PHARMACIA

Pharmacia 71: 1–7
DOI 10.3897/pharmacia.71.e117823

Research Article

# Effect of *Equisetum ramosissimum* Desf. on body weight and Leptin/Ghrelin in standard and high-fat diet

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**Received** 25 December 2023 ♦ **Accepted** 6 March 2024 ♦ **Published** 26 March 2024

**Citation:** Issa R, Abu Samak M, Abbas MM, Al-Qaisi T, Obeidat R, Ghanim B, Qinna N (2024) Effect of *Equisetum ramosissimum* Desf. on body weight and Leptin/Ghrelin in standard and high-fat diet. Pharmacia 71: 1–7. https://doi.org/10.3897/pharmacia.71.e117823

## Abstract

The necessity of maintaining healthy habits, diet and minimal medical complications is challenging. The current study investigates *Equisetum ramosissimum* on levels of leptin, ghrelin, and their ratio in association with body weight changes in animals receiving standard and high-fat diet. Ethanolic extract was prepared by maceration and total phenols and flavonoids contents were determined using Folin-cicalto and $AlCl_3$ methods. Study animals were fed with either standard or high-fat diets prior treatment with *E. ramosissimum* extract (500mg/kg). Body weights were monitored and serum concentrations of leptin, ghrelin and their ratios were determined. The plant extract comprised total phenols and flavonoids of 0.03 ± 0.01 mg/mg as Gallic acid, and 0.04 ± 0.03 mg/mg as quercetin, respectively. Findings revealed significant decrease in body weights of standard-diet animals and restoration of weight to normal after treatment with extract along with significant increase in serum ghrelin concentrations with strong correlation R and $R^2$ values in respect to body weight. As well, significant reduction in serum leptin/ghrelin ratio was positively correlated with body weight and serum leptin levels. *E. rasmossessium* extract possessed promising characteristics on being introduced in dietary supplements and weight control regimens. The current study presented the influence of extract in decreasing body weight in animals fed with standard diet. Moreover, it restored the weights of obese animals which received the extract after gaining weight and being on high-fat diet. Further preclinical studies are warranted to reveal the translational potentials of *E. rasmossessium* extract and its vital effect on adipokines.

## Keywords

Branched horsetail, leptin, ghrelin, diet, metabolic syndromes

## Introduction

In terms of known global pandemics, metabolic syndromes and cardiovascular diseases score highly in records of clinically distressed patients. The principal and most well-known metabolic diseases include, but are not limited to, insulin resistance and glucose intolerance, abdominal obesity, hypertension, low high-density cholesterol, and hypertriglyceridemia (Cătoi et al. 2018). The pathophysiology of these metabolic syndromes is often influenced

by adipokine hormones, specifically leptin and ghrelin. Adipokine hormones are well-known to modulate energy expenditure, as well as glucose and lipid metabolism, and are therefore claimed as potential predictive clinical markers for metabolic diseases (Ghadge and Khaire 2019). Studies of the correlation between adipokine concentrations and the calculated body mass index (BMI) of an individual have documented that levels of leptin and ghrelin are directly related to BMI. Meanwhile, not only is obesity directly affected by leptin and ghrelin disturbances but so also is the progression of diabetes. Thus, concentrations of adipokine hormones offer a basis for early intervention in diabetic and obese patients (Sitar-Taut et al. 2021).

Leptin is a hormone released by the adipose tissue and indirectly functions as a suppressor of appetite through signalling feedback concerning food intake and energy disbursement. In addition, leptin is reported to influence glucose homeostasis, immune response, pathogenesis of hypertension, atherosclerosis, and cancer (Arabi et al. 2019). On the other hand, ghrelin, also known as a hunger hormone, is secreted in several organs including the stomach and functions as a potent appetite stimulator. Not only does it increase in fasting periods but it also promotes weight gain. Nevertheless, ghrelin has also been reported to possess anti-inflammatory properties (Lyra Jr et al. 2019). Furthermore, decreased ghrelin levels have been reported in different pathophysiological conditions including obesity, type 2 diabetes, and other conditions with metabolic disturbances (Pulkkinen et al. 2010). Leptin and ghrelin are hormones with opposite effects on energy homeostasis and fitness. Therefore, the 'leptin over ghrelin ratio' has been suggested to be a marker and predictor in energy restriction treatment (Labayen et al. 2011). A recent study investigated the importance of the leptin/ghrelin ratio as a biomarker in dietary-induced hyperlipidemia in female mice, and found that animals fed with an excess fructose and cholesterol diet had significantly lower leptin levels and a subsequent drop in the leptin/ghrelin ratio. In addition, the animals showed low total body fat deposition. On the other hand, in another study model, specifically for dyslipidaemia, the levels of ghrelin were found generally higher than leptin (Riger et al. 2018). The level of the leptin/ghrelin ratio is reported to be altered in animals in a fasting state, in comparison to the levels after intake of varying macronutrient contents (Adamska-Patruno et al. 2018).

