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Systematic Review and Metanalysis of the *in vitro* Studies Examining the Erosive Effect of Energy Drinks Consumption on Dental Tissues

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ABSTRACT

***Background*: Considering the high market share of energy drinks and the number of existing studies examining their erosive properties this systemic review and metanalysis aim at investigating a selected number of relevant *in vitro* studies**

***Materials and Methods:* This systematic review focuses on a number of *in vitro* studies selected via the utilization of a configuration tool and their strength of evidence provided and subsequent procedures. Further selection occurred in order to perform a metanalysis.**

***Results*: The systematic review involved assessment of reporting quality, performance bias and other type of bias, all following a configuration of criteria established to serve this purpose. Three grades were used to describe the assessment. In the metanalysis a number of studies were selected that satisfied the requirements set. Results highlighted the erosive properties of the energy drinks.**

***Conclusion*: All studies included in this systemic review and metanalysis, showcased the erosive properties of the energy drinks and the requirement for further research.**

Keywords: Dental, dental erosion, dentistry, energy drinks, erosive properties, low pH, tooth wear.

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I. Introduction

The term *"energy drink"* defines a beverage containing stimulant ingredients, most notably caffeine. Usually, energy drinks are carbonated, and the sweetening agent can be sugar or substitutes. The sugars can be in the form of glucose or derivatives, including glucuronolactone and ribose [1]. They may also contain supplements (e.g., vitamin B complex), taurine, herbal extracts, guarana, ginseng, ginkgo biloba, and even amino acid derivatives such as carnitine, etc. [2]. Energy drinks are enhancers of other activities [3]. They typically contain 80 to 141 mg of caffeine per 8 ounces (236.588 ml), equivalent to 5 ounces (147.868 ml) of coffee-initially, energy drinks were aimed at the population of athletes as possible consumers. However, the energy drinks industry grew and expanded in various niche markets, increasing in numerous social groups. Nowadays, energy drinks are targeted at teenagers and young adults aged 18 to 34 due to the "on-the-go" lifestyle and receptiveness to these trends and advertisement goals [4]. As was demonstrated by [5] and [6] the popularity of energy drinks is evident among younger individuals, with 34% of 18 to 24 years old ones being regular users of energy drinks. A report published by [7] manifested that one-half of college students consume at least one energy drink per month to compensate for lack of sleep or to combine with alcohol. It is not uncommon to evidence misconceptions about energy drinks leading to an equation with sports drinks [8]. The main ingredient in most energy drinks, providing their energizing properties, is caffeine. Sports drinks mainly aim at rehydration and "calories providence," and this goal cannot be achieved with caffeine [9]. On the other hand, energy drinks have higher carbohydrate concentrations, though low-calorie products have been launched [10]. The profound expansion of the energy drinks industry has sparked the need for scientific research concerning their impact on human health. As a result, numerous *in vitro* and *in situ* studies have revealed essential facts in dentistry. For example, dental erosion is a pathological state that, according to accumulated scientific evidence, is related to the consumption of energy drinks. *Dental erosion* is defined as the pathologic chronic loss of dental tissues due to the chemical influence of intrinsic or/and extrinsic acidic substances without bacteria involvement [11]. It is a multicausal condition usually combined with other forms of tooth wear, such as attrition caused by contact between teeth and abrasion caused by any material with abrasive capability [12]. The chemical demineralization occurs when the pH drops under the critical 5.5 value [13]. In this state, the erosive agent protons attack the hydroxyapatite crystals, specifically phosphate, carbonate, and hydroxyl ions. The consequential dissolution of hydroxyapatite leads to the release of calcium

