

RESEARCH ARTICLE

The muscular effect of Cobra energy drink on toad (*Bufo marinus* = *Rhinella marina*) gastrocnemius fatigue time and contraction force

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ABSTRACT


Background: Cobra energy drink is one of the most popular beverages in the Philippines used by many for its energy-boosting effects. It is known to contain ingredients such as caffeine, B-vitamins, taurine, and ginseng, which are all said to improve muscle performance. Caffeine helps aid muscle contraction through the opening of calcium ion channels, while B vitamins aid in the production of adenosine triphosphate needed by the muscles during exercise. In addition to this, taurine helps maintain contractile functions while ginseng protects the muscles from exercise-induced oxidative stress. **Aims and Objectives:** The objectives of the study are to determine the effect of Cobra energy drink on the contraction force and fatigability of the gastrocnemius muscle of toad. **Materials and Methods:** Cobra energy drink was administered in four different concentrations (100% v/v, 75% v/v, 50% v/v, and 25% v/v). Each treatment concentration had four replicates each and a total of 16 toads were used. For each toad, both legs were used, one leg for the treatment and the other one for the control. The factors measured are as follows: Mean force, maximum force, and fatigue time. These parameters were measured using the PowerLab/4ST. **Results:** Based on the results gathered, 100% v/v showed an increase in muscle contractility by garnering a positive result for all three factors while the rest of the treatments gave negative means for all three factors. Means for 100% v/v were considered statistically significant from all the other treatments while the rest showed no significant differences. Moreover, no significant differences were detected from all treatments in fatigue time including the 100% v/v. However, the results imply that as the treatment concentration increased, muscle contractility also increased. 100% v/v showed the most promising results in muscle contraction and fatigability due to it gaining positive values for all three parameters while the other treatments gave negative results. **Conclusion:** It can be said that Cobra energy drink had a beneficial effect on the muscle in terms of muscle contractility due to the significant differences detected in muscle contractility and fatigability.

KEY WORDS: Energy Drink; Gastrocnemius; Muscle Contraction; Muscle Fatigue; Toad

INTRODUCTION

Cobra energy drink is one of the most popular energy drinks available in the Philippines. Despite the recent emergence

of other local energy drinks, it still remains the best-selling energy drink in the country. Some of the reasons why it is consumed are to counteract sleepiness, increase energy, maintain alertness while studying and driving, also to reduce symptoms of hangover.^[1] A 350 mL bottle of Cobra energy drink contains 134 mg caffeine, 1.36 mg Vitamin B1, 7 mg Vitamin B3, 1.35 mg Vitamin B6, 0.70 mg Vitamin B12, 153 mg taurine, and 17.5 mg of ginseng. B vitamins are part of the five essential vitamins that aid in boosting muscle strength, muscle recovery, and muscle toning.^[2] B vitamins improve athletic performance by optimizing the body's capacity to utilize oxygen. It is also essential in

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metabolizing carbohydrates, fats, and proteins.^[3] Another important ingredient found in Cobra energy drink is caffeine. Despite considerable researches on the effects of caffeine, the role of caffeine as a performance-enhancing drug is still controversial. Some of the reports are conflicting, which is in part due to how the experimental studies were designed and what methods were used. Although some researchers say that caffeine negatively affects muscles, it is said to improve alertness and reduce fatigue when given in proper doses.^[4] Cobra energy drink also contains ginseng, a herbal supplement often used by athletes for proposed performance-enhancing benefits such as increased energy, stamina, and recovery.^[5] Although there is a lack of study to support claims regarding the beneficial effects of ginseng on athletes, promoters claim that ginseng can improve athletic performance by reducing muscle fatigue and aiding recovery.^[5] Another component of Cobra energy drink is taurine. Taurine is a conditionally essential amino acid, classified between essential amino acids that the body cannot produce (e.g., lysine, tryptophan) and non-essential amino acids that the body can produce (e.g., alanine, cysteine). Taurine is synthesized in the liver and brain through cysteine or methionine. This process requires several enzymatic steps, all of which require Vitamin B6 as a cofactor.^[6] Taurine is often added to various products besides from energy drinks because it contains antioxidant properties that fight off free radicals and protect the body. Not only does it improve athletic performance but also it can contribute to heart health and lower chances of diabetes.^[7]