The interest in identifying natural products or chemicals that enhance the body to modulate the levels of either leptin, ghrelin or both is increasing. Chemical compounds which have been obtained from natural sources, including saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters, are plant constituents that have diverse medicinal properties (Talib et al. 2019; Savaya et al. 2020; Ubaydee et al. 2022). Recently, a study showed that white grape juice extracts were reported to reduce fat accumulation through the modulation of ghrelin and leptin expression in an *in vivo* model of overfed zebrafish, indicating that natural compounds could offer a potential insight into regulation of these hormones in metabolic diseases (Montalbano et al. 2021).

*Equisetum ramosissimum* Desf. is a native and rare plant known as "branched horse-tail". A recent study reported high prevalence of phenolic and flavonoid contents in its airborne parts (Savaya et al. 2020), rendering it as a phytochemical product with potential antioxidant properties. The plant species *E. ramosissimum* belongs to the family Equisetaceae, which is documented to possess a very potent anti-diabetic and antihyperlipidemic action (Safiyeh et al. 2007; Soleimani et al. 2007). Moreover, the plant was also reported to reduce oxidative stress in the context of some diseases, including atherosclerosis, ischemic cardiac disease, aging, and even infectious diseases (Boeing et al. 2021).

The *E. ramosissimum* plant has been used in folk medicine for treatment of various conditions, including reducing body weight and controlled some metabolomics diseases. The use of the extract is widely communicated between local herbalists for the treatment of different conditions and as an ingredient in herbal mixtures used for diabetes, lipidaemia and obesity. Therefore, the current study was conducted to answer whether *E. ramosissimum* plant extract would affect serum levels of leptin and ghrelin hormones and their ratios in an *in vivo* high-fat diet (HD) rat model. To our knowledge, it is a first to investigate the effect of this extract on metabolic hormones concerning body weight.

## Materials and methods

### Preparation and extraction of *E. ramosissimum*

The species of *E. ramosissimum* was collected from Mujib Biosphere Reserve (84 Km from Amman –FH4V+6PQ, Dead sea road, Sweimeh, Jordan) after authentication by a professional taxonomist at the Nature Conservation Monitoring Centre (Amman, Jordan) (RCSN herbarium no. E.r-5/7/2017). The shoot (ariel) part of the plant was dried under shade and stored thereafter at room temperature. On demand, the dried plant was crushed using a commercial blender (Philips HL7756/00 Mixer Grinder, 750W, USA) to obtain an acceptable particle size for further processing. A maceration method using an ethanolic medium (100% EtOH) was used for phytochemical extraction based on an in-house validated method. Briefly, 0.3% (w/v) of plant powder was soaked in ethanol for 24 h with constant agitation at room temperature. The mixture was then paper-filtered and evaporated at 60 °C using a rotary evaporator (R-300, Buchi, USA) at 90 rpm producing an extraction yield of 40.3% (w/w dry weight) for ethanolic extract. Extracts were stored dry conditions at room temperature 22 °C.