ions. The key factors determining hydroxyapatite's demineralization are the pH, phosphate concentration, calcium concentration, titratable acidity, and fluoride proportion in the erosive solution. Clinically, dental erosion initially appears as smoothed, silky, glazed enamel surface. Further progression may lead to shallow concavities or rounding and grooving of the edges or the cusps of the tooth surfaces [14]. Biological factors may potentially be beneficial or aggravating in the erosive procedure. Such factors are the quality of dental hard tissues, the orthodontal position of teeth, and the saliva flow, composition, and buffer capacity. In addition, it has been suggested that the biofilm properties can impair the diffusion of acids aggravating the erosion [15]. The more outstanding picture drawing dental erosion's exogenous fundamental causal elements could be highlighted in the properties of diet, environment, medication, and lifestyle [13]. The scientific findings illuminate in the diet section, where energy drinks are categorized as possible erosive agents. In a study conducted by [16] it was revealed that the examined energy drinks demonstrated pH values below 4.0. In Korea researchers, Choi and Shin measured pH in over 158 beverages and found none had a pH value above 4.0 [17]. While pH determines free protons in a solution, titratable acidity refers to the total concentration of free protons and undissociated acids in a solution that can react with a strong base and be neutralized. The latter and the buffering capacity provide a holistic approach to studying the erosive procedure [18]. As was demonstrated in [19] energy drinks have a high erosive potential as they present with low pH and a high non-reducing sugar content. In addition, it has been demonstrated that time exposure to the erosive agent contributes to the aggravation of the condition. Reference [20] has suggested in a clinical study that the pattern of oral pH differed between subjects with and without erosion after drinking an acidic beverage. This may be related to observed differences in drinking habits, which could have influenced the erosion pattern of these subjects. On the other hand, [21] has conducted a study that concluded that consumption of cola-type drinks, method of drinking, amount of palatal plaque on anterior teeth, and salivary urea concentration are factors associated with dental Erosion. In another *in vitro* study, [22] revealed that Coca Cola classic, Gatorade, and Red Bull with/without fluoride presented with the highest post-treatment roughness measurements, and these readings were compared to the ones of Starbucks coffee and two types of water. This Systemic Review examines all *in vitro* studies focusing on energy drinks and their erosive potential with the utilization of criteria that narrowed the amplitude to the extent of interest. The keywords used were "*dental AND erosion AND energy AND drinks".* The period was from 01/01/1985 till 01/01/2021. MEDLINE via PubMed and Scopus were the leading platforms used. The grey literature reports were also included as theses, dissertations, product reports, and unpublished studies through ClinicalTrials.gov, OpenGrey.eu, and ISRCTN registry. In addition, the research expanded in various well-established scientific journals, archives, and fora. The language selected was English. Furthermore, the reference lists of selected studies were scrutinized in search of relevant papers.

II. Study Selection Stages

A. Stage One

In stage 1, keywords utilization revealed various studies that two investigators independently evaluated. The initial evaluation involved an assessment of the title and abstract. Studies that met the inclusion criteria were aggregated for further investigation in the following stage. The inclusion criteria consisted of at least one energy drink in the study and English as the publication language or if a formal English translation accompanied the study. In Table I the number of studies initially selected via the approved sources is segregated based on the source. Sources that had not fruitful results are not mentioned in Table I.

In addition to Table I in Table II the selected studies are categorized based on their type. The assessment led to four categories.

TABLE I: Studies Segregation based on the Source

Pubmed	4
Scopus	14
Pubmed and Scopus	12
Drug Invention Today	1
BMC Public Health	1
British Dental Journal	1
Nutrition Journal	1
International Journal of Health Sciences Qassim University	1
World Journal of Stomatology	1
Journal of Dentistry	1
European Archives of Paediatric Dentistry	1
Journal Dental Hygiene Science	1
Open Access Macedonian Journal of Medical Sciences	1
Journal of American Dental Association	1
Journal of International Society of Preventive and Community Dentistry	1
Total number of studies	42

TABLE II: Categorization of Selected Studies in accordance with Their Type

In vitro	24
In vivo	8
Review	3
Observational/Cross Sectional	7
Total number of studies	42

B. Stage 2

In stage 2 the paper inclusion criteria were adjusted to meet the differences deriving from each category of studies and the goals of the current systemic review. In this manner, finalising the inclusion procedure, 2 criteria were introduced, aiming to narrow the selection, and simultaneously forging the basis of homogeneity. The reviewers decided to proceed with the *in vitro* studies yet filtrating them even further as have already been mentioned. *Reporting quality,* examined the number of teeth deployed, origin of teeth, state of teeth, period of teeth storage, storage and handling of teeth, temperature, brands of drinks used, storage and handling of drinks and ethical approval. *Performance bias* examined the equipment used for teeth preparation, the equipment used for experiment, the equipment used for acidity measurements and the number of examiners. *Other types of bias* examined whether funding was granted by private sector. Each part was assessed using three scores: *Specified* when full details were included in the study, *partly specified* when data were partly

missing and *not specified* when relevant data were absent.

