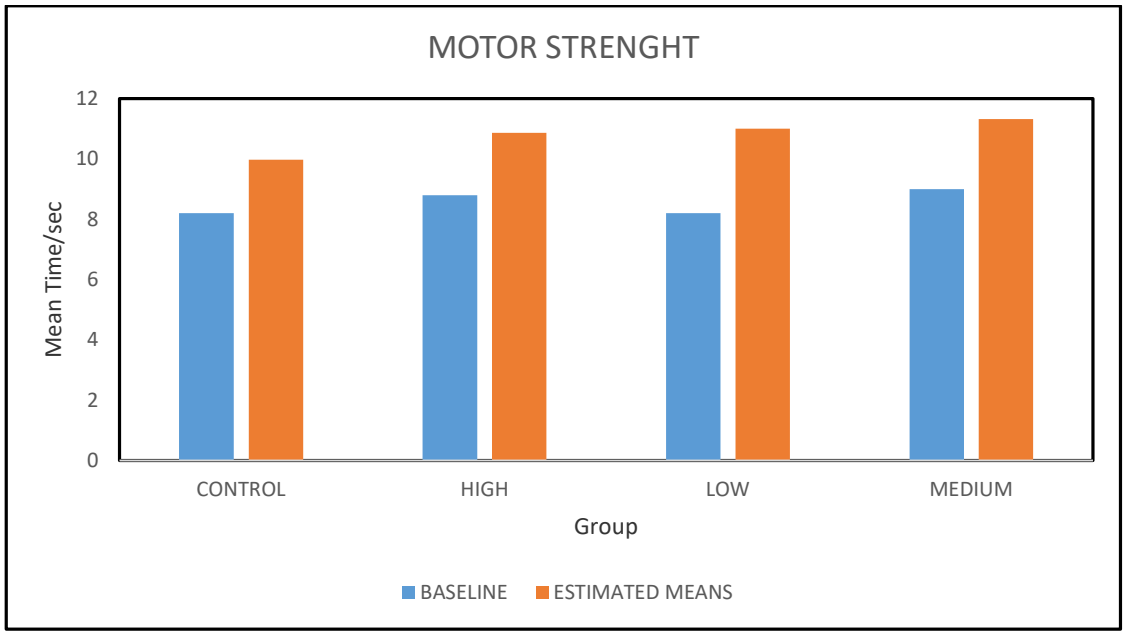

Figure 2: Mean Time taken for the animal to release the horizontal following administration with initial Baselines. The Graph of motor strength based on estimated means compared to the initial baseline showed an improvement across all groups but more prominent in the low and moderate dose

Table 3: Showing Mean Time taken for the animals to walk on the walk beam from starting point to the end following administration of the PEDAC.

	Control	High Dose	Low Dose	Medium Dose
Day 1	6.0±2.2	6.6±1.1	5.2±1.6	4.8±1.4
Day 3	4.4±0.5	5.6±2.1	5.2±1.3	4.6±0.9
Day 5	4.0±0.7	5.2±1.1	5.4±1.8	4.2±0.8
Day 7	4.6±1.1	4.8±1.5	4.4±1.1	4.6±1.1
Day 9	5.2±0.8	4.2±1.3	4.6±0.5	4.0±1.0
Day 11	5.0±0.7	4.0±0.7	4.0±0.7	3.2±0.8
Day 13	5.2±0.8	4.0±1.0	4.0±1.0	3.4±0.5
Day 15	4.6±1.1	3.2±0.8	3.8±1.3	3.0±1.0
Day 17	4.8±1.6	2.8±0.8	3.2±0.8	3.0±0.7
Day 19	5.0±1.0	3.0±1.2	2.6±0.9	2.8±0.8
Day 21	4.0±0.7	3.2±1.3	2.4±0.5	2.6±0.9
Day 23	4.8±0.8	3.0±1.0	2.6±0.5	2.8±0.8
Day 25	4.2±0.9	2.6±0.9	2.6±0.9	2.8±0.8
Day 26	3.8±1.3	3.0±1.0	2.2±0.4	3.0±1.0
Day 27	3.8±1.3	2.8±0.4	2.6±0.9	3.0±1.0
Day 28	4.4±0.9	3.0±0.7	2.4±0.5	2.2±0.4

