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AESTHETIC RADIOFREQUENCY COMBINED WITH ORAL SUPPLEMENTATION WITH Rosmarinus officinalis CHANGES ANTHROPOMETRIC MEASURES IN WOMEN: RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Introduction: Aesthetic radiofrequency treatment is a therapeutic method focused on reducing adiposity, of reducing body weight, concomitant with the use of aesthetic techniques using herbal medicines and medicinal plants. One of the plants still little explored by health professionals is Rosmarinus officinalis. Objective: To evaluate the impact of aesthetic radiofrequency treatment, combined with the use of standardized dry extract R. officinalis, on anthropometric variables in healthy women. Materials and Methods: Thirty-two healthy women were subjected to radiofrequency cosmetic treatment for 4 weeks. These women were divided into Control Group (CG) and Intervention Group (IG). In CG (n=8), placebo capsules were included in the diet. The intervention group was divided into: Group A (n=8) 100 mg day^{-1}; Group B (n=8) 500 mg day^{-1}; Group C (n=8): 1,000 mg day^{-1}. Anthropometric data regarding abdominal skinfolds, abdominal circumferences, and bioimpedance were collected before and after the interventions. Results: The aesthetic treatment with radiofrequency did not change the parameters evaluated. Significant changes were observed for the use of R. officinalis, through the significant increase in skinfolds, for all doses evaluated, as well as reduction in abdominal circumference with the use of 500 mg. Conclusion: Aesthetic radiofrequency treatment did not result in significant changes in anthropometric variables. Oral supplementation with R. officinalis extract can increase abdominal skinfolds in a linear, dose-dependent way. In addition, oral supplementation can significantly change abdominal circumferences.

Key words: Radiofrequency Therapy. Rosmarinus. Anthropometry. Skinfold Thickness. Abdominal Circumference.

RESUMO

Radiofrequência estética combinada à suplementação oral com Rosmarinus officinalis altera as medidas antropométricas nas mulheres: ensaio clínico randomizado

Objetivo: Avaliar o impacto do tratamento estético com radiofrequência, associado ao uso de extrato seco padronizado de Rosmarinus officinalis, nas variáveis antropométricas de mulheres saudáveis. Materiais e Métodos: Trinta e duas mulheres saudáveis foram submetidas a tratamento cosmético por radiofrequência durante 4 semanas. Essas mulheres foram divididas em Grupo Controle (GC) e Grupo Intervenção (GI). No GC (n=8), cápsulas de placebo foram incluídas na dieta. O grupo intervenção foi dividido em: Grupo A (n=8) 100 mg dia-1; Grupo B (n=8) 500 mg dia-1; Grupo C (n=8): 1.000 mg dia-1. Dados antropométricos referentes a dobras cutâneas abdominais, circunferências abdominais e bioimpedância foram coletados antes e após as intervenções. Resultados: O tratamento estético com radiofrequência não alterou os parâmetros avaliados. Mudanças significativas foram observadas para o uso de Rosmarinus officinalis, através do aumento significativo das dobras cutâneas, para todas as doses avaliadas, bem como redução da circunferência abdominal com o uso de 500 mg. Conclusão: O tratamento estético com radiofrequência não resultou em mudanças significativas nas variáveis antropométricas. A suplementação oral com extrato de Rosmarinus officinalis pode aumentar as dobras cutâneas abdominais de forma linear e dependente da dose. Além disso, a suplementação oral pode alterar significativamente as circunferências abdominais.

Palavras-chave: Terapia por Radiofrequência. Rosmarinus. dobra cutânea. Circunferência abdominal.

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INTRODUCTION

Aesthetic radiofrequency treatment is a widely used therapeutic method for sagging facial and body skin and improving body contour (Tagliolatto, 2015) and has shown benefits for patients seeking to reduce adiposity(Nojomi et al., 2016).

