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ORIGINAL ARTICLE

Adverse effects of deoxycholic acid in submandibular glands, submental, inguinal and subplantar regions: a study in rats

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Abstract

Objective We aimed to evaluate the effects of the deoxycholic acid (DCA) in the submental and subplantar regions of rats, and to histologically analyze the changes caused in the submandibular glands, soft tissues of the paw, and inguinal adipose tissue.

Material and methods Sixty male Wistar rats were divided into DCA and control (CG) groups. DCA was injected in the submental, inguinal, and subplantar regions, and saline was injected in the CG. The animals were euthanized after 24 h and at 7 and 21 days.

Results The DCA group showed edema in the submental region in 24 h and in the paw in all experimental times. In the paw there were also erythema and ulceration in 7 days, and alopecia after 21 days. At 21 days, a few animals also showed erythema and ulceration in paw; however, there was no significant difference from CG. Histological analysis of the paw showed an intense inflammatory process, with a predominance of neutrophils, lymphocytes, and plasma cells in 24 h and 7 days. In the adipose tissue, we observed loss of architecture and inflammatory infiltrate, followed with a lower number of adipose cells, and at 21 days, fibroplasia. In the submandibular glands we observed inflammatory infiltration, loss of tissue architecture, and fibrosis.

Conclusions DCA produces a significant inflammatory process in the structures. It can cause skin ulcerations and, in salivary glands, it causes loss of tissue architecture and fibrosis.

Clinical relevance There has been growing increase in the use of DCA for aesthetic purposes by health care providers. Due to the presence of important anatomical structures in the submental region, constant vigilance is required to report new adverse effects.

Keyword Deoxycholic acid · Submandibular gland · Submental fat · Adipose tissue · Side effects

Introduction

Deoxycholic acid (DCA) is a secondary endogenous bile salt capable of emulsifying and solubilizing dietary fats. In 2007, DCA was produced by the pharmaceutical industry to reduce supraplatysmal fat, as an alternative to aesthetic surgical procedures in this region [1–3]. Its action is milder in protein-poor tissue, such as skin and muscles [4]. Local adverse effects after application of DCA are listed, such as swelling, hematoma, pain, numbness, erythema, and induration [1, 5–8]. Studies also list, less frequently, the occurrence of lesions of the marginal mandibular nerve, skin ulceration, vascular events, and alopecia [1, 7, 8]. The majority of these effects resolve within the treatment interval of 28 days, the time needed to the next session.

Up to six sessions are indicated, with injections of 0.2 mL per point, spaced 1 cm apart. A total dose per session should not exceed 10 mL, an amount considered safe [1, 9, 10]. After the injection of DCA, there is an increase in its plasma concentrations, which return to endogenous levels by 12 h. There are no significant changes in the levels of total cholesterol, total triglycerides, free fatty acids, C-reactive protein, or interleukin-6 after subcutaneous application of DCA [11].

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DCA was the first pharmaceutical intervention approved by the Food and Drug Administration (FDA) for the reduction of submental fat, in 2015 [12]. This substance was already found on the market for cardiovascular therapies, treatment of lipomas, and then for cosmetic purposes, in combination with phosphatidylcholine [13, 14]. There has been growing increase in the use of DCA for aesthetic purposes by health care providers in recent years. Due to the presence of important anatomical structures in the submental region, constant vigilance is required to report new adverse effects, as safety is an essential requirement for non-surgical therapeutic agents for fat removal [15]. Thus, the present study aimed to clinically evaluate the effects of the application of DCA in the submental and subplantar regions, of rats, and also to histologically analyze the changes caused by this substance in the submandibular gland, soft tissues of the subplantar region, and inguinal adipose tissue. We chose to histologically evaluate the submandibular salivary glands of rats, chosen for their anatomical location, close to the region of application of DCA. The submandibular gland can be mistaken with fat deposits, especially in cases of ptosis of the gland, which occurs more frequently with age [16, 17]. There are no studies demonstrating the action of DCA if accidentally applied to salivary gland tissue. We also applied the substance in the animals' inguinal region for histological investigation of adipose tissue, since there is little fat in the submental region of the rat [18]. The paw region was chosen due to the ease of assessing clinical signs [19].

Materials and methods

The study was approved by the Scientific Committee of the School of Health and Life Sciences of Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil and by the Ethics Committee on Animals Use (CEUA) of the same institution (PUCRS, protocol number # 9073).

The sample consisted of 60 male rats (*Rattus norvegicus*, Wistar strain), which were 60 to 90 days old with a mean weight of 300–400 g at the beginning of the experiment. They were housed in standard microisolators (four per cage) at 22 °C with a 12-h light/dark cycle (lights on at 7:00 am and off at 7:00 pm). During the experiments, a standard diet of rat chow (Nuvilab, Colombo, Paraná, Brazil) and filtered water were provided ad libitum. After 10 days of acclimatization, the animals were randomly divided into two groups, DCA group ($n=30$), that received DCA application (10 mg/mL) (Biometil®, São Bento do Sul, Santa Catarina, Brazil) and control group (CG) ($n=30$), who received saline solution. Each group was divided into three subgroups of 10 animals according to the experimental time of 24 h, 7, or 21 days (Fig. 1).

Deoxycholic acid application

The rats were anesthetized with deep-inhalation anesthesia using 4% isoflurane until the loss of protective reflexes. Immediately afterward, the application sites were cleaned with cotton soaked with 70% alcohol and the injection points were marked on the hair with an appropriate pen.

