

## EFFECTS OF ENERGY DRINKS ON SERUM VEGF AND bFGF LEVELS DURING WOUND HEALING

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### ABSTRACT

**Introduction:** Energy drinks consist of many substances like caffeine, taurine, sucrose/glucose, vitamins and some herbal supplements like ginseng, ginkgo biloba and guarana and are used by millions of people world-wide. The study aims to investigate the effect of energy drinks on blood vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) levels.

**Material and method:** Thirty male adult Wistar albino rats were randomly assigned to two groups, an experimental group (EG) and the control group (CG). The Red Bull (3.57 mL/kg/day equivalent to a person weighing 70 kg consuming 250 mL per day) was given by oral gavage to the EG and the same volume of water was given by oral gavage to CG. The full thickness 5 cm diameter skin incisions were performed on the back of the rats. The blood samples were taken from the rats (n=5 in each group) on the 3rd, 7th and 14th days of the experiment. The biochemical blood analysis was performed at the end of the experiment.

**Results:** The 3rd day, mean VEGF values of EG and CG were statistically different. Similarly, on the 14<sup>th</sup> day, mean bFGF values of EG and CG were statistically different. On the 7<sup>th</sup> day, there were no statistically differences between mean VEGF and bFGF values of EG and CG.

**Discussion:** The ingredients of energy drinks may affect the wound healing positively or negatively with many mechanisms described in literature. Our results confirmed that energy drinks may accelerate angiogenesis in early and late stages of the proliferation phase of wound healing. Further investigations may reveal more reliable results.

**Key words:** Energy drink, VEGF, bFGF, wound healing.

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### Introduction

Energy drinks consist of many substances and are used by millions of people world-wide. They contain a combination of ingredients that increase cell metabolism. Caffeine, taurine, sucrose/glucose, vitamins and some herbal supplements like ginseng, ginkgo biloba and guarana are frequently added materials into energy drinks<sup>(1,2)</sup>. Reducing fatigue, improving concentration, and increasing mental and physical performances are the presented benefits of energy drinks by manufacturers<sup>(1)</sup>. Television, internet, and market advertisements aimed at adolescents, young people, students, athletes, and hard-working people increased energy drink sale around

the world in the past few years<sup>(2,3)</sup>. Some studies revealed that more than 30-50% of young adults, adolescents and college students consume energy drinks<sup>(2,4,5)</sup>. The stimulating effects of energy drinks on the central nervous system and cellular energy rate have been shown in many studies<sup>(1,6,7)</sup>.

Wound healing is a normal, complex, and multicellular physiological process of the body and many factors can interfere with the stages of this procedure, such as infection, age, sex hormones, diabetes, stress, oxygenation, and nutrition<sup>(8)</sup>. The wound healing regulation is under the control of a complex signalling network which involves many growth factors, like epidermal growth factor family, transforming growth factor beta family, VEGF,

fibroblast growth factor (FGF) family, platelet-derived growth factor, connective tissue growth factor, and tumour necrosis factor alpha family<sup>(9)</sup>.

Blood vessels are essential to deliver the nutrients to the other tissues<sup>(10)</sup>. Angiogenesis is the widely used wound healing parameter in literature. VEGF is important and promotive in the early events of the angiogenesis, such as endothelial cell migration and proliferation during wound healing<sup>(11, 12)</sup>. VEGF is accepted as the one of the most important regulators of the physiological and pathological angiogenesis, and it is extensively used as angiogenic cytokine in literature<sup>(13, 14)</sup>. Promoting angiogenic factors, like VEGF and bFGF, might have beneficial effects on ischemia<sup>(10)</sup>.

The FGF family has 23 members, but the three most important members that are involved in cutaneous wound healing are FGF2, FGF7, and FGF10<sup>(15)</sup>. FGF2, known as bFGF, is a promoter of angiogenesis and lymphangiogenesis and increases in acute wound stages that granulation tissue formation, re-epithelialization, and tissue remodelling<sup>(16)</sup>. bFGF levels are decreased in chronic wounds<sup>(17)</sup>. bFGF is the most widely used FGF peptide when compared with the other FGF's<sup>(18)</sup>.