Medicinal plants are an alternative used to assist in the process of reducing body adiposity, concomitant with the use of aesthetic techniques, such as aesthetic radiofrequency treatment (Costa et al., 2020).

These plants, combined with food reeducation, have shown positive results in reducing body fat levels (Oliveira et al., 2017). One of the plants still little explored for adiposity is Rosmarinus officinalis, which has important therapeutic properties (Bao et al., 2020; Oliveira, Veiga, 2019).

The effects of R. officinalis leaf extract on mice have shown that it can limit weight gain induced by a high-fat diet (Harach et al., 2010).

There are no studies in the literature associating aesthetic radiofrequency treatment with the use of herbal medicines, and the effect of using R. officinalis for reducing body measurements is still unclear.

Thus, the objective of this study was to evaluate the impact of aesthetic radiofrequency treatment, combined with the oral use of standardized dry extract of R. officinalis, on anthropometric variables in healthy women.

MATERIALS AND METHODS

Experimental design and participants

This is an experimental, longitudinal, analytical, prospective, randomized, double-blind clinical trial, in which oxidative stress markers were analyzed.

The study was approved by the Research Ethics Committee of the Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), under the number 4,461,079/2020; Brazilian Registry of Clinical Trials (ReBEC): RBR-86m4sns; Universal Trial Number (UTN) U1111-1274-6255.

The sample consisted of 32 women, between 18 and 50 years old, with localized abdominal fat. They were randomly invited through local media and included in the research after signing a Free and Informed Consent Term.

Women who did not present comorbidities, did not undergo non-invasive cosmetic procedures in the last 30 days, and were not on a restrictive diet for weight reduction were included.

Those who were incapable natural persons, pregnant or breastfeeding; use anti-inflammatory drugs, herbal medicines, or weight-loss products; had allergy or intolerance to Rosmarinus officinalis; or sensitivity or allergy, or contraindications to the procedures were excluded from the study. Obese women, with body mass index (BMI) ≥ 30 (World Health Organization, 1995), calculated by the formula BMI = weight (kg) / height^2 (m) (Brasil, 2011), or had undergone invasive cosmetic procedures in the previous 12 months were also excluded.

Women were randomized considering age as the matching criterion.

Rosmarinus officinalis

R. officinalis capsules were produced from a standardized dry extract purchased from a supplier authorized by Brazilian national health agencies.

The manipulation of herbal and placebo capsules was carried out at the University Pharmacy of the UNIJUÍ, in compliance with good handling practices. Starch was used as excipient to produce the capsules and placebo.

The quality control recommended by the Brazilian Pharmacopoeia and required by the Collegiate Board Resolution (RDC) 67/2007 was carried out for each batch of capsules produced; the average weight, upper and lower limits, and coefficient of variation were calculated.

Capsule jars were delivered to participants on the day of baseline assessments and blood collection, containing 30 capsules in each jar. The participants were instructed to take a daily dose, in the mornings.

Interventions

The study was carried out in a multidisciplinary private clinic in Ajuricaba, RS, and in a clinic school in Ijuí-RS, Brazil. The protocol was applied between December 2020 and June 2021.

Participants of the study were divided into two groups: control (CG) and intervention (IG). Women of both groups were subjected to application of 12 aesthetic radiofrequency sessions, 3 times a week, for 4 weeks.

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Radiofrequency was applied to the abdominal region, with temperatures between 39 and 43 °C, subdivided into 4 regions of 100 cm^2 for 10 minutes in each region. A bipolar applicator with concentric field and frequency of 640 KHz was used. This procedure was carried out by the researcher.

The pharmacological intervention using R. officinalis was carried out in the same period as the aesthetic treatment (4 weeks). In the control group (n=8), placebo capsules (starch) were included in the diet. The intervention group was divided into 3 subgroups: Group A (n=8), consisted of supplementation with R. officinalis at 100 mg day^{-1} in the diet and radiofrequency; Group B (n=8), which consisted of supplementation with R. officinalis at 500 mg day^{-1} in the diet and radiofrequency; and Group C (n=8), which consisted of supplementation with R. officinalis at 1,000 mg day^{-1} in the diet and radiofrequency.