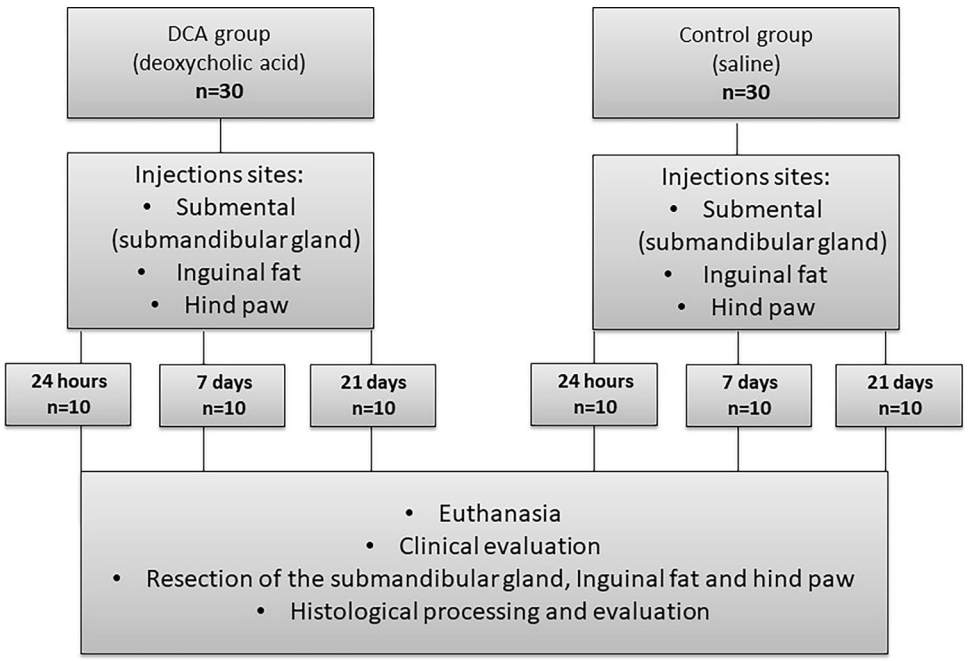

Fig. 1 Flowchart representing the stages of the study

The DCA was injected with a 1-mL syringe and 30-gauge needle (0.3 × 13 mm). Each animal received a total of 1 mL of the substance. The needle was inserted perpendicularly at each of the marked injection site, penetrating 2 to 3 mm into the tissue, and 0.1 mL of DCA was applied per point. Four points were applied in the submental region, including the left submandibular gland; four points in the inguinal region, with 0.5 cm of distance between points; and two points were applied in the subplantar region in the hind paw, one in each fat pad. The injections were performed unilaterally, on the animals' left side (Fig. 2). The dose of DCA used in the present study of 0.1 mL (1 mg) per point corresponds to half of the dose used in humans (0.2 mL per point = 2 mg per point). This was settled by a pilot study based on Schuller-Petrovic et al. [14] and El-Goweli et al. [20], considering the dose of DCA and volume of the substance in each anatomic area and similar findings in both species.

In the CG, the same technique was used as in the DCA group, but saline solution was injected in the same quantities. Ten animals from each group were euthanized after 24 h, 7, and 21 days. Euthanasia was performed with an overdose of deep-inhalation anesthesia using 4% isoflurane.

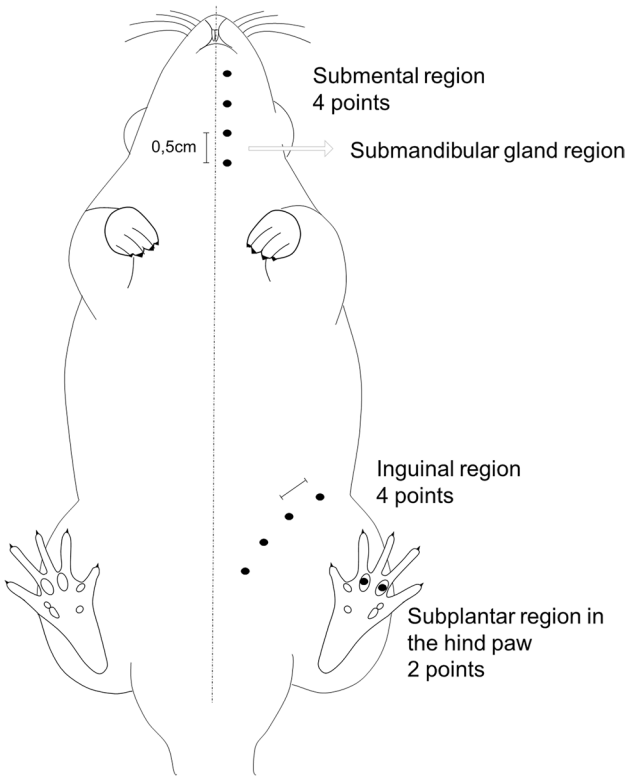

Fig. 2 Illustration of the injection points in the rats. Four points were applied in the submental region and four points in the inguinal region, with 0.5 cm of distance among them. Two points were applied in the subplantar region in the hind paw, one in each fat pad. The volume of 0.1 mL of DCA or saline was applied per point. All points were performed on the animals' left side

Clinical and macroscopic evaluation

The clinical evaluation was carried out in the submental and subplantar regions. Immediately after euthanasia, evaluation at the injection sites was performed and the following changes were recorded as present or absent: erythema/flushing, edema, ulceration, suppuration, nodule formation, hematoma, and alopecia in comparison with the right side of the animal. The reactions observed were also recorded using photography (Canon EOS 60D, Tokyo, Japan). The inguinal region was excluded from clinical evaluation due to the difficulty in observing clinical signs at this anatomical site.