Muscle fatigue is influenced by physical activity that diminishes the maximum optional strength of muscle.^[8] It could occur due to outlying modifications at the level of the muscle. However, it could also be due to that central nervous system if it has not successfully operated the motor neurons sufficiently. Thus, optional activation normally lessens throughout maximum optional isomeric functions that are a central fatigue evolve and motor unit firing percentages decreases. There are other central changes that cause muscle fatigue such as changes in proprioception, tremor and synchronization, and synkinesis and muscle rotation.^[9] The ingestion of energy drinks before, during, or following exercise can affect the performance or training adaptation. Energy drinks contain a number of nutrients that affect mental and physical performance, but its primary ergogenic nutrient appears to be caffeine or carbohydrate. Furthermore, consumption of low-calorie energy drink during training or weight loss trials could provide ergogenic benefit or promote a small amount of fat loss.^[10]

All in all, Cobra energy drink claims to improve mental and physical performance which will help the consumer achieve more in a day. With that being said, more and more Filipinos from all walks of life consume cobra on a daily basis. It is not drink only athletes patronize but also students and people from the working sector of the society. In this study, the researchers aimed to know how Cobra energy drink affects

the muscle performance *in vitro* using the gastrocnemius muscle of toad. The administration of different treatment concentrations along with various laboratory procedures is necessary in determining the effectivity of Cobra energy drink on the muscle contraction of the gastrocnemius. The use of animals is necessary for this experiment as fatigue was experimentally induced to the gastrocnemius muscle to see what will be the effect of the different concentrations of Cobra energy drink.

MATERIALS AND METHODS

Collection of Samples

A total of 16 toads (*Bufo marinus*) regardless of gender, size, and weight were used in the experiment. Cobra energy drink was administered in four concentrations (100% v/v, 75% v/v, 50% v/v, and 25% v/v) with the use of a medicine dropper. Four mature toads were used for each treatment concentration. For each toad, one leg was used for the control and one leg for the treatment for a total of 16 toads (32 legs). All in all, 16 legs were used for all four treatments (four legs per treatment) and 16 legs for the control.

Materials

The PowerLab/4ST ML760 served as the main data-gathering tool. Ringer's solution was used to prevent muscle dry up. Ringer's solution contains 6.5 g of sodium chloride (NaCl), 0.14g of potassium chloride (KCl), 0.12 g of calcium chloride (CaCl₂), 0.2 g of sodium bicarbonate (NaHCO₃), and 0.01 g of sodium pyrophosphate.

Cobra energy drink is in liquid form; therefore, concentrations were computed through percentage. Treatment 1 contained 100% (v/v) pure cobra concentrate (100 mL Cobra energy drink). Treatment 2 contained 75% (v/v) cobra concentrate (75 mL Cobra energy drink + 25 mL Ringer's solution), treatment 3 contained 50% (v/v) cobra concentrate (50 mL Cobra energy drink + 50 mL Ringer's solution), and treatment 4 contained 25% (v/v) cobra concentrate (25 mL Cobra energy drink + 75 mL Ringer's solution).

Laboratory Analysis

The toad was first double-pithed. After pithing, the legs of the toad were skinned until the gastrocnemius muscle was exposed. The gastrocnemius muscle was then isolated by cutting off the femur with the gastrocnemius muscle and Achilles tendon left attached.

The isolated gastrocnemius muscle was then attached to the femur clamp with the Achilles' tendon attached to a muscle force transducer. The electrodes of the induction stimulator of the powerlab were attached to the belly of the muscle. While attached to the muscle force transducer, the muscle

was treated with its respective treatment concentration using a medicine dropper. The four gastrocnemius muscles used for treatment 1 were given 100% cobra concentrate. Another four gastrocnemius muscles were given 75% cobra concentrate for treatment 2. The four muscles used for treatment 3 were given 50% cobra concentrate and another four muscles were given 25% cobra concentrate which served as treatment 4. 16 gastrocnemius muscles were used as control samples and were treated with Ringer's solution. The muscle was then stimulated to induce muscle contractions. Continuous dropping of the respective treatment was done before and during the induced contractions until the muscle fatigued. The PowerLab setup was set to an output range of 10 V, amplitude of 1 V, baseline of 0 V, and range of 200 mV for all samples.

Results for muscle fatigue (seconds) and height of contraction (mV) were then recorded and documented.