Based on these additional criteria, the selection scheme led to 13 *in vitro* studies being included in the final assessment. These studies are:

- Acidic beverages increase the risk of in vitro tooth erosion [23].
- Analysis of the erosive effect of different dietary substances and medications [24].
- Effect of Carbonated Beverages, Coffee, Sports and High Energy Drinks, and Bottled Water on the in vitro Erosion Characteristics of Dental Enamel [22].
- Erosive effect of energy drinks alone and mixed with alcohol on human enamel surface. An *in vitro* study [25]
- Erosive potential of energy drinks on the dentine surface [16]
- Erosive potential of sports beverages on human enamel *in vitro* [26]
- *In Vitro* assessment of dentin erosion after immersion in acidic beverages: surface profile analysis and energy - dispersive X-Ray fluorescence spectrometry study [27]
- Influence of beverage composition on the results of erosive potential measurement by different measurement techniques [28]
- Influence of various acidic beverages on tooth erosion. Evaluation by a New Method [29]
- Properties and modification of soft drinks in relation to their erosive potential *in vitro* [30]
- A study on the enamel erosion caused by energy drinks [31]
- Testing the effect of aggressive beverage on the damage of enamel structure [32]
- The erosive potential of soft drinks on enamel surface substrate: An *In Vitro* scanning electron microscopy investigation [33]

C. *Stage 3: Assessment Bias Risk*

The methodological assessment of the included studies was structured in a predefined manner. For this purpose, the reviewers (CR, VK) independently developed a configuration based on the criteria they selected to evaluate each study.

First, the criteria were organized into the categories of reporting and management. Following these, scoring was selected to estimate the bias risk, ranging in three sections. *Specified* described the case of detailed presentation of data, *partly specified* when data were not fully presented, and *not specified* when lack of data was present, as has been described above. The criteria displayed in this scheme were developed since no systematic tool for this purpose was detected in the bibliography.

III. Meta Analysis

A. *Stage One: Inclusion Criteria and Study Selection*

Organizing the selected studies which were included in the systemic review brought the investigators to the issue of comparative data and the feasibility of this task. As a result, the 13 studies that had undergone the systemic review were selected to proceed with meta-analysis because data were eligible for this calculation. The criteria can be viewed in Table III. The remaining 10 papers did not specify pH for examined energy drinks, or any measurements were not accompanied by SD calculation. In addition, not every study has focused on a predefined set of energy drinks. Although this feature did not affect the bias assessment, it was a prerequisite to proceeding with meta-analytic comparisons. The initial investigations through the studies pot revealed possible candidates. The further evaluation led to specific issues regarding the configuration of the appropriate studies to be candidates for meta-analysis. The population of fizzy drinks and beverages examined in the *in vitro* studies, acquired from previously mentioned sources, was not exact. The precondition to performing a meta-analysis was to detect a common denominator, and for this purpose, *Red Bull* was present in 8 out of 10 preselected studies. However, only 3 studies had Standard Deviation (SD) computed, a compulsory feature to perform meta-analysis in this case. As shown in *table 5* a set of criteria was arranged based on the requirements associated with meta-analysis and the three studies meeting these criteria.

TABLE III: Inclusion Criteria for Meta-Analysis

Inclusion criteria	Yes	No
SD calculated for pH measurements	[16], [31], [32]	
At least one energy drink being present in common in all selected studies	[16], [31], [32]	

B. *Stage 2: Selection of Studies and Data Derived*

During the procedure of attaining data from the selected studies, the mean pH value of *Red Bull* and SD were obtained, and as a control, the measured mean pH of distilled water and the subsequent SD. Since the studies were *in vitro,* researchers segregated the total teeth population into groups for each experimental group. Reference [16] had populations of 10 (n=10) for each group, [31] had n=15 and [32] had n=40. These numbers were used to perform the meta-analytic computing.

C. *Stage 3: Statistical Analysis and Software*

The Review Manager 5.3 by Cochrane was used for the statistical analysis, focusing on SD, and mean comparisons.

IV. Results of Risk of Bias

The selected studies were evaluated using the reporting calibration and risk of bias configuration tool as presented in the methods section. The selected studies were enumerated and estimated. All the features selected to be evaluated and used as a comparison measure fulfilled the criteria. These features could be objectified and present the appropriate data to achieve an evaluation of the studies. The results can be viewed in Fig. 1. As can be sighted in Fig. 1 concerning the Reporting quality all the selected studies provided data concerning the number of teeth used and the origin of those teeth. However, 23.08% of the studies did not specify the state of those teeth (e.g., whether they were healthy or with carries), and 7.69% provided a partial specification. On the other hand, 53.85% did not specify the storage period, and 7.69% did not provide data regarding the storage and handling of those teeth (where under what conditions etc.),

while 15.38% delivered partial specification. Furthermore, the same percentage-15.38%-did not supply specific temperature data, and 38.46% provided the partial specification. When the description of the brands of drinks selected was evaluated, only 7.69% of the studies lacked specific data. However, 23.08% did not specify the storage and handling of the drinks, and 30.77% provided a partial specification. Finally, 69.23% did not specify whether ethical approval was attained in the reporting quality section.