Processing of specimens

After clinical analysis, surgical resection of the hind paw, submandibular gland, and subcutaneous adipose tissue of the inguinal region of the left side of the animal was performed. The specimens were fixed separately in 10% buffered formalin. After 24 h, the pieces were subjected to routine histological processing and embedded in paraffin and 180 blocks were prepared from the 60 animals, one for each region of analysis. Sections 5 µm thick were obtained and stained with hematoxylin and eosin (H&E).

Histological analysis

The histological slides containing the submandibular gland tissue were initially qualitatively analyzed for subsequent capture of three equidistant fields in an Olympus BX-43 binocular light microscope (Olympus, Tokyo, Japan), connected to a computer with an Olympus DP-73 digital camera. For capture, a 400X objective with a resolution of 1600 × 1200 was used, and the images were saved in tiff format on a computer using the Olympus Cellsens Standard software. Histomorphometric analysis of the submandibular glands was performed using the manual point counting technique of Image-Pro Plus 4.1 software (Media Cybernetics, Bethesda, MD, USA). A grid of 300 points was superimposed on the image to quantify the corresponding morphological structure [21]. In this technique, each point of the grid is counted setting which variable (normal tissue, inflammation, vacuolization, loss of tissue architecture, edema, and fibrosis) it matches. After the selection of the points, the software provides the absolute and relative (%) values for each variable in the image [22]. The proportion of normal tissue, inflammation, vacuolization, loss of tissue architecture, edema, and fibrosis in submandibular gland was determined by the relative values provided by the software. For each sample, an average was established for each variable after the analysis of the three fields from every slide. The analyses were performed by a single blinded and calibrated examiner. For calibration, 20 microscopic fields were evaluated in duplicate

with an interval of 7 days, in no predetermined order. The intraexaminer agreement was assessed by the intraclass correlation coefficient, and the value obtained from the average of each item was 0.9.

The slides containing the soft tissue of the hind paw region and inguinal adipose tissue were analyzed qualitatively, describing the histological changes observed.

Statistical analysis

The data were analyzed using descriptive statistics. To analyze the clinical signs of the paw and submental regions, Fisher's exact test was used to compare the dichotomous variables between the groups. The Mann–Whitney test was applied to compare histological variables in the submandibular gland between groups. To compare the glandular histological changes within each group, between different experimental times, the Kruskal–Wallis test was used. The value established to reject the null hypothesis was $P \leq 0.05$. SPSS software version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Results

Clinical and macroscopic analysis

In the submental region, the DCA group showed a higher frequency of edema 24 h after the procedure compared to the CG ($P = 0.000$, Fisher's exact test). There was no difference regarding this variable in the experimental times of 7 and 21 days. Erythema, ulceration, suppuration, nodules, hematoma, or alopecia were not observed in this region (Table 1).

Paw analysis showed that the DCA group had a higher frequency of edema at the three experimental times ($P < 0.05$), a higher frequency of erythema and ulceration 7 days after the procedure ($P = 0.000$), and a higher frequency of alopecia 21 days after the procedure ($P = 0.000$). Even though erythema and ulceration were seen in the paw of a few animals of DCA group 21 days after the procedure, there was no significant difference in comparison with control group ($P > 0.05$). Although DCA was injected into the subplantar region, the ulcerations occurred on the dorsal side of the animals' paw. No suppuration or nodules were observed in this region (Table 1; Fig. 3).

Histological analysis

In the soft tissue of the paw (Fig. 4), the entire sample of the DCA group presented, within 24 h, edema,

Table 1 Comparison of clinical variables between groups according to experimental time in the submental and subplantar regions

Variables	24 h				*P*	7 days				*P*	21 days				*P*
	DCA		Control			DCA		Control			DCA		Control		
	N	%	*N*	%		*N*	%	*N*	%		*n*	%	*N*	%	
Submental region															
Erythema	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Edema	10	100	0	0	0.000	0	0	0	0	a	0	0	0	0	a
Ulceration	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Suppuration	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Nodules	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Hematoma	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Alopecia	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Subplantar region															
Erythema	2	20	0	0	0.237	8	80	0	0	0.000	1	10	0	0	0.500
Edema	10	100	1	10	0.000	10	100	0	0	0.000	4	40	0	0	0.043
Ulceration	0	0	0	0	a	10	100	0	0	0.000	2	20	0	0	0.237
Suppuration	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Nodules	0	0	0	0	a	0	0	0	0	a	0	0	0	0	a
Hematoma	5	50	3	30	0.325	0	0	0	0	a	1	10	0	0	0.500
Alopecia	3	30	0	0	0.105	0	0	0	0	a	9	90	0	0	0.000

[a]The statistic was not determined because the variable is a constant. *DCA* deoxycholic acid group. Fisher's exact test