Anthropometric measurements

An anamnesis form was prepared for the participants for evaluating weight, body water, lean mass index, and fat mass index, which were assessed using a bioimpedance scale.

Circumference measurements were taken following the Guidelines for Collection and Analysis of Anthropometric Data in Health Services (Brasil, 2011).

The following points were evaluated to standardize abdomen measurements: just below the breasts; 5 cm above the umbilicus; over the umbilicus; 5 cm below the umbilicus; and waist, at the smallest abdominal circumference.

The abdominal skinfold was measured with the aid of an adipometer to quantify the fat in this region of the body. Three points were measured: horizontal skinfold located laterally at 3 cm from the umbilicus and 1 cm below the center of the umbilicus; Vertical skinfold located laterally at 2 cm from the umbilicus; skinfold located laterally at 5 cm from the umbilicus. The measurements were taken following the recommendation of Machado (2008). Measurements were carried out by a trained aesthetic cosmetologist.

Statistical analysis

The data was analyzed using scientific formulas in the Microsoft Excel program. Analyses were carried out using the GENES Software (Quantitative Genetics and Experimental Statistics, version 2015.5.0).

Data normality was evaluated using the Kolmogorov-Smirnov test. Continuous data were described as mean ± standard deviation (SD) or median (interquartile range), and categorical data were described as absolute and relative frequency.

The association of quantitative variables was assessed using mean comparison test with paired data by the Student's t test. A 5% significance level was used for all tests.

The data were subjected to analysis of variance to assess the main effects and the interaction between use of R. officinalis and exposure to radiofrequency. Then, linear regression analysis ($Y = b0 \pm b1x$) was carried out for assessing the efficiency of the use of R. officinalis to change anthropometric variables, by the value of the linear coefficient = intercept (b0), that indicates the point of departure of the variable in the regression and the slope (b1), which determines the growth or reduction rate in the variable y by the dose of R. officinalis.

RESULTS

The study included 32 healthy women who had localized abdominal fat. The mean age of the participants was 34 years, with standard deviation of 8.97. The average body mass index (BMI) was 24.30 kg m^{-2}, with standard deviation of 2.41, with the sample in the normal weight range (WHO, 1995).

Considering the interventions carried out in the research (supplementation with Rosmarinus officinalis and abdominal aesthetic treatment with radiofrequency), the result of the analysis of variance (Table 1) indicated that the variation due to the doses of the bioactive were significant; however, the radiofrequency treatment, represented by the data collections (0, 07, 14, 21, and 28 days) and the interaction between the sources of variation was not significant.

Skinfolds 1, 2 and 3 showed statistical significance for the use of R. officinalis and no significance for the aesthetic procedure. As for the circumferences tested, the measurements over the umbilicus and below the umbilicus showed statistical significance for the use of R. officinalis and no significance for the aesthetic treatment (Table 1).

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Table 1 - Analysis of variance of anthropometric measurements of healthy women as a function of Rosemary (R. Officinalis) doses and radiofrequency treatment, Rio Grande do Sul, 2020, 2021.

Source of Variation	DF	Medium Square			
		Skinfold 1	Skinfold 2	Skinfold 3	Waist
Bioactive Doses (BD)	4	4.47*	6.39*	5.54*	33.69^{ns}
Radiofrequency treatment (RT)	3	0.22^{ns}	0.05^{ns}	0.18^{ns}	6.19^{ns}
BD × RT	12	0.09^{ns}	0.02^{ns}	0.04^{ns}	2.04^{ns}
Error	140	0.64	0.45	0.79	28.96
Total	159	-	-	-	-
General Average		2.87	2.38	2.79	74.38
Coefficient of Variation (CV)		27.85	28.3	31.93	7.23