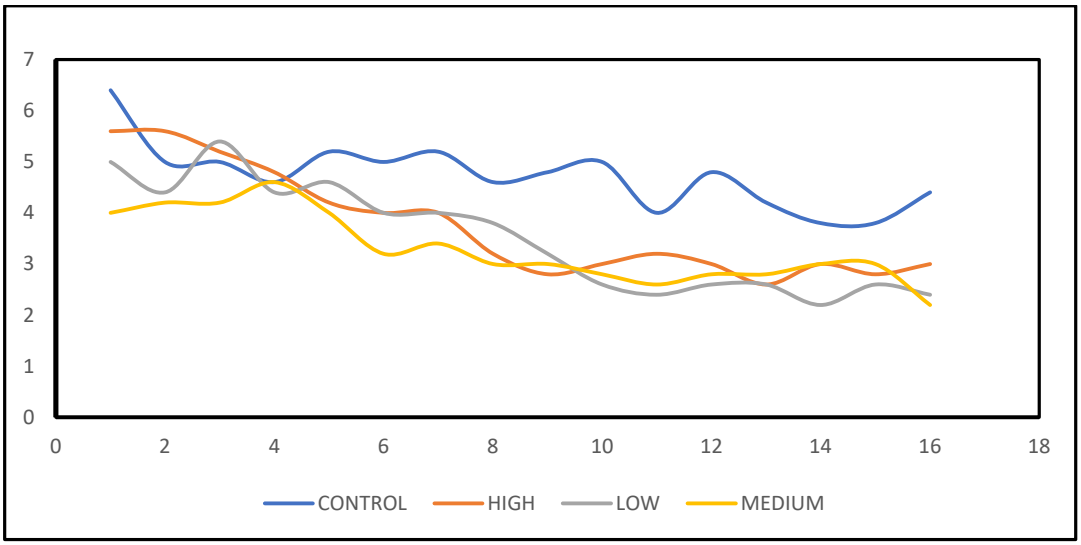

Figure 3: Mean Time taken for the animals to on the walk beam from starting point to the end following administration for 28 days.

Table 4: Showing estimated mean time(s) taken to release the horizontal rod for 28 days (walk beam)

GROUP	Mean	SEM	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	4.613	0.62	4.236	4.989
H/Dose	3.813	0.59	3.436	4.189
L/Dose	3.575	0.58	3.198	3.952
M/Dose	3.375	0.34	2.998	3.752

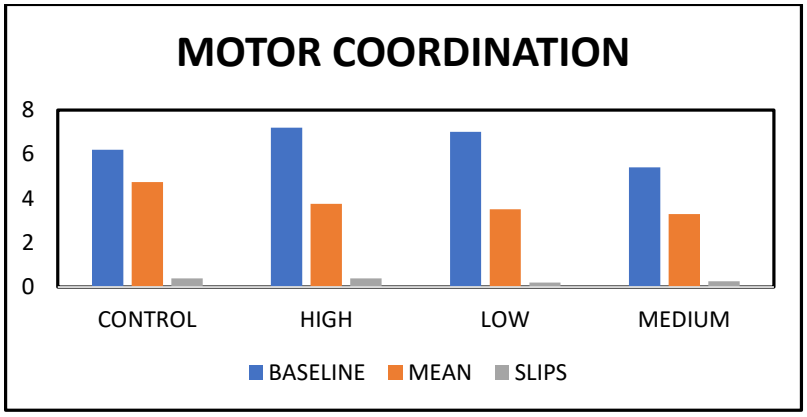

Figure 4: Mean Time taken for the animal to walk on the walk beam from starting point to the end and number of slips following administration with initial Baselines.

Discussion

The main objectives of this study were to examine the sub-chronic effects of energy drinks in combination with coffee, in proportions of 5 ml/kg, 7.5 ml/kg, and 10 ml/kg of energy drink mixture with caffeine at 9 mg/ml, on motor skills, namely motor strength and motor coordination. The mixture of the two beverages, statistically increased motor strength and motor coordination in the forehand grip in the low dose (11.013±0.3) and medium dose (11.313±0.3) groups, with a significant difference compared to control (10.013±0.2) group, whereas the high dose (10.08±0.2) group showed increased grip time compared to baselines but no significant difference compared to the control. On the walk beam scores, the time taken to walk from start to finish was significantly lower and reduced compared to baseline measures and the control group across all test groups. The dose-effect relationship indicates an improved grip time across the test groups, with optimum effects seen in the low and moderate dose groups.

In the context of this study, one possible reason for the observed increased performance in motor activity could be linked to the animals' 10 days of training on the beam walk and foregrip test prior to testing, as well as the duration of the test (Zuo *et al*., 2014).