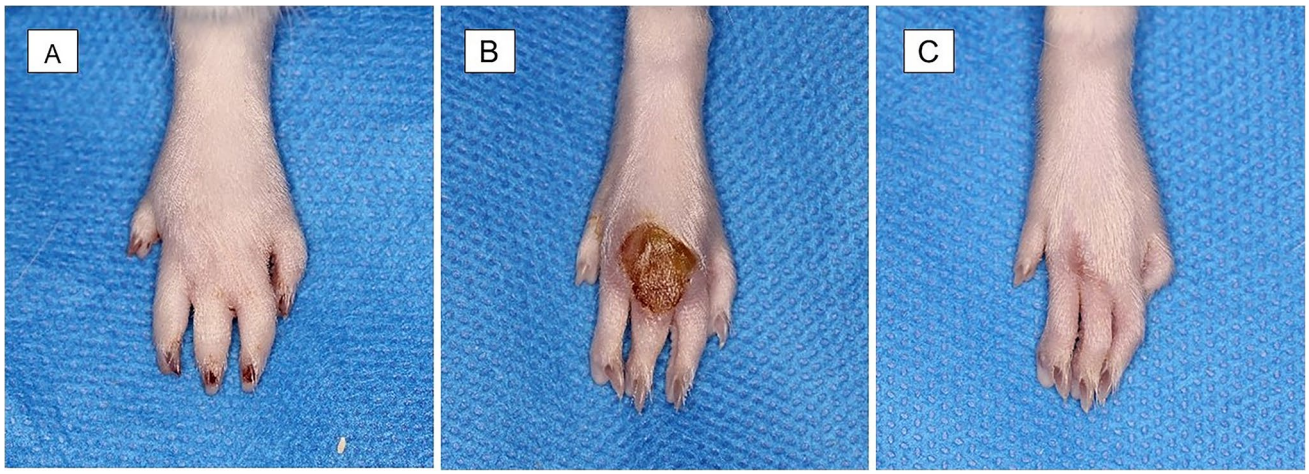

Fig. 3 Clinical images of paw dorsal side. Deoxycholic acid group 24 h (A), 7 days (B), and 21 days (C)

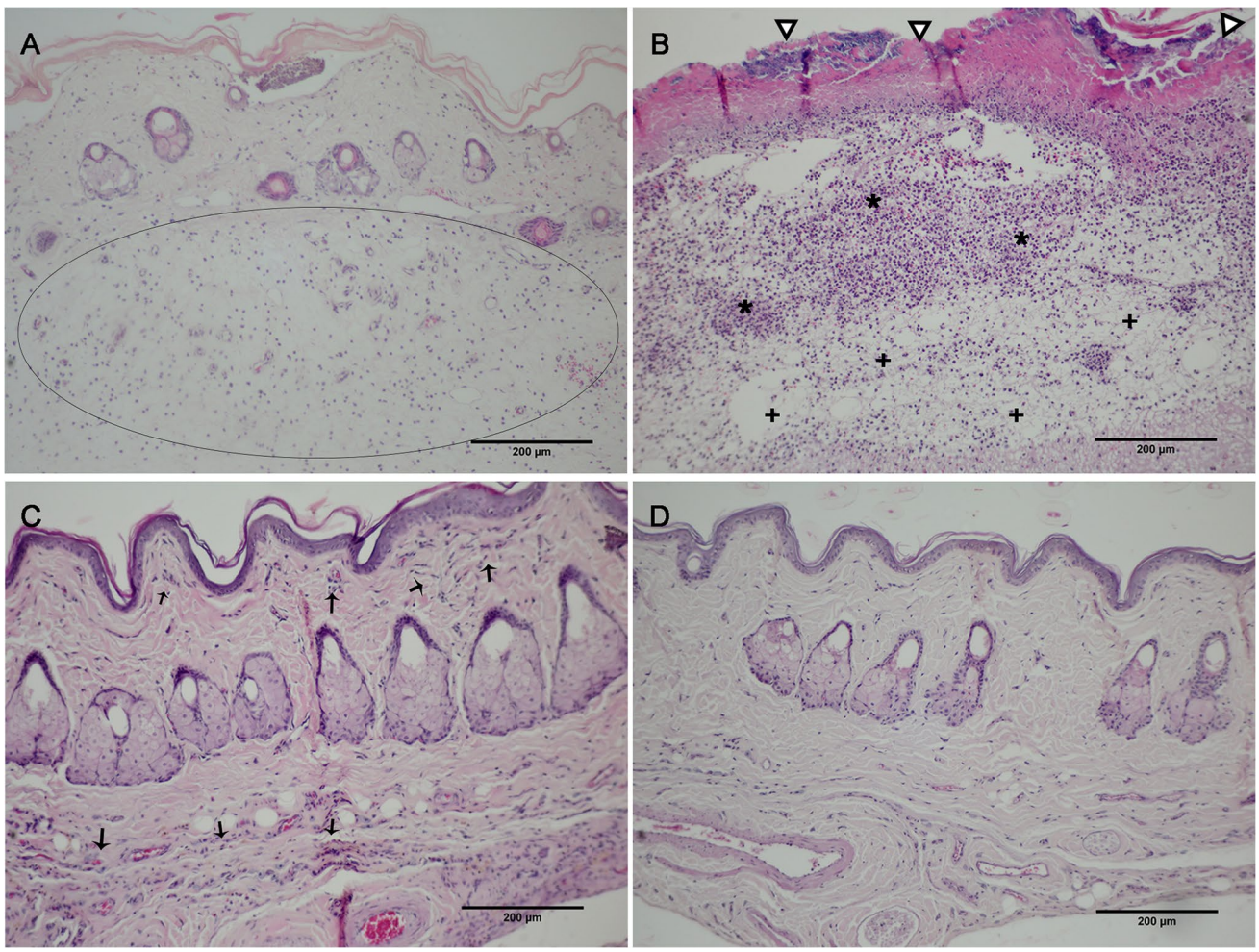

Fig. 4 Microscopic appearance of soft tissue of the hind paw after application of deoxycholic acid (H&E stain, 100X). Intense inflammatory infiltrate and edema (inside circle) at 24 h (A), intense inflammatory infiltrate (*), edema (+), and ulcer area (arrow head) at 7 days (B) and scarce inflammatory infiltrate (arrow) at 21 days (C). No significant alterations 24 h after application of saline solution (D)

inflammatory infiltrate composed of neutrophils, lymphocytes, and plasma cells and atrophy of the lining epithelial tissue. In addition, morphological changes in the sebaceous glands were observed in two samples, such as degeneration and inflammatory infiltration. Within 24 h, hemorrhagic areas and hyperemia were also observed in this tissue in three and five samples, respectively. In the CG, in which saline solution was injected, there were hemorrhagic areas in two samples. The other samples had no alterations.