Regarding the performance bias assessment results, 38.46% of the studies did not specify the equipment used for teeth preparation. In comparison, 7.69% did not supply data regarding equipment used in the experimental steps, and the same percentage did not specify the equipment used for acidity measurements. Finally, 100% of the studies included in this paper did not include data on the number of examiners involved in the preparation and practical steps.

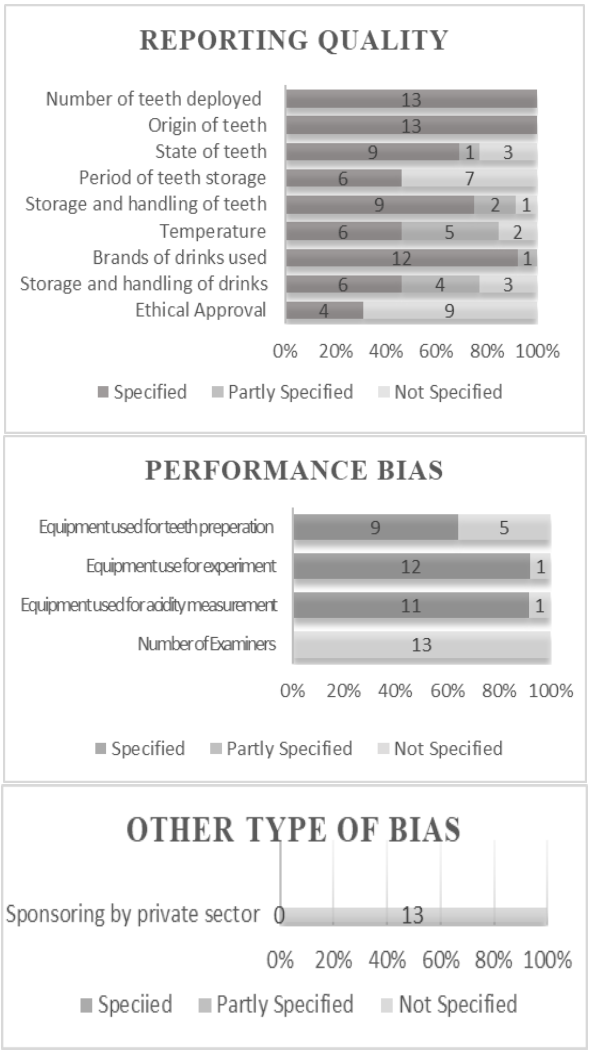

Fig. 1. Reporting Quality, Performance Bias, and Other Type of Bias. Results were demonstrated in percentages for all 5 selected studies.

V. Results of Meta Analysis

The three selected studies utilized different equipment to proceed with pH measurement, but this equipment is standardized and calibrated, so all values have been considered objective for this meta-analysis. Reference [31] employed the P25; pH Meter EcoMet, Seoul, Korea, [32] used AFM of JSPM-5200 and [16] used the pH meter AT-350, Sao Paulo, Brazil.

As can be viewed in Table IV, the mean values of *Red Bull* pH and the Standard Deviations on the energy drink section, while the control division contains the distilled water pH values and Standard Deviations. When evaluating the Forest Plot in Fig. 2 we can see a prominent placement of all pH values on the left side, which consists of indisputable evidence of the energy drinks erosive potential. When performing a Funnel Plot, these reading emphasize the theoretical and practical interpretation of the erosive properties associated with energy drinks, specifically *Red Bull.* The Funnel Plot can be viewed in Fig. 3.

Data presented in the Funnel Plot demonstrate the mean between the computation outcomes and further solidify the conclusion that energy drinks have acidity and, in this case, *Red Bull, which* is an intense and aggravating agent in dental erosion.

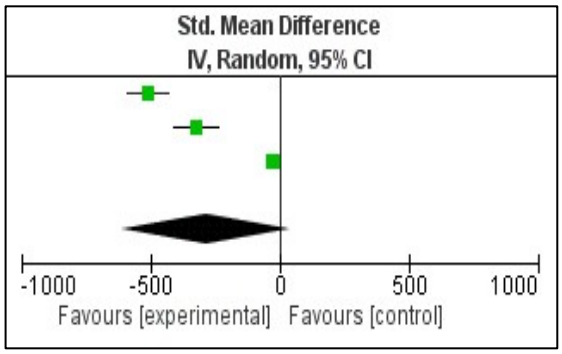

Fig. 2. Forest Plot.