## Phytochemical analysis of Phenols and Flavonoids

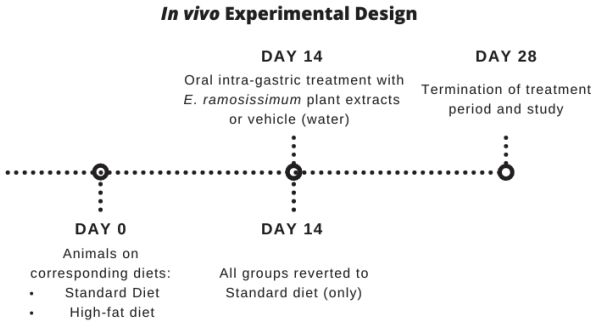
In order to determine the total phenol content within the extract, the Folin-Ciocalteu method was used as described earlier (Zahra et al. 2015). Briefly, plant extract was dissolved in methanol (0.002% w/v) and then $Na_2CO_3$ solution (5% v/v in distilled water) and Folin reagent (20% v/v in distilled water) were added to initiate the reaction medium. Reaction was maintained covered under aluminium foil for 1 h at room temperature. Then, the solution was quantified against gallic acid as a reference standard through determining the UV spectroscopy (Hitachi U-1800, UK) readings at 760 nm.

For the determination of total flavonoid content, a colorimetric method, based on the formation of a complex flavonoid–aluminium was employed, as described previously (Pękal and Pyrzynska 2014). Briefly AlCl3 solution (0.5 mL, 2 %, w/v) was added to 1 mL of the extract sample, and subsequently 0.5 mL of water was added. Then, the mixture was mixed and incubated for 10 mins at room temperature prior to spectral analysis. The solution was quantified against quercetin as a reference standard through determining the UV spectroscopy readings at 510 nm.

## *In vivo* experimental design

Eight-week old male Wistar rats were housed and acclimated at the animal house of Applied Science University, Amman Jordan. Handling and treatment procedures were conducted after acquisition of ethical approval from the Institutional Review Board approval no. 2021-PHA-40 (Date: 5/12/2021), and in accordance with the guidelines of its Institutional Animal Ethical Committee. Animals were maintained under conditions of 12 h light/ dark cycle, ambient room temperature (25 ± 2 °C), and with *ad libitum* access to water.

In our previous published work, an in vivo study on healthy standard diet-fed animals and an induced hyperlipidaemia model, it was concluded that the extract alone and in combination with Atorvastatin had a significant ($P<0.05$) reducing effect on serum lipid profile (Al-Bayati et al. 2023). These findings revealed the potential advantages of the extract alone and suggested further exploration of its mechanisms. Therefore, the current investigation focused on the action of extract on body weight variation and modulation of Leptin/Ghrelin concentrations post treatment, in both standard and high fat diet. Being a crucial element of the study, diet was served under specific parameters as described previously (Irudayaraj et al. 2013; Riger et al. 2018). Animals were randomized into 4 groups (n = 6) housed as 4 animals per cages and provided with corresponding diets for 14 days prior to treatments, specifically 2 groups receiving standard diet (SD) and 2 receiving high-fat diet (HD). The HD, containing 30% of lamb fat (50 g/kg/day) mixed with standard rodent chow, is considered rich in cholesterol and capable of inducing body weight gain. Then, after confirmation of the obesity model with at least 20–30% increase in weight, groups were treated once daily with either plant extracts (500 mg/kg) or vehicle (water) using oral intra-gastric tube for another 14 days (total study period of 28 days) (Alebous et al. 2016). Based on our previous study a lower dose of plant extract was employed (200 mg/kg) and was found to be effective in lowering the lipid profile of animals on high-fat diet, therefore in order to induce a prominent effect, a higher safe dose was used in the current study (Al-Bayati et al. 2023). Study timeline described in Scheme 1.




**Scheme 1.** Experimental in vivo study design.

## Serum concentration of Leptin and Ghrelin

In order to determine the concentrations of leptin and ghrelin in treated animals, serum samples were collected from all rats at the termination of the study. On the 28$^{th}$ day of the study, animals were fasted overnight and prepared for sampling under 2% isoflurane anaesthesia. Blood samples were collected from the orbital plexus using heparinized capillary tubes into plain mini-collect tubes. Animals were sacrificed by cervical dislocation after completion of sampling. Serum was separated after centrifuging the blood tubes at 8,000×g for 10 mins at ambient temperature. Serum samples were quantified for leptin and ghrelin concentrations using colorimetric assays and in accordance with the manufacturer's instructions for each kit, Abcam (ab100773, UK) and MyBiosource (MBS731169, US), respectively.