Data Analysis/Statistical Tool

Control values were subtracted from treatment values to obtain actual data values. Values obtained were analyzed using one-way analysis of variance (ANOVA) to detect differences among treatments. Treatments at $P < 0.05$ were considered statistically significant. Tukey's test and ANOVA were both used to look for means that are significantly different from each other. All statistical analysis was performed using STATA v.12.

RESULTS

The different graphs generated by the PowerLab were analyzed and interpreted using the LabChart Reader software to obtain the values for maximum contraction, force mean, and fatigue time. Data for the three factors were needed to understand the muscle contraction and fatigability of the gastrocnemius muscle for each treatment concentration. The mean values were obtained by subtracting the control values from the treatment values. The mean values were then subjected to one-way ANOVA and Tukey's test to detect differences among means [Table 1].

The negative control values are as follows: 28.6172 mV for maximum contraction, 15.9464 mV for force mean, and 451 s for fatigue time. Since the negative control was not treated with Cobra energy drink, the values obtained for the negative

control are considered normal values. To properly compare the effect of the different treatments on the muscle, the control values were subtracted from the treatment values. The newly obtained differences were then subjected to one-way ANOVA and Tukey's test. Treatments with positive values imply that these values are higher than the normal, while negative values imply that the values obtained are lower than the normal.

Based on the results, treatment 1 (100% cobra concentrate) can be considered as the most effective concentration garnering a positive result for all three parameters. This suggests that treatment 1 gave higher than normal values for all three parameters; therefore, it is beneficial in increasing muscle contraction and fatigability. The values obtained from treatment 1 also showed a significant difference from all the other treatments in almost all factors. The least effective concentration is 25% cobra concentrate garnering negative values for all three parameters, therefore, implying that lower doses may result to detrimental effects on muscle contraction and fatigue.

DISCUSSION

According to the label, a 350 mL bottle of Cobra energy drink contains 134 mg caffeine, 1.36 mg Vitamin B1, 7 mg Vitamin B3, 1.35 mg Vitamin B6, 0.70 mg Vitamin B12, 153 mg taurine, and 17.5 mg of ginseng. Although there are no studies that tackle the effects of Cobra energy drink on muscle contraction and fatigability, Cobra energy drink contains mainly caffeine yet other ingredients are B vitamins, taurine, and ginseng, which are said to improve muscle performance. In this experiment, Cobra energy drink did not show favorable effects when given in smaller doses. However, when administered in 100% concentration, Cobra energy drink showed promising effects in terms of muscle contractility and fatigability.

Caffeine is widely used and consumed by people. It comes from dietary sources such as beverages and food but most of it from coffee and tea. Coffee and other caffeine-containing beverages were introduced in Europe few hundred years ago and now the consumption of these beverages occupies a significant place in the national cultures.^[11] Caffeine is said to reduce leg muscle pain during exercise. With moderate or large doses of caffeine, it can improve performance of athletes

Table 1: Mean and standard deviation of each cobra treatment concentration

Treatment (%)	Maximum contraction (mV)	Force mean (mV)	Fatigue time (s)
1 (100)	16.1345 ^a ±18.9011	7.9933 ^a ±5.2215	451±790.3447
2 (75)	-4.525 ^{ab} ±1.7154	-3.5936 ^b ±3.9621	-125±139.5349
3 (50)	-5.6 ^b ±2.1602	-4.3637 ^b ±4.4203	-25.75±66.1079
4 (25)	-16.125 ^{ab} ±7.3808	-5.764 ^b ±2.3924	0±91.1783

*Different superscripts within columns indicate statistically significant difference ($P < 0.05$)

during endurance tests.^[12] The ingestion of caffeine in the form of energy drink tends to improve physical performance and a dose of energy drink of at least 3 mg/kg of caffeine may necessary improve lower body and upper body muscle power and strength.^[13] For the reason that caffeine provides valuable performance-enhancing effects on stimulated intermittent high-intensity team sport performance, it became a potentially useful supplement for games such as rugby, football, soccer, hockey, basketball, and tennis. Although mechanisms of caffeine are not fully understood, it is perceived that it influences several processes in the central nervous system to reduce fatigue and permit a higher level of motor drive and motor skills.^[4] However, in a study done by Rosser *et al.*,^[14] it was found that caffeine increases skeletal muscle contraction through increasing calcium ion release. In addition of being a food constituent, Lieberman *et al.*^[15] stated that caffeine is also a common analgesic.^[16-19]