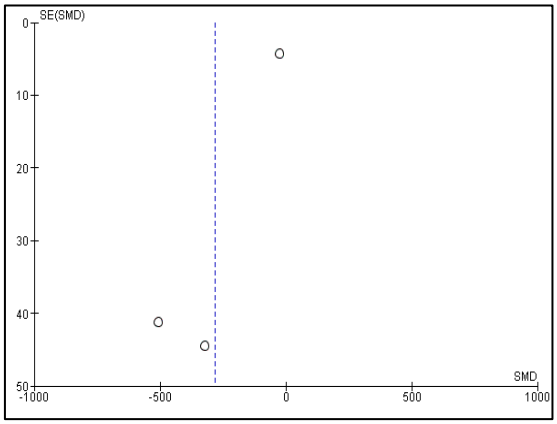

Fig. 3. Funnel Plot.

TABLE IIII: Meta–analysis Configuration

	Energy drink			Control				Std Mean difference IV, Random, 95% CI
Study or subgroup	Mean	SD	Total	Mean	SD	Total	Weight	
[32]	3.32	0.006	40	7.55	0.01	40	33.1%	-508.01 [-588.75, -427.28]
[31]	3.63	0.007	15	6.475	0.01	15	33.0%	-320.70 [-407.77, -233.64]
[16]	3.81	0.21	10	8.17	0.12	10	33.8%	-24.42 [-32.91, -15.93]
Total (95% CI)			65			65	100%	-282.57 [-608.83, -15.93]

Heterogeneity, $Tau^2 = 81908.84$; $Chi^2=178.85$, df=2 ($p<0.00001$); $I^2= 99\%$
Test for overall effect: Z=1.70 (p=0.09)

VI. Discussion

Although many studies have examined the erosive properties of soft drinks, there has been limited research on the erosive attributes of energy drinks, especially if the scientific examination involves shedding light on further comprehension of the outcomes deriving from such studies. Scrutinizing the selected *in vitro* studies for this systemic review revealed several exciting data. It has been well known that beverages have acidic pH hence an erosive character [23]. In addition to this, soft drinks contain sugar that comprises the primary nutritious source metabolized by the dental plaque, a fact that leads to acidic derivatives being released with subsequent pH fall and demineralization of dental tissues [13]. Examining the relevant studies to the criteria set for this review showcased that a differentiation concerning the approaches and methodology of various researchers exists and creates several obstacles when it comes down to performing a meta-analysis. However, the meta-analysis highlights that energy drinks are acidic, and their acidity is intense since pH values are way lower than the critical 5.5, initializing the erosive procedure [13]. *Red Bull* was used as a common denominator between the selected three studies, providing adequate data to perform the meta-analysis. Being an energy drink, which holds a prestigious position in the world market [34], demonstrates the extent of energy drinks proliferation in everyday dietary habits. Many *in vitro* studies have moved even further to assess dental tissue alterations and the manifestation of erosion when immersed in energy drinks solutions. Reference [29] and [30] have utilized computing the weight loss associated with acidic environment and mineral expulsion.

On the other hand, [22], [24], [26], [27] and [31] have used relevant strategies of surface microhardness measurements. Additionally, [32] used AFM nanometer, [33] utilized Electron Microscopy Investigation, [28] proceeded with Profilometric analysis, [16] and [25] performed their investigations with Scanning Electron microscopy. Finally, [23] used Polarized Light microscopy. This polyphony in the scientific investigation concerning the erosive properties of beverages and to this systemic review's interest, energy drinks, solidifies the scientific view but creates slim potentialities of meta-analysis. The relevantly moderate number of studies, with fundamental differentiates in procedures and methodology, on the other hand, incommode the meta-analytic initiative. Nonetheless, the conclusion is that energy drinks are intensively erosive commodities, which gain a more significant proportion in the world market yearly. Therefore, the scientific community must carry on with the further investigation, emphasizing this health issue constantly.

VII. Conclusion

The investigation of *in vitro* studies satisfying the criteria of the presence systemic review, revealed a relatively moderate bias, while the metanalysis led to fortify the data for the erosive properties of energy drinks. However further studies are required to create comparable and correlated data under regimes that could be examined in future metanalysis.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

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