The treated rats were hyperactive after the administration of the combined two beverages, as reported by a recent study (Klosterman, 2006; Krahe *et al*., 2017). This observed hyperactivity is attributed to the psychoactive properties of caffeine, which is the most important active substance in energy drinks and coffee (McLellan and Lieberman, 2012). In contrast to other studies using free access through drinking water, our method of oral gavage, which resembles the delivery of caffeine pills or high-caffeine energy drinks with concentrated doses, could be the reason for this observed behaviours and difference (Olopade *et al*., 2021).

Finding from this study represents the first attempt in assessing the changes in motor skills following the ingestion of an energy drink combined with coffee. Findings from this study is partially related to a meta-analysis on energy drinks alone and physical performance in human trials (Souza *et al*., 2017). Additionally, findings from this study is align partially with a recent study on the effects of chronic caffeine ingestion on motor function and brain regions (Olopade *et al*., 2021). Since Alford and his colleagues (Alford *et al*., 2001) were the first authors to investigate the outcomes of caffeine-containing energy drinks on physical performance, many authors have researched this subject matter due to the novelty of these beverages, using the most popular energy drink, Red Bull, which contains 80 mg/serving of 250 ml (Del Coso *et al*., 2012). Their research indicated improved physical performance where the caffeine dose is about 3mg/kg in relation to effects on lower and upper body muscles, which agrees with the present study.

Using the hang test in this study to assess muscular strength, a noticeable difference exists between the treatment groups, specifically in the low dose (3.575±0.58) and medium dose (3.375±0.34) in comparison with control (4.613±0.62) which agrees with the results by Beck *et al*. (2006), who examined caffeine's effect on muscular endurance on bench press in male volunteers in high dose. A study by Forbes *et al*. (2007) using Red Bull Wingate cycle test rivals this claim with respect to gender inclusion and higher dosing. Forbes used a dosing of 2 mg/kg caffeine and observed an outstanding increase in upper body muscular endurance, which partially relates to our findings indicating a significant increase in motor strength in the low and moderate dose group compared to control.

Beam walk test results showed that the test group had a remarkable improvement in motor function, coordination, and speed which is consistent with a previous study by Tallis *et al*.,

2015, which found that physiological concentrations of caffeine can improve muscle performance by increasing the force, work, and power that muscles can generate.

Conclusion

This study highlights the positive impact of energy drinks mixed with coffee on motor skills over a 28-day period. Dose-dependent improvements were observed, particularly in motor strength and coordination. These findings emphasize the potential benefits of this combination for enhancing motor function.

Recommendation

Research is needed to quantify the actual amount of caffeine present in energy drinks and to explore possible interactions with other ingredients. Further research is needed to be conducted on the toxicity of this mixed PEDAC on liver, kidney, brain, heart, and other body organs. These pharmacological investigations will enhance our understanding of the effects of mixed PEDAC on rodents and will trigger further investigation on higher animals and human being.

Reference

Alford, C., Cox, H., Wescott, R. (2001). The effects of Red Bull energy drink on human performance and mood. *Amino Acids*, 21(2), 139-50.

Aslam, H. F., Mughal, A., Edhi, M. M., Saleem, S., Rao, M. H., Aftab, A., *et al*. (2013). Assessment of pattern for consumption and awareness regarding energy drinks among medical students. *Archives of Public Health*, 71, 31.

Beck, T.W., T.J. Housh, R.J. Schmidt, G.O. Johnson, D.J. Housh, J.W. Coburn, M.H. Malek (2006). The acute effects of a caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities. *J. Strength Cond. Res*. 20:506-510.

Carter, R.J., Morton, J., Dunnett, S.B. (2001). Motor coordination and balance in rodents. Curr Protoc Neurosci.

Cormack, S.J, Newtown, R.U., McGulgan., M.R., Doyle, T.L.A. (2008). Reliability of measures obtained during single and repeated countermovement jumps. *Int J Sports Physiol Perform*. 3(2), 131-144.

Costantino, A., Maiese, A., Lazzari, J., Casula, C., Turillazzi, E., Frati, P., & Fineschi, V. (2023). The Dark Side of Energy Drinks: A Comprehensive Review of Their Impact on the Human Body. *Nutrients, 15*(11), 3922.

Del Coso, J., Salinero, J.J., González-Millán, C. *et al.* (2012). Dose response effects of a caffeine-containing energy drink on muscle performance: a repeated measures design. *J Int Soc Sports Nutr* **9**, 21

Ferreira, S.E., Abrahao, K.P., Souza-Formigoni, M.L.O. (2013). Expression of behavioural sensitization to ethanol is increased by energy drink administration. *Pharmacology Biocheistry and Behaviuor*, 110; 245-248.