## Statistical analysis

The statistical analysis of data was done using SPSS, version 27.0 (Chicago, IL, USA). One-way ANOVA test using Tukey's post hoc was used to obtain statistical significance between all study groups, and student t-test was made to compare data of treated and non-treated groups of the same diet. Data are presented as mean values with standard deviation (SD). All experiments were run in triplicates unless stated otherwise.

# Results

## Phytochemical analysis

Methanol extract of *E. ramosissimum* was examined for phenol and flavonoid content. The extract presented a yield of 0.032 ± 0.01 mg/mg phenolic content in dry extract, calculated as gallic acid equivalence. As for the flavonoid content, a yield of 0.44 ± 0.03 mg was calculated in the dry extract as quercetin equivalence, as shown in Fig. 1.

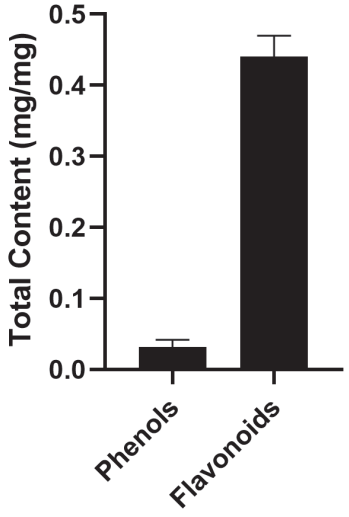



**Figure 1.** Total Phenol and Flavonoid content in *E. ramosissimum* extract. Extracts are quantified against equivalent quantities of reference standards, specifically Gallic acid and quercetin, respectively.

## Percent change in Body Weight

After confirming the weight-gain animal model, animals were treated with *E. ramosissimum* extract and weights were recorded after 14 days of treatment. As shown in Fig. 2, a decrease in weight was observed in animals treated with the plant extract. The drop in body weight after treatment with plant extract was considered statistically significant in animals receiving a standard diet ($p$-value<0.001). Despite the statistical insignificance, a decrease in body weight was also noticed in animals that received the high fat diet.

## Serum Leptin and Ghrelin concentrations

As shown in Fig. 3, the differences in serum ghrelin concentrations are marked between animals treated with high-fat diet, as well as all animals treated with the plant. The increase in ghrelin levels was considered statistically significant between the SD and SDP groups, which indicates a direct effect of the plant extract. On the other hand, a similar increase was also noticed in animals receiving a high-fat diet ($p$<0.05), regardless of whether they were treated with the plant extract or not. However, and although not considered statistically significant, animals on the high-fat diet had lower levels of ghrelin in comparison to those on a standard-diet which had been receiving plant extract.

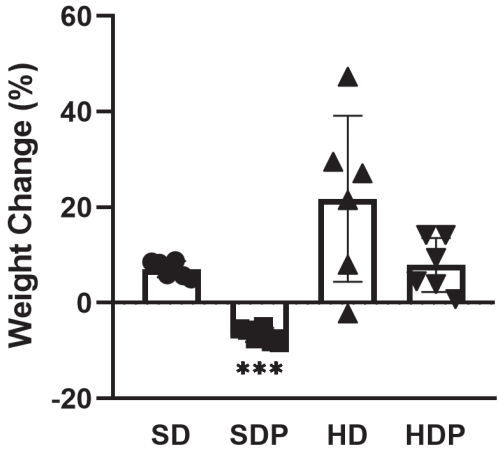



**Figure 2.** Percent Body Weight change in healthy and obese animals treated with *E. ramosissimum* extract. Comparison of weight change was made between: Standard Diet (SD); SD receiving Plant extract (SDP); High-fat Diet (HD); and HD receiving Plant extract (HDP). ***: $p$<0.001 in comparison to SD, as calculated through t-test.