Seven days after the injection of DCA, all paw samples persisted with an inflammatory infiltrate composed of neutrophils, lymphocytes, and plasmocytes. Eight samples still had edema, two had hyperemia, and six had hemorrhage. In nine samples, areas of ulceration, microabscesses, and vascular neoformation were observed. In areas where there was no ulceration, the epithelium was sometimes thicker and more keratinized, sometimes atrophic. In the CG, no changes were observed in the 7-day period.

At 21 days after the application of the DCA, a significant reduction in the histological changes previously described was observed. A mild inflammatory infiltrate was present in nine of the samples. In one sample there was still ulceration, microabscesses, vascular neoformation, and fibroplasia. There were also no histological changes in the 21-day CG.

In the adipose tissue of the inguinal region (Fig. 5), 24 h after the application of the DCA, we observed loss of tissue architecture, decreased amount of unilocular adipose cells. Multilocular adipose tissue was also observed. Six samples showed a predominant inflammatory infiltrate of neutrophils. In 7 days there was a decrease in the amount of unilocular adipose cells, less cell volume, in addition to a greater amount of multilocular adipose tissue. In five samples there was fibroplasia, vascular neoformation, and

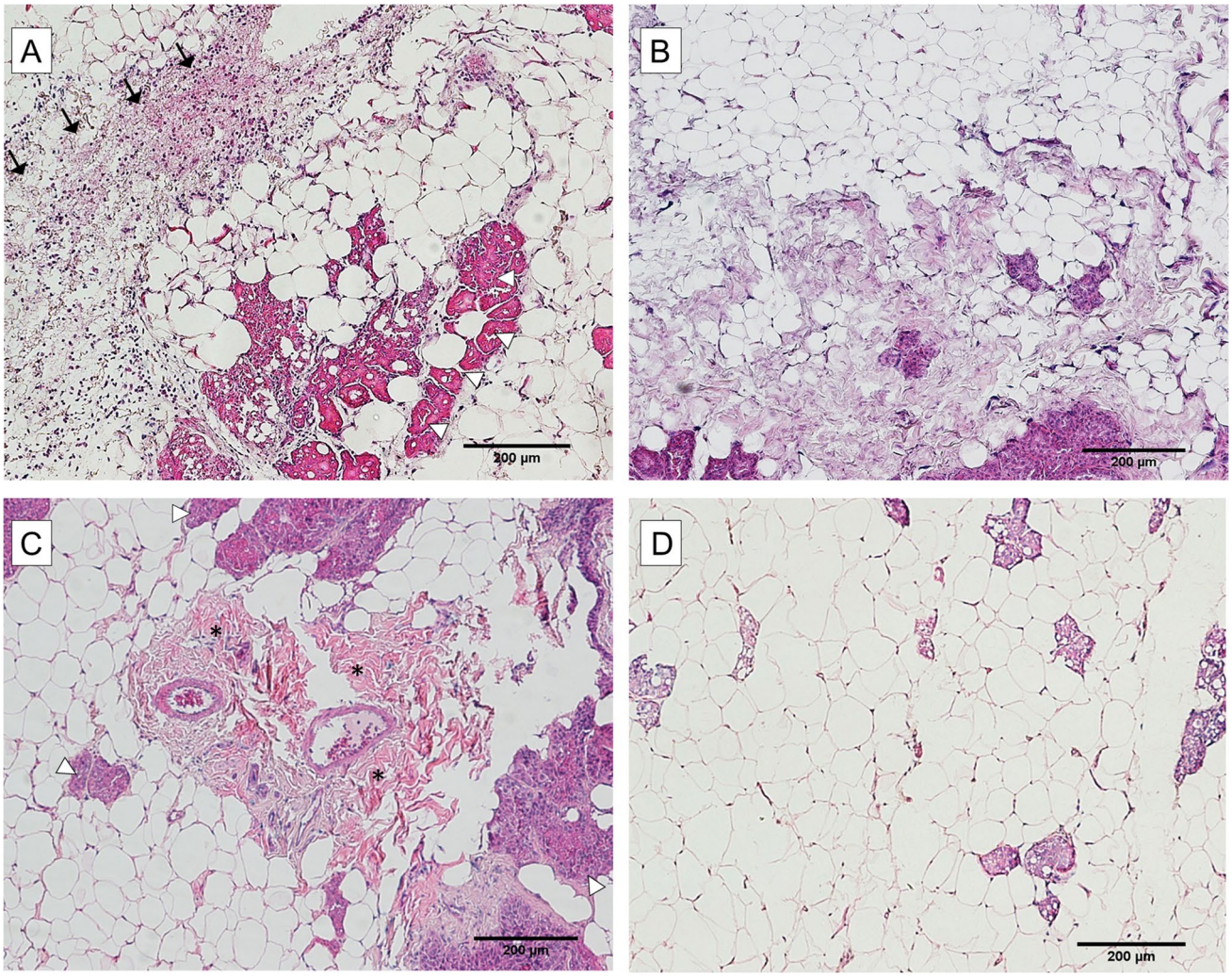

Fig. 5 Microscopic analysis of adipose tissue (H&E stain, 100X). Deoxycholic acid group, 24 h showing inflammatory infiltrate (arrow) and degeneration of multilocular adipose tissue (arrow head) at 24 h (A). Loss of tissue architecture is seen at 24 h (A), 7 days (B), and 21 days (C). Fibroplasia (*) is seen at 21 days (C). Control group 7 days without changes in morphology (D)

lymphoplasmocytic infiltrate. At 21 days all samples presented areas with loss of architecture of the adipose tissue, as well as areas with preserved architecture. Fibroplasia and vascular neoformation were observed in nine and three slides, respectively.