Source of Variation	DF	Medium Square			
		Below the breast	Above the umbilicus	Over the umbilicus	Below the umbilicus
Bioactive Dose (BD)	4	34.78^{ns}	65.9^{ns}	155.13*	86.8*
Radiofrequency treatment (RT)	3	0.72^{ns}	7.35^{ns}	11.06^{ns}	5.5^{ns}
BD × RT	12	0.90^{ns}	1.58^{ns}	4.1^{ns}	0.49^{ns}
Error	140	24.37	42.36	48.8	34.21
Total	159	-	-	-	-
General Average		78.16	76.8	84.3	89.5
Coefficient of variation (CV)		6.31	8.47	8.28	6.5

Legenda: DF = degrees of freedom; * = significant by the F Test ($p<0.05$); ns: not significant.

According to Table 1, the supplementation with R. officinalis cause variation in skinfolds and circumferences, while the aesthetic treatment with radiofrequency did not result in changes, as shown by the not significant means found for the radiofrequency treatment.

The statistical significance found for skinfolds was due to increases in their thickness as the R officinalis dose was increased. The use of the bioactive was significant for increasing skinfold 1, when compared to the placebo. This variable presented no difference between doses (100, 500, and 1,000 mg day^{-1}). Skinfolds 2 and 3 presented increases when using supplementation with R officinalis and differences between supplementation doses 0 or 100 mg and larger doses (500 mg and 1,000 mg) (Table 2).

Table 2 - Correlations between statistically significant measurements of healthy women and doses of R officinalis, Rio Grande do Sul, 2020, 2021.

Rosmarinus officinalis (mg)	Skinfold 1 (cm)	Skinfold 2 (cm)	Skinfold 3 (cm)	Over the umbilicus (cm)	Below the umbilicus (cm)
0	2.49 b	1.95 b	2.40 b	84.31 a	89.47 a
100	2.73 a	2.15 b	2.56 b	86.10 a	90.73 a
500	3.00 a	2.58 a	3.01 a	81.50 b	87.52 b
1,000	3.26 a	2.82 a	3.19 a	85.31 a	90.54 a

Means followed by the same letter in the columns are not different from each other by the Scott-Knott Test ($p<0.05$).

The comparison between the use of the 500 mg dose and the other doses tested presented statistical differences for circumferences over the umbilicus and below the umbilicus. The circumference decreased to values below the placebo values when using supplementation with 500 mg of R. officinalis.

The results for the doses of 100 mg and 1,000 mg indicated increases in circumference measurements.

Considering the trends of skinfold and circumference means, regression equations were applied. The result of the analysis of variance indicated that variations in doses and

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skinfolds were significant. However, statistical significance was found only for skinfolds 1, 2 and 3 (Table 3). The data fitted to first-degree linear equations, denoting a tendency of increases in skinfold as the R. officinalis doses was increased.

Table 3 - Regression equation of anthropometric measurements of healthy women subjected to radiofrequency as a function of different doses of Rosmarinus officinalis, Rio Grande do Sul, 2020, 2021.

Source of Variation	Equation $y=a\pm bx\pm cx^2$	R^2	Optimal dose	y
	Skinfold 1			
Linear	$2.59+0.00071x^*$	94.08	-	-
Quadratic	$2.54+0.0012x-0.00000051x^{2ns}$	97.3	-	-
	Skinfold 2			
Linear	$2.03-0.00085x^*$	94.3	-	-
Quadratic	$1.97+0.001x-0.000000x^{2ns}$	99.8	-	-
	Skinfold 3			
Linear	$2.48+0.00078x^*$	92.65	-	-
Quadratic	$2.40+0.0016x-0.00000086x^{2ns}$	99.98	-	-
	Umbilicus			
Linear	$84.47-0.00034x^{ns}$	0.63	-	-
Quadratic	$85.55-0.012x+0.000012x^2$	57.59	-	-
	Below the umbilicus			
Linear	$89.52+0.00011x^{ns}$	0.11	-	-
Quadratic	$90.36-0.0096+0.0000097x^2$	60.96	-	-

Legenda: R^2 = coefficient of determination; y = maximum expression; ns = not significant by the t test ($p<0.05$); * = significant by the t test ($p<0.05$).