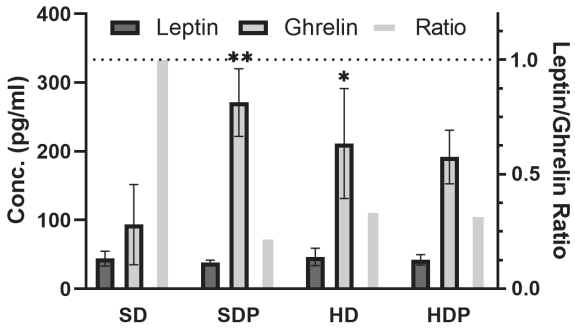



**Figure 3.** Serum concentrations of leptin and ghrelin and their corresponding ratios. Serum concentrations of leptin and ghrelin and their corresponding ratios were quantified for animals receiving Standard Diet (SD); SD receiving Plant extract (SDP); High-fat Diet (HD); and HD receiving Plant extract (HDP). Ratios of leptin/ghrelin were calculated and presented in normalized values to the standard body levels (right y-axis), specifically per animals receiving SD. **: $p$<0.01; *$p$<0.05 in comparison to SD.

## Correlation of body weight change and Leptin/Ghrelin ratio

As shown in Table 1, a marked statistical correlation between the BW and the evaluated biomarkers of serum levels for leptin, ghrelin and their ratios was observed. The correlation of body weight change and Leptin/Ghrelin

**Table 1.** The difference in correlation factor, coefficient determination, and significance p-values between change in body weight and its relation to serum levels of leptin, ghrelin and their ratio in study groups (n = 6).

| | Correlation factor (R) | | | | Coefficient determination ($R^2$) | | | | *p*-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SD** | **SDP** | **HD** | **HDP** | **SD** | **SDP** | **HD** | **HDP** | **SD** | **SDP** | **HD** | **HDP** |
| **Leptin** | **0.13** | **0.7** | -0.63 | -0.2 | 0.02 | **0.5** | **0.39** | 0.04 | 0.8 | 0.12 | 0.18 | 0.7 |
| **Ghrelin** | 0.07 | **-0.58** | -0.63 | -0.89 | 0.01 | 0.34 | **0.39** | **0.79** | 0.89 | 0.23 | 0.18 | 0.18 |
| **L/G ratio** | -0.24 | -0.14 | **0.44** | **0.74** | **0.06** | 0.02 | 0.19 | **0.54** | 0.66 | 0.79 | 0.38 | 0.1 |

Groups: Standard Diet (SD); SD receiving Plant extract (SDP); High-fat Diet (HD); HD receiving Plant extract (HDP)

ratio was made based on calculating the correlation factor (R), the coefficient determination ($R^2$), and significant p-values (<0.05). Despite animals treated with plant extract after consuming standard-diet having showed strong correlation between change of body weight and leptin/ghrelin concentration, based on high R and $R^2$ values, animals fed with a high-fat diet showed a stronger correlation between changes of body weight and levels and ratios of leptin and ghrelin. Nevertheless, animals treated with the plant extract after being on the high-fat diet also showed a higher significant correlation between the leptin/ghrelin concentration and change of body weight, in comparison to all study groups.

## Discussion

The natural collaboration between leptin and ghrelin in maintaining appetite, weight control and energy homeostasis remains an important topic that needs further understanding. Adiposity and appetite regulating hormones, including leptin, ghrelin, insulin and others, are key biotargets in the fields of nutraceutics, herbal medicines and general physical health. The investigation of nutraceuticals has great potential value for limiting or preventing excess weight gain and its accompanying metabolic syndrome.

Extracts collected from different species of *Equisetum* have been reported to be rich sources of phenolic compounds and flavonoids that have pharmacological properties (Boeing et al. 2021). In addition, alkaloids, phytosterols, tannins, and triterpenoids of the *Equisetum* genus are among its most well-known phytochemical constituents (Mimica-Dukic et al. 2008). Horse tail species have been reported to have haemostatic, diuretic, antifungal, antiviral, antibacterial, antioxidant, and anticancer properties (Boeing et al. 2021). Hypoglycaemic effects were also reported in some species of horse tail plants such as *E. arvense*, *E. myriochaetum*, and *E. giganteum* (Angel et al. 2020; Hegedűs et al. 2020; Vieira et al. 2020). The prevalence of high phenolic and flavonoid content in the study species, aligns with a suggestion of its potential in controlling diabetes as well as for lowering cholesterol levels and body weight (Hegedűs et al. 2020). Several compounds such as kaempferol, luteolin, myricetin, quercetin, rutin, caffeic acid derivative, tannins, ferulic acid, linoleic acid, saponin, and phytosterol, which have been isolated from an alcoholic extract of *E. rasmossessium*, are well-documented to possess positive effects on metabolic diseases (Revilla et al. 2002; Afifi and Kasabri 2013; Batir-Marin et al. 2021).