The histomorphometric analysis of submandibular gland tissue is shown in Tables 2 and 3, where the results of the quantification of the histological variables are presented. Table 2 presents the results comparing both groups in each experimental time (24 h, 7, and 21 days). Table 3 shows a comparison of the three times of the experiment inside each group separately. In the histomorphometric analysis of the submandibular gland tissue (Fig. 6), we observed that the DCA group had a higher proportion of inflammation, loss of tissue architecture, and edema when compared to the CG in the three experimental times ($P < 0.05$). In addition, at 21 days of experiment, the DCA group exhibited a higher proportion of fibrosis when compared to the CG

Table 2 Comparison of histological variables (%) observed in the submandibular gland between groups according to experimental time

Variables	24 h		*P*	7 days		*P*	21 days		*P*
	DCA group	Control group		DCA group	Control group		DCA group	Control group	
	Median (P25; P75)	Median (P25; P75)		Median (P25; P75)	Median (P25; P75)		Median (P25; P75)	Median (P25; P75)	
Inflammation	2.778 (1.250; 4.861)	0.000 (0.000; 0.139)	**0.000***	2.000 (1.667; 2.472)	0.000 (0.000; 0.028)	**0.000***	0.444 (0.000; 0.778)	0.000 (0.000; 0.028)	**0.008***
Vacuolization	5.000 (3.083; 8.639)	0.945 (0.722; 1.25)	**0.000***	6.333 (4.389; 8.917)	0.167 (0.667; 1.389)	**0.000***	0.500 (0.167; 0.917)	1.000 (0.611; 1.500)	**0.034***
Loss of architecture	75.944 (66.444; 82.417)	0.000 (0.000; 0.000)	**0.000***	80.111 (69.861; 83.750)	1.500 (1.000; 2.111)	**0.000***	77.111 (68.833; 86.528)	0.000 (0.000; 0.639)	**0.000***
Normal tissue	0.000 (0.000; 5.639)	98.833 (98.667; 99.278)	**0.000***	7.778 (3.167; 15.000)	97.722 (96.111; 98.194)	**0.000***	10.278 (6.417; 24.778)	98.444 (97.611; 98.944)	**0.000***
Edema	10.778 (8.833; 14.806)	0.000 (0.000; 0.056)	**0.000***	0.833 (0.417; 1.444)	0.000 (0.000; 0.000)	**0.000***	0.389 (0.000; 1.056)	0.000 (0.000; 0.361)	**0.048***
Fibrosis	0.000 (0.000; 0.000)	0.000 (0.000; 0.000)	1.000	0.000 (0.000; 0.000)	0.000 (0.000; 0.000)	1.000	3.278 (1.056; 6.972)	0.000 (0.000; 0.056)	**0.001***

Mann–Whitney test. *Indicates significant difference between groups. *DAC* deoxycholic acid

Table 3 Comparison of histological variables (%) of salivary gland according experimental time within each group

Variables	Deoxycholic acid group				Control group			
	24 h	7 days	21 days	*P*	24 h	7 days	21 days	*P*
	Median (P25; P75)	Median (P25; P75)	Median (P25; P75)		Median (P25; P75)	Median (P25; P75)	Median (P25; P75)	
Inflammation	2.778^{a} (1.250; 4.861)	2.000^{a} (1.667; 2.472)	0.444^{b} (0.000; 0.778)	**0.000***	0.000 (0.000; 0.139)	0.000 (0.000; 0.028)	0.000 (0.000; 0.028)	0.768
Vacuolization	5.000^{a} (3.083; 8.639)	6.333^{a} (4.389; 8.917)	0.500^{b} (0.167; 0.917)	**0.000***	0.945 (0.722; 1.25)	0.667 (0.167; 1.389)	1.000 (0.611; 1.500)	0.375
Loss of architecture	75.944 (66.444; 82.417)	80.111 (69.861; 83.750)	77.111 (68.833; 86.528)	0.791	0.000^{a} (0.000; 0.000)	1.500^{b} (1.000; 2.111)	0.000^{a} (0.000; 0.639)	**0.000***
Normal tissue	0.000^{a} (0.000; 5.639)	7.778^{ab} (3.167; 15.000)	10.278^{b} (6.417; 24.778)	**0.018***	98.833^{a} (98.667; 99.278)	97.722^{b} (96.111; 98.194)	98.444^{ab} (97.611; 98.944)	**0.002***
Edema	10.778^{a} (8.833; 14.806)	0.833^{b} (0.417; 1.444)	0.389^{b} (0.000; 1.056)	**0.000***	0.000 (0.000; 0.056)	0.000 (0.000; 0.000)	0.000 (0.000; 0.361)	0.451
Fibrosis	0.000^{a} (0.000; 0.000)	0.000^{a} (0.000; 0.000)	3.278^{b} (1.056; 6.972)	**0.000***	0.000 (0.000; 0.000)	0.000 (0.000; 0.000)	0.000 (0.000; 0.056)	0.126