According to researchers, B vitamins such as B-6 and B-12 play an important role in muscle endurance. B-6 has an important role in the metabolic pathways that is required for exercise while B-12 assists with DNA synthesis which is necessary for the formation of red blood cells. B vitamins act as coenzymes in the metabolic pathways causing the breakdown of carbohydrates, fats, and proteins into energy needed for exercise.^[3] Spradley *et al.*^[20] examined that if B vitamins together with other ingredients such as caffeine, beta-alanine, branched chain amino acids, and creatine ingested together, it may work synergistically to enhance various aspects of exercise performance. The findings of the investigation show that the ingredients combined may improve perceived feelings of energy, focus, and alertness. It also shows that it may reduce feelings of fatigue which can increase the performance of muscular endurance and reaction time. Another thing is that B-complex vitamins such as thiamine, riboflavin, and Vitamin B-6 have several important functions in the body, which includes energy production, hemoglobin synthesis, adequate immune function, and building and the repair of muscle tissue. These micronutrients play an important role in sustaining the health of physically active individuals.^[21]

Taurine or 2-aminoethanesulfonic acid is a sulfonated β -amino acid which is derived from diet or synthesized from the cysteine mainly in the liver. It is highly concentrated in the heart and liver as well in the central nervous system including the brain stem and the hippocampus. Its role is in osmoregulation, membrane stabilization, neuroprotection, neuromodulation, and regulation of cellular calcium level. According to Giles *et al.*,^[1] the intake of taurine opposed the effects of caffeine on mood, including reducing feelings of vigor and increasing caffeine withdrawal symptoms. Hence, when taking energy drinks, taurine helps balance the effect of caffeine. In recent studies, the physiological roles of taurine in heart and skeletal muscle focus on the maintenance of contractile function, osmoregulation, conjugation,

antioxidant activity, and protein phosphorylation.^[22] It is found that taurine is essential for normal contraction of skeletal muscle because it facilitates calcium-dependent excitation-contraction processes.^[23]

Ginseng has been used over a thousand of years because of the belief that it promotes longevity. It has both stimulatory and inhibitory effects on the central nervous system, and it may modulate neurotransmission. Ginseng also has antineoplastic and immunomodulatory effects.^[24] Most pharmacological action or effects of ginseng are attributed to ginsenosides.^[25] It is able to act on a wide range of tissues. Since ginsenosides are amphiphilic in nature, it has the ability to become intercalated in the membrane environment. *In vivo* study reported that ginseng administration decreased the free radical generation in skeletal muscles after exhaustive exercise of mice. The long-term administration of ginseng extract was able to protect the muscle from exercise-induced oxidative stress-independent fiber type.

CONCLUSION

A 350 mL bottle of Cobra energy drink contains 134 mg caffeine, 1.36 mg Vitamin B1, 7 mg Vitamin B3, 1.35 mg Vitamin B6, 0.70 mg Vitamin B12, 153 mg taurine, and 17.5 mg of ginseng. Coffee contains about the same amount of caffeine as Cobra energy drink; however, Cobra energy drink does more than a regular cup of coffee because it contains other ingredients such as B vitamins, taurine, and ginseng. All these ingredients work together to get that performance-boosting energy. Caffeine increases muscle contraction through the opening of calcium channels while B vitamins help produce energy needed by the muscles when exercising. Taurine, on the other hand, maintains contractile functions by facilitating calcium-dependent excitation-contraction processes. Finally, ginseng aids in the protection of muscles from exercise-induced oxidative stress.

Significant differences among the different concentration means were detected in the data for muscle contraction, but differences among means measuring fatigue time were found to be non-significant. This shows that Cobra energy drink has an effect on muscle contraction but does not necessarily affect fatigue time. In addition to this, a trend can be observed on the effect of Cobra energy drink on muscle contraction, but none could be derived from fatigue time due to the inconsistency of the results. Based on the data gathered, as treatment concentration increases, muscle contraction becomes more positive.

Due to the detection of significant differences among means, Cobra energy drink can be considered beneficial in terms of muscle contraction when given in 100% concentration. However, since there was no significant difference among the means of fatigue time, it can be concurred that Cobra energy drink is not necessarily beneficial in terms of muscle fatigability. With that being said, higher concentrations of

Cobra energy drink are preferred since it increases the force of contraction.

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