Introducing the use of *E. rasmossessium* extract as a potential candidate in regulating targets involved in metabolic diseases has not previously been studied. Therefore, the current study focused on observing the potential of the extract on animals of high weight, and its capacity in stimulating body weight loss. Identifying the suspected underlying mechanism, we focused on evaluating the correlation of serum levels of leptin, ghlerin and their calculated ratios. Despite the levels were considerably lower than treated animals fed on SD, the direct effect of the *E. rasmossessium* extract on leptin levels in animals fed on HD was statistically insignificant, which is in accordance with previously published results (Schmid et al. 2005). Ghrelin appears to be inversely related to body weight (increases in standard diet rats that lose weight with treatment, Fig. 2) but directly related to body weight in rats that gain weight with high fat diet and show elevated ghrelin regardless of treatment (increased leptin with increased body weight with high fat diet). Therefore, it could be suggested that levels of ghrelin are increased in animals treated with the plant extract after being on a standard diet and are slightly decreased in others on a high-fat diet. Despite the fluctuating levels of ghrelin in HD animals, they remain higher than in animals of the same diet that were treated with the plant extract. Therefore, in order to ascertain the effect of plant extract on lowering ghrelin in animals on HD, a larger sample population study will be conducted. The contrary data of having the plant extract affect ghrelin levels based on the nature of the diet suggests that the plant extract could have a neutralizing influence on levels ghrelin. In line with these results, higher baseline ghrelin levels have been shown to be accompanied by weight loss (Garcia et al. 2006; Rosenbaum et al. 2019), and fasting ghrelin levels correlate inversely with food intake (Salbe et al. 2004).

Conclusions on leptin levels and its correlation with body weight loss could be completed through calculating and understanding its ratio in comparison to levels of ghrelin. Findings of the study revealed a direct effect of *E. rasmossessium* extract on body weight, regardless of whether exposed to a standard or high-fat diet. Nevertheless, its potential for reducing the body weight of high weight animals was also observed. These data highlighted the differences in the correlations between body weight

and the measured biomarkers within the two sets of groups: standard and high-fat diet fed animals. Treatment with the plant extract showed that leptin levels are strongly correlated to body weight in animals on a standard diet. On the other hand, the correlation was considered statistically weak when levels of leptin were compared in animals fed with a high-fat diet. Several studies have investigated the role of leptin on the changes in energy metabolism that occur during weight loss. In obesity, the existence of an endogenous leptin-resistance mechanism limits its impact on weight, interpreted as an energy-sparing mechanism operating in obese animals (Labayen et al. 2011; de Git et al. 2018; Barham et al. 2021). The reverse correlation between body weight and serum leptin levels is now well recognized in human and animal studies (Labayen et al. 2011; Arabi et al. 2019; Montalbano et al. 2021). In the same context, the reduction of leptin and leptin/ghrelin ratio reflects an improvement in leptin resistance that accompanies loss of body weight which has been considered as a key factor in improving insulin resistance (Pulkkinen et al. 2010; Riger et al. 2018).

## Conclusion

*E. rasmossessium* extract shows promising characteristics of being introduced in dietary supplements, weight control regimens and some medical interventions. The current study presents the influence of *E. rasmossessium* extract in decreasing body weight in animals receiving standard diet and limiting weight gain in animals on high-fat diet. Further preclinical studies are warranted to reveal the clincial outcomes of *E. rasmossessium* extract and its vital influence on adipokines, specifically leptin and ghrelin.

## Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## Competing interests

The authors declare no potential conflicts of interests with respect to the research, authorship, and/or publication of this article.

## Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

## Acknowledgments

The authors appreciate the Pharmacological and Diagnostic Research Centre at Al-Ahliyya Amman University, Jordan, as well as the efforts of botanist and taxonomist Anas Sabarini at the Nature Conservation Monitoring Centre for authenticating the species and facilitating its collection within Mujib Biosphere Reserve of the Royal Society for the Conservation of Nature (RSCN), Jordan.

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