Kruskal–Wallis test. Different letters on same row indicate a significant difference between the experimental times. * Indicates significant difference between the groups

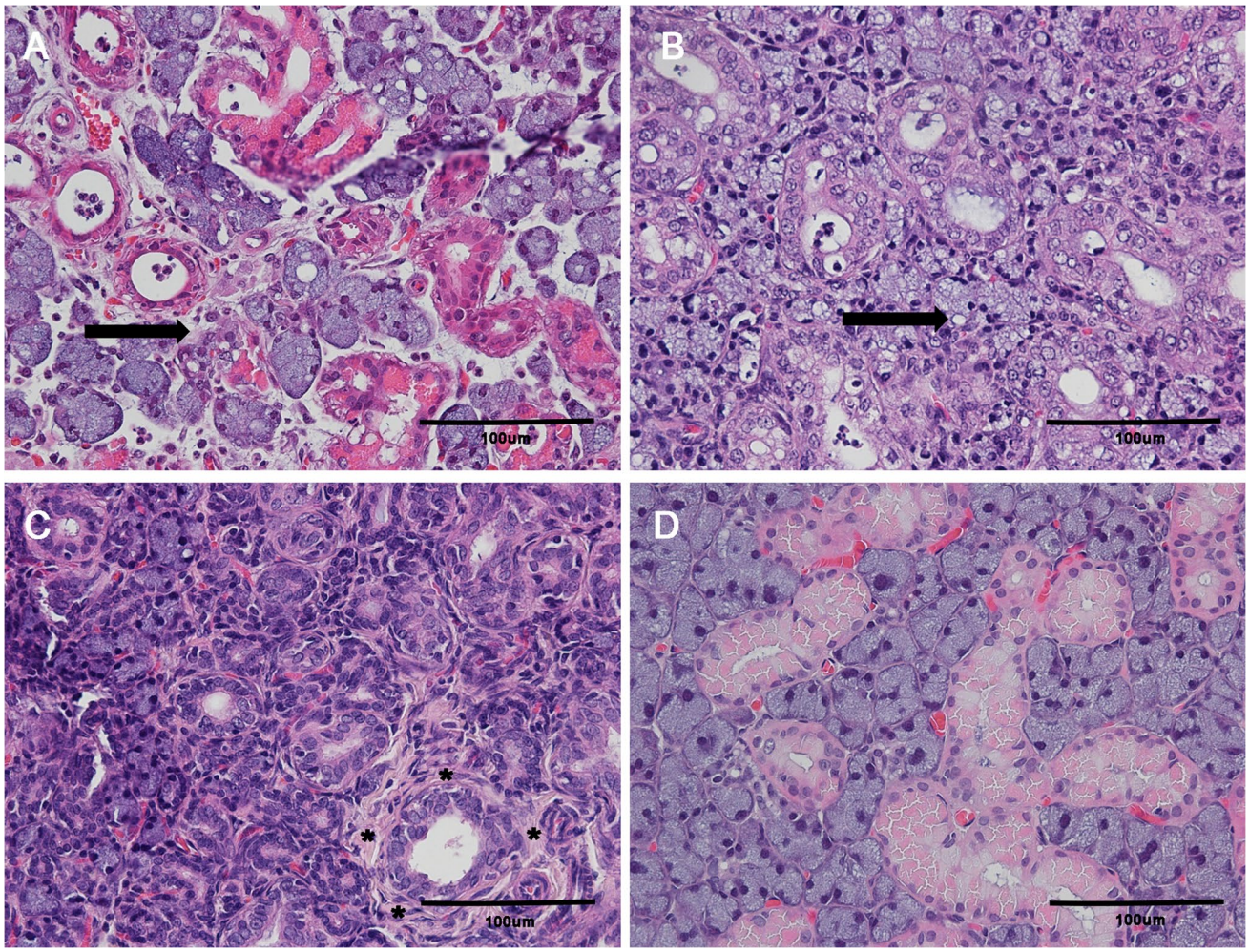

Fig. 6 Histological examination of salivary gland (400X) (H&E stain). Deoxycholic acid group 24 h showing edema and inflammatory infiltrate arrow (A), 7 days after injection showing inflammatory infiltrate and vacuolization (arrow) (B), and 21 days loss of tissue architecture and fibrosis (*) (C). Control group 21 days without changes in morphology (D)

($P=0.001$). Regarding vacuolization, a higher proportion was observed in the DCA group 24 h and 7 days after the procedure ($P=0.000$), and a lower proportion 21 days after the procedure ($P=0.034$). The CG showed a higher proportion of normal tissue than the DCA group ($P=0.000$) and slight vacuolization in 21 days (Table 2).

When comparing the glandular histological variables within each group, according to the experimental time, it was found that in the DCA group, inflammation and vacuolization underwent a significant reduction in the 21-day period ($P=0.000$). The edema was significantly higher 24 h after the procedure than the other experimental times ($P=0.000$). Still in the DCA group, the proportion of fibrosis was higher 21 days after the procedure ($P<0.000$). The loss of tissue architecture remained high during all experimental periods, with no significant difference ($P=0.791$). The proportion of normal tissue reached a median of 10% at 21 days, a value significantly higher than that of the evaluation performed 24 h after the application of the DCA ($P=0.018$). At 7 days, the CG showed a higher proportion of tissue architecture loss ($P=0.000$) and a lower proportion of normal tissue compared to other experimental times ($P=0.002$) (Table 3).