No significant changes were found in the analysis of body composition: fat mass, lean mass, BMI, weight, and hydration (Table 4).

Table 4 - Analysis of anthropometric measurements of healthy women subjected to radiofrequency treatment and different doses of Rosmarinus officinalis, Rio Grande do Sul, 2020, 2021.

Dose (mg)	Fat (%)			Fat (%)			IMC (Kg m^{-2})		
	Initial	SL	Final	Initial	SL	Final	Initial	SL	Final
0	34.88	ns	35.27	28.01	ns	27.66	24.96	ns	24.80
100	35.70	ns	36.40	26.83	ns	26.42	24.32	ns	24.33
500	36.05	ns	37.16	26.18	ns	25.23	23.90	ns	23.86
1,000	37.90	ns	36.58	25.1	ns	26.21	24.10	ns	24.21
	-		36.35	-		26.38	-		24.30
SD	-		4.07	-		2.07	-		2.41
1	-		40.42	-		28.45	-		26.71
X-1	-		32.28	-		24.31	-		21.89

Dose (mg)	Weight (kg)			Hydration (%)		
	Initial	SL	Final	Initial	SL	Final
0	66.13	ns	65.68	53.31	ns	53.7
100	68.03	ns	67.96	53.98	ns	53.96
500	63.82	ns	63.70	54.56	ns	54.45
1,000	63.50	ns	63.81	53.93	ns	54.26
	-		65.29	-		54.09
SD	-		8.46	-		2.51
+1SD	-		73.75	-		56.60
-1SD	-		56.83	-		51.58

Legenda: SL = significance level; by the Student's t test; ns = not significant; SD = standard deviation; +1SD = mean plus one standard deviation; -1SD = mean minus one standard deviation.

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DISCUSSION

The main contribution of this study is related to effects of Rosmarinus officinalis supplementation on anthropometric measurements.

As shown in Table 1, the aesthetic procedure with radiofrequency had no significance in altering the variables, and the supplementation with R. officinalis was solely responsible for the results of changes in skinfolds and abdominal circumferences.

Thus, the application of only the aesthetic procedure with radiofrequency did not result in significant changes in anthropometric variables (abdominal circumference and skinfold) in women with localized fat.

Contrastingly, the studied plant extract resulted in significant changes in the variables studied. Significant thickening of skinfolds was found for all doses evaluated, and it was dose dependent, with a linear tendency to increase as the R. officinalis dose was increased.

This is explained by evidences of the effect of R. officinalis, which increases skin hyaluronic acid contents and, consequently, results in thickening of the skin, as shown in the present study. Although R. officinalis extract was used in the present study, an in vitro study showed that lavender and R. officinalis essential oils also significantly increased ($p<0.05$) hyaluronic acid contents (Machado et al., 2020).

Hyaluronic acid is a glycosaminoglycan polysaccharide present in the extracellular matrix of the skin. It has hydration, lubrication, and stabilization functions of these tissues (Alster, West, 2000). Hyaluronic acid in tissues is degraded by reactive oxygen species (Heldin et al., 2019).

Considering the antioxidant properties of R. officinalis, a relationship between decreases in reactive species and increases in hyaluronic acid can be found due to non-degradation and possible accumulation of this acid, explaining the increases in skinfolds. However, further specific studies are needed to prove this hypothesis.

Complementarily, the increases in skinfolds are explained by physiological factors of the cutaneous integument; thus, the thickening of skinfolds is not due to abdominal subcutaneous fat, since no change in fat mass was found.