Forbes, S.C., D.G. Candow, J.P. Little, C. Magnus, and P.D.Chilibeck (2007). Effect of Red Bull Energy Drink on repeated Wingate cycle performance and bench-press muscle endurance. *International Journal of Sport Nutrition and Exercise Metabolism* 17:433-444.

Fray, S. D., Johnson, R. K., Wang, M. Q. (2005). Food sources and intakes of caffeine in the diets of persons in the United States. *Journal of the American Dietetic Association*, 105, 110–113.

Fulgoni III, V. L., Keast, D. R., Lieberman, H. R. (2015). Trends in intake and sources of caffeine in the diets of US adults: 2001–2010. *American Journal of Clinical Nutrition,* 101, 1081–1087.

Khayyat, L.I., Amina, E.E., Maisaa, M.A and Jehan, M.S. (2013). Comparative study on the effect of energy drinks on haematopoietic system in Wistar albino rats. *Journal of Environmental Biology*, 35, 883-891.

Klosterman, L. (2006). The Facts about Caffeine (Drugs). Benchmark Book.

Krahe, T. E., Filgueiras, C. C., Quaresma, R. S., Schibuola, H. G., Abreu-Villaça, Y., Manhães, A. C., & Ribeiro-Carvalho, A. (2017). Energy drink enhances the behavioral effects of alcohol in adolescent mice. *Neuroscience Letters*, 651, 102-108.

McLellan, T.M. and Lieberman H.R. (2012). Do Energy Drinks Contain Active Components other than Caffeine? Journal Article, 70(12); 730-744.

Nancy, G., Karima, B., Jennifer, B., Andrew, D.K and Sebasteien L. (2021). Effects of caffeinated energy drinks on cardiovascular responses during exercise in health adults: a systematic review and meta-analysis of randomized controlled trials. Applied Physiology, Nutrition and Metabolism.

National Agency for Food and Drug Administration and Control (2014). Some important facts about Energy Drinks. Available on 4th April, 2014. Retrieved on 23rd January, 2024.

Nehlig, A. (1999). Are we dependent upon coffee and caffeine? A review on human and animal data. *Neuroscience and Biobehavioral Reviews*, 23, 563–576.

OECD. Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. Organization for Economic Co-operation and Development, Paris, France; 2008.

Olopade, F. E., Femi-Akinlosotu, O. M., Adekanmbi, A. J., Ighogboja, O. O., & Shokunbi, M. T. (2021). Chronic caffeine ingestion improves motor function and increases dendritic length and arborization in the motor cortex, striatum, and cerebellum. *Journal of Caffeine and Adenosine Research*, *11*(1), 3–14.

Paul, H.P., Tinh, N.L., Holly, J.C. and Amber, S. (2011). Assessment of Motor Balance and Coordination in Mice using the Balance Beam. *Journal of Visualized Eperiments*, (49): 2376.

Reissig, C., Strain, E. C., & Griffiths, R. R. (2009). Caffeinated energy drinks: A growing problem. *Drug and Alcohol Dependence*, 99, 1-10.

Seifert, S. M., Schaechter, J. L., Hershorin, E. R., & Lipshultz, S. E. (2011). Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics*, 127, 511–528.

Smith, A. E., Fukuda, D. H., Kendall, K. L., Stout, J. R. (2010). The effects of a pre-workout supplement containing caffeine, creatine, and amino acids during three weeks of high-intensity exercise on aerobic and anaerobic performance. *Journal of the International Society of Sports Nutrition*, 7, 10.

Smith, J.P., Hicks, P.S., Ortiz, L.R., Martinez, M.J and Mandler, R.N. (1995). Quantitative measurement of muscle strength in the mouse. *Journal of Neurosci Methods*; 62, 15-19.

Souza, D. B., Del Coso, J., Casonatto, J., & Polito, M. D. (2017). Acute effects of caffeine-containing energy drinks on physical performance: A systematic review and meta-analysis. *European Journal of Nutrition*, 56(1), 13–27.

Tallis, J., Duncan, M. J., & James, R. S. (2015). What can isolate skeletal muscle experiments tell us about the effects of caffeine on exercise performance? *British Journal of Pharmacology*, 172(22), 3703-3713.

Tanne, J. H. (2012). New York attorney general investigates energy drink makers. *BMJ*, 345, e6108.

Vaughan Kelly (2023). How the beans, brewing method, and grind impact the amount of caffeine in your coffee. *Food and Wine*. Published 11th July, 2023. Retrieved 4th January, 2024.

Zuo, Y., Chen, C., and Gilmore, A. (2014). Study Motor Skills Learning by Single-pallet Reaching Tasks in Mice. *Journal of Visualized Experiments*. (85): 51238.