Discussion

The facial harmonization and aesthetics market is constantly updating and increasingly discovering new procedures. Since the emergence of DCA and its use for cosmetic purposes in the submental region, studies have been carried out to elucidate the mechanisms of action, side effects, and dose adequacy [1, 23]. There are few studies investigating the side effects of DCA in animal model. In the clinical examination of the submental and subplantar regions, we observed significant edema after the application of DCA. This finding is in agreement with the adverse effects already

described in the literature, being the most frequent after the application of DCA [24, 25]. In the submental region, no other clinical changes were observed, and edema regressed spontaneously in subsequent evaluations. However, in the animals' paw, edema remained significantly greater at all experimental times. In addition, erythema and extensive ulcerated lesions were observed at the 7-day evaluation. The presence of ulcers or necrosis was described after the beginning of the commercialization of DCA as an adverse event of less occurrence, and it was viewed as an event arising from superficial application [8, 10]. However, in the present study, DCA was injected into the subplantar region and ulcers occurred on the dorsum of the animals' paw, indicating that they were not associated with superficial injection. We can infer that the animal's paw has scarce adipose tissue, with little keratinized epithelium on the dorsal side. In addition, even though the paw received a lower dose of DCA when compared to the other regions, to the anatomical issue of the subplantar region, the amount of DCA may have been high, resulting in the occurrence of an important inflammatory process and atrophy of epithelial tissue, followed by ulcers, which affected the whole sample at 7 days and part of the sample at 21 days. A preclinical study showed that DCA has a direct effect over epithelial cells causing decrease of cell migration [26]. According to the authors, it may impair epithelial wound healing, which corroborates our findings. This adverse effect was substantial in the present study and occurred only in the paw, demonstrating the care needed with regard to the amount of DCA to be administered and the imminent risk of this alteration. Another clinical change observed in the paw was alopecia, which occurred at 21 days in previously ulcerated areas. This might have resulted from the ulcer healing process. However, three cases of alopecia were observed after 24 h, which occurred before ulceration. Some authors proposed two hypothesis for the alopecia seen after this treatment: inflammation, which can cause damage to hair follicles, or superficial injection, which can destroy the hair bulb [7, 27, 28]. In the present study, significant edema and inflammatory process was seen in the paws of entire sample of DCA, which corroborates the first hypothesis. In addition, important morphological changes such as degeneration and inflammatory infiltration were observed in the sebaceous glands, which probably contributed to the alopecia process. A few series of cases of alopecia in men was published after DCA went on the market. Some cases have shown spontaneous resolution and others not [7, 27, 28]. Our study would require a longer experimental time to determine whether or not there would be resolution of alopecia.

The histological changes we observed in the inguinal adipose tissue after DCA treatment were in accordance with the findings of a series of authors, who described an adipocyte rupture process, followed by a local inflammatory response [3, 29, 30]. These histological changes are essential for fat reduction to occur in the region of DCA application.

In the submandibular gland, 24 h after DCA injection, significant histological changes were observed, including edema, vacuolization, inflammation, and loss of tissue architecture, and at 21 days, tissue fibrosis. Inflammation and edema are similar to the effects already described in other tissues after the application of DCA and also showed significant regression in the 21-day evaluation. However, two important histological changes observed in the 21-day period were the loss of tissue architecture, which was as much as 77%, and fibrosis. That is, at 21 days, loss of tissue architecture was not reversed, and there was still mild fibrosis. The fact that the gland did not show improvement regarding this condition, raises questions about the glandular changes induced by DCA. As we did not assess glandular function in this study, there are doubts about the relationship of the histological changes observed with functional changes. The histological changes observed in the glandular tissue of the control group may be a consequence of mechanical trauma resulting from the insertion of the needle and injection of saline solution in the region.

A recent study by Shridharani and Chandawarkar [25] proposed to expand the DCA injection area to improve results in patients who have fat distributed in the cervical region, increasing the area of lipolysis. Thus, in addition to adipose tissue, submandibular glands and digastric muscles, which contribute to submental and submandibular volume, could be affected during treatment. El-Gowelli et al. [20] investigated the effect of DCA, combined with phosphatidylcholine, when injected repeatedly, close to the neurovascular bundle of the femoral region of rats. Local inflammation was observed invading the skeletal muscles, with consequent myofiber necrosis, myophagocytosis, and muscle degeneration, with deposition of collagen fibers. Damage to nerve tissue has also been described, with thickening of the walls of intraneural blood vessels and intense inflammation. Our study and the results of El-Gowelli et al. [20] warn about the risks of reaching the anatomic structures adjacent to the adipose tissue of the submental region.

The use of DCA brings a series of adverse effects already described, transient or not [1, 4, 11]. In a scenario where there is a lack of knowledge on the part of the health care provider for the local anatomy and the properties of the substance, reactions will appear with greater frequency and intensity. Thus, professionals must be aware of the anatomy of the face, correct injection technique, and ideal amount of agent to be applied to avoid unwanted effects [10]. In addition to the clinical and histological effects already described in adipose tissue, we observed significant microscopic changes in submandibular glands in the experimental conditions of this study. We note the need for further studies

in the area to assess the glandular complications resulting from accidental injection.

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Declarations

Ethics approval The study was approved by the Scientific Committee of the School of Health and Life Sciences of Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil and by the Ethics Committee on Animals Use (CEUA) of the same institution (PUCRS, protocol number 9073).

Conflict of interest The authors declare no competing interests.

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