Regarding the circumferences, the dose of 500 mg showed statistical effect on the reduction of circumferences over the umbilicus and below the umbilicus, which is explained by the antioxidant action attributed to the R. officinalis extract; in addition, the adiposity reduction process can be explained by the relationship between free radicals and adipocytes.

Low contents of reactive oxygen species potentiate adipocyte differentiation, and efforts to attenuate reactive oxygen species can be deleterious to adipose cell development (Hauck et al., 2019).

This effect of reducing circumferences was found only for the dose of 500 mg, considering that this dose has an antioxidant effect on local subcutaneous adipocytes.

Cyclic diterpene diphenols, carnosolic acid, and carnosol are among the most representative antioxidant constituents in R. officinalis (Nieto et al., 2018).

Zhao et al., (2015) investigated preventive effects of carnosic acid, one of the main bioactive components of R. officinalis extract, on high-fat diet-induced obesity and metabolic syndrome in mice and found that the supplementation significantly reduced body weight gain, body fat percentage, liver triglycerides, and free fatty acid levels, compared to untreated mice (Zhao et al., 2015).

Contrastingly, the present study was carried out in humans, presenting greater complexity in the evaluation of these variables due to multiple non-controlled factors, and focused on the healthy human beings.

There were no significant changes in anthropometric measurements related to fat mass, lean mass, weight, BMI, and hydration, and the supplementation with R. officinalis presented no systemic lipolytic effect on healthy humans.

There are few studies about effects of R. officinalis on reducing adiposity, but most of them refer to biochemical variables and not to specific anthropometric parameters. A study on complementary therapy with R. officinalis infusion in water (2 g $liter^{-1}$ day^{-1}), 40 patients diagnosed with type 2 diabetes mellitus, and intervention performed for 90 days, showed a significant reduction in BMI anthropometric parameters and waist-to-hip ratio (Quirarte-Báez et al., 2019).

R. officinalis has hepatoprotective effects (Ramadan et al., 2013; Raskovic et al., 2015) that are related to stimulating mechanisms of immune, antioxidant, and

digestive enzymes and may result in increases in adiposity in humans.

The effects of supplementation with R. officinalis at doses of 100 mg and 500 mg on fat mass presented no statistical significance. The group without supplementation presented an increase in the values, which can be explained using the bioactive, because of the effect of recovery of liver functions and, consequently, increases in appetite.

The effect of the dose of 1,000 mg presented opposite results, with decreases in fat mass when comparing initial and final means. It may be connected to saturation of receptors that occurs at higher doses; in this situation, the plant may not have antioxidant effects related to liver protection.

The results did not show significance for body measurements as the study conducted by Quirarte-Báez et al., (2019).

However, an important difference between the studies is that the research in the present study was conducted with healthy women. Another limitation of the present work is that there was no assessment of eating habits and physical exercises, which are factors that directly affect the studied variables.

A promising perspective for further researches is the use of other routes for administration of the plant. R. officinalis supplementation showed to be a skinfold thickener and its mechanisms of action for this effect need to be better elucidated. Considering the local effects on skinfolds and subcutaneous adipocytes, topical use of R. officinalis may have potential for changes in anthropometric measurements; however, additional studies are needed to evaluate its absorption in membranes and dermis, since oral administration was used in the present study. Moreover, the novelty of the results found and how promising they are, in the perspective of new treatments, is highlighted.

CONCLUSION

The aesthetic procedure with radiofrequency did not result in significant changes in variables of body composition: lean mass, fat mass, BMI, weight, and hydration.

The procedure had no effect on skinfolds and circumferences of healthy women subjected to 12 aesthetic sessions for one month. Oral supplementation with standardized dry extract of Rosmarinus officinalis can increase abdominal skinfolds in a linear, dose-dependent way. Oral supplementation with R. officinalis extract can significantly change abdominal circumferences.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no potential conflicts of interest.

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