Energy drinks containing many nutrients may affect the wound healing process. In a literature search, we did not uncover any experimental work on the effects of energy drinks on blood VEGF and bFGF levels during wound healing period. The study aims to investigate the effects of energy drinks on blood VEGF and bFGF levels during acute wound healing.

## Material and method

In the current study, all the experimental procedures were applied in the experimental animal facility of Abant İzzet Baysal University. The Institutional Animal Ethics Committee of Abant İzzet Baysal University approved the study.

Thirty male adult Wistar albino rats (200-250 g), maintained under standard housing conditions (room temperature  $25\pm 3^{\circ}$  C, humidity 60-65%, 12:12 h dark-light cycle) and consuming a standard diet and water ad libitum during the procedure, were used for the study. The rats were randomly assigned to two groups, an experimental group (EG) and the control group (CG). The Red Bull (3.57 mL/kg/day equivalent to a person weighing 70 kg consuming 250 mL per day) was given by oral gavage to the EG and the same volume of

water was given by oral gavage to CG starting one day before from the surgical procedure. The rats were anesthetized using an intraperitoneal injection of Brema®-Ketamin10%, 60–80 mg/kg (Bremer Pharma GMBH, Bremerhaven, Germany) and Alfazyne® 25, 8-10 mg/kg (Alfasan International B.V, Woerden, Holland). The dorsum of the rats were shaved with an electrical clipper, and their skin disinfection was performed by alcohol (70%) and rinsed with sterile water. A full-thickness paravertebral linear incision (5 cm) wound model was created with a sterile surgical blade at a distance of 1.5 cm from the midline of the vertebral column under aseptic conditions. The edge of the wound was closed with a sterile strip plaster. Thereafter, the animals were individually caged. The blood samples were taken from the rats (n=5 in each group) on the 3<sup>rd</sup>, 7<sup>th</sup> and 14<sup>th</sup> days of the experiment. The biochemical blood analysis was performed at the end of the experiment. VEGF and bFGF levels in the blood serum were measured by a specific enzyme-linked immunosorbant assay method with EIAab (Wuhan EIAAB Science Co., Ltd. Wuhan, China).

The data was statistically analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The results of the bFGF and VEGF mean values in EG and CG were compared using the Mann-Whitney U test.

## Results

The 3<sup>rd</sup> day EG mean VEGF value was  $26.39 \pm 4.65$  pg/mL and the mean VEGF value of CG was the  $18.50 \pm 1.99$  pg/mL. The difference was statistically significant ( $p=0.016$ ) (Table 1). The bFGF mean values of EG and CG were  $8.70 \pm 2.23$  pg/mL and  $7.41 \pm 0.96$  pg/mL. The difference was not statistically significant ( $p=0.690$ ) (Table 2).

The 7<sup>th</sup> day VEGF mean values of EG and CG were  $16.85 \pm 2.96$  pg/mL and  $15.44 \pm 2.33$  pg/mL. The difference was not statistically significant ( $p=0,857$ ) (Table 1). The mean bFGF values of EG and CG were  $6.25 \pm 0.18$  pg/mL and  $8.48 \pm 2.55$  pg/mL. The result was not statistically significant ( $p=0,229$ ) (Table 2).

The 14<sup>th</sup> day VEGF mean values of EG and CG were  $14.48 \pm 1.17$  pg/mL and  $15.31 \pm 0.86$  pg/mL. The difference was not statistically significant ( $p=0.190$ ) (Table 1). The mean bFGF values of EG

and CG were  $7.73 \pm 1.16$  pg/mL and  $6.48 \pm 0.15$  pg/mL and the difference was statistically significant ( $p=0.016$ ) (Table 2).

		vegf			p
		mean	±	SD	
Day 3	EG	26,39	±	4,65	0,016
	CG	18,50	±	1,99	
Day 7	EG	16,85	±	2,96	0,857
	CG	15,44	±	2,33	
Day 14	EG	14,48	±	1,17	0,190
	CG	15,31	±	0,86	

**Table 1:** The comparison of EG and CG mean values of VEGF shows the statistically significant difference at 3<sup>rd</sup> day.

Mann whitney u test ( $p<0,05$ )

		bFGF			p
		mean	±	SD	
Day 3	EG	8,70	±	2,23	0,690
	CG	7,41	±	0,96	
Day 7	EG	6,25	±	0,18	0,229
	CG	8,48	±	2,55	
Day 14	EG	7,73	±	1,16	0,016
	CG	6,48	±	0,15	

**Table 2:** The comparison of EG and CG mean values of bFGF shows the statistically significant difference at 14<sup>th</sup> day.

Mann whitney u test ( $p<0,05$ )

## Discussion

The current study was motivated by the idea that the ingredients of the energy drinks may affect the angiogenesis markers in serum. Energy drinks contains various nutrients like sucrose, glucose, water, B vitamins, taurine, and especially a high-level of caffeine<sup>(19)</sup>. Caffeine (1, 3, 7-trimethylxanthine) is a natural purine alkaloid and the most consumed psychoactive substance in the world. The induction of apoptosis and suppression of cell proliferation in several cancers are the different pharmacological actions of caffeine<sup>(20, 21)</sup>.

A recent study claims that caffeine suppresses the expression/secretion of VEGF-A in cancer associated fibroblasts and inhibits their pro-angiogenic effect<sup>(22)</sup>. An experimental study claims that high daily caffeine intake may disturb the early stages of

hard tissue wound healing, but in late stages it has no effect on bone density<sup>(23)</sup>.

Taurine is an amino acid containing sulphur and an antioxidant that shows antineoplastic activity by suppressing the angiogenesis<sup>(24)</sup>. It is found in almost all mammal organs and plays physiological and pathological roles with antiapoptotic, anti-inflammatory, and antilipid effects<sup>(25)</sup>. Kontny et al. revealed that taurine chloramine inhibited spontaneous and bFGF-triggered proliferation of fibroblast-like synoviocytes isolated from rheumatoid arthritis patients<sup>(26)</sup>. Taurine has also positive effects on brain damage in traumatic brain injury and markedly decreases the level of VEGF in a week<sup>(27)</sup>. High concentration of oxygen free radicals cause tissue damage, but low concentration of oxygen free radicals promotes VEGF, expressing in keratinocytes<sup>(28, 29)</sup>. Taurine is capable of inhibiting oxygen free radicals during the inflammation phase in the wound healing process and may affect the VEGF expression.

Ginkgo biloba is made from the dried leaves of the Ginkgo tree and has cardiovascular protective effects. Its extract is used for the treatment of vertigo, tinnitus, and peripheral arterial diseases in Europe<sup>(30)</sup>. Ginkgo biloba extract also downregulates VEGF protein and VEGF mRNA expression in rat aortic endothelial cells<sup>(31)</sup>. Kisli et al. showed the ginkgo biloba extract enhances the stability of colonic anastomoses<sup>(32)</sup>. The plant extracts, including ginkgo biloba, ginseng, and guarana, which are the ingredients of energy beverages, have been used as topical agents for accelerating wound healing widely<sup>(33)</sup>.

In light of the literature mentioned above, it may be considered that energy drinks can reduce the serum levels of VEGF and bFGF and slow down the angiogenesis, depending on whether there is a high concentrate of caffeine and taurine. In relation to this, a neoplastic effect may be mentioned. Controversially, it may be thought that the nutrients, like taurine and herbal extracts, which energy beverages contain, may regulate the wound healing process. Angiogenesis is seen in the proliferation phase of wound healing. In our study, on the 3<sup>rd</sup> day, the mean VEGF value of the EG was statistically higher than the mean CG value. It may be considered that energy drinks accelerate angiogenesis in the inflammation phase and the early stage of the proliferation phase of the wound healing process. At the end of the first week, there were no statistical differences between the EG and CG means of VEGF and bFGF. It can be thought that there is no effect from energy drinks on angiogenesis at the peak of the pro-

liferation phase of soft tissue wound healing. The only marker, bFGF, had a statistically higher mean value in the EG than in the CG on the 14<sup>th</sup> day. It may be claimed that energy drinks may be effective on angiogenesis at the late stage of the proliferation phase. The results showed us that the energy beverages may have positive and accelerative effects on wound healing by promoting angiogenesis. Further investigations should be considered and performed to confirm the results of our study.

## Conclusion

This study is one of the first studies investigating the difference of the serums of VEGF and bFGF levels, depending on energy drink consumption during the wound healing process. The results confirmed that energy drinks may accelerate angiogenesis in early and late stages of the proliferation phase of wound healing. Further investigations may reveal more reliable results.

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