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Ultrasound assessment of botulinum toxin-A (BOTOX) injection into adult Arabian dromedary camels' lips: efficacy in detection and localization

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Abstract

Background Ultrasound imaging has become an invaluable tool in veterinary medicine, particularly in guiding injections and visualizing soft tissue structures. Recently, ultrasonography has been used in camel practice to detect cosmetic fillers, particularly in aesthetic evaluations. However, previous applications lacked a controlled experimental background. This study is the first to experimentally assess the effectiveness of ultrasound in detecting and localizing botulinum toxin (BOTOX) injections in camels, using two different doses, from the time of injection until the toxin's effect becomes undetectable.

Objective The objective of this study was to assess the efficacy of ultrasound in detecting and localizing botulinum toxin injection sites in camels' lips.

Methods This study involved 18 adult Arabian dromedary camels (9 Magateer and 9 Majaheem breeds) from the Camel Research Center, King Faisal University. The camels, aged 4.3 ± 1.3 years and weighing 405.5 ± 20.6 kg, were randomly assigned to three treatment groups (6 camels each). Treatments included 100 IU and 200 IU of botulinum toxin type A, and a control of 5 ml sterile saline, administered to the upper and lower lips. Morphological changes, inflammation, and lip thickness were assessed weekly for two months. Ultrasound examinations and hematological and biochemical analyses were conducted at specified intervals. Data were analyzed using two-way repeated-measures ANOVA and Tukey's test, with significance set at $p < 0.05$.

Results Morphological assessments revealed significant changes in the lips of camels treated with 200 IU BOTOX, showing the highest shape change scores (3 ± 0) compared to 100 IU BOTOX (1.75 ± 0.87) and control (0.4 ± 0). Inflammation and tissue reactions were more pronounced in the BOTOX-treated groups, with higher scores in the 200 IU group. Lip thickness increased significantly in the BOTOX groups, with the greatest thickening in the 200 IU group (2.4 ± 0.54 mm). Ultrasonographic findings showed structural changes and increased tissue thickness, peaking on Day

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7 and gradually normalizing by Day 54. Hematological and biochemical profiles showed no significant differences between the treated and control groups.

Conclusions The study demonstrates the effect of BOTOX on camel lip morphology and tissue characteristics, with higher doses (200 IU) causing more significant and prolonged changes. Both morphological scoring and ultrasonographic evaluation effectively monitored these effects, including the timeframe of BOTOX detection and when it became undetectable.

Keywords Aesthetic, BOTOX, Camel, Cosmetic, Ultrasound

Background

The dromedary camel holds substantial economic and cultural value in the Gulf region and south Asia [1], where it serves as a key source of milk and meat production. Recently, the popularity of camel racing and beauty contests has risen across Arabian Gulf countries, particularly in the United Arab Emirates, Saudi Arabia, Kuwait and Qatar. These beauty contests, central to traditional camel festivals, celebrate prized camels primarily dromedaries according to breed-specific beauty standards [2, 3]. Judging criteria for camel beauty typically include body size and structure, the shape of the hump, coat quality, and specific facial features. For example, a broad head with a prominent nose bridge, expressive eyes, and long lashes are highly valued, as are small, proportionate ears positioned close to the head. Additional aesthetic features, such as well-shaped, fuller nose and lips, are also emphasized in some contests [3–5]. Due to significant financial rewards, fraud has been reported in these competitions [3]. Common unethical practices include the use of stimulants or BOTOX, fillers injections to temporarily alter camels' posture and muscle tone, plastic surgery to reshape features such as lips and ears, and cosmetic enhancements like injections, particularly around the nose, lips, and hump, to enhance appearance. These cosmetic modifications are among the most frequent forms of fraud detected in high-profile camel beauty contests [3, 5–7]. Organizers place significant emphasis on fraud detection by employing veterinary specialists who use all available methods, including x-ray imaging, to identify the use of fillers, and other injectable substances in the lips and facial structures of camels [2]. "BOTOX" is a brand name for an anti-wrinkle treatment derived from botulinum toxin, a potent neurotoxin produced by the bacterium *Clostridium botulinum*, which can naturally occur in contaminated meat products. It blocks nerve signal transmission to muscles, causing paralysis, and severe poisoning can result in respiratory failure and death. In controlled, highly diluted doses, botulinum toxin is used therapeutically to treat medical conditions such as cervical dystonia, torticollis, blepharospasm, hyperhidrosis, strabismus, and chronic migraines. When injected into facial muscles, temporarily paralyzes them, smoothing facial skin. This effect typically lasts two to six months, during which the body gradually regenerates nerve endings [8, 9]. This study aimed to assess the effectiveness of ultrasound as a diagnostic tool for detecting botulinum toxin type A injection in dromedary Arabian camels' lips.

Methods

This study included eighteen adult Arabian dromedary camels (Camelus dromedarius) of different breeds (9 Magateer and 9 Majaheem breeds); sourced from the herd owned by the Camel Research Center, King Faisal University, Al-Ahsa, Saudi Arabia. The camels are intended for research and consent to participate was required. All camels included in this study were clinically healthy, free from any apparent diseases or abnormalities, and maintained under standard management and nutritional conditions throughout the study period. The group consisted of nine intact males and nine non-pregnant females. The mean ± SD age of the animals was 4.3 ± 1.3 years, with a mean weight of 405.5 ± 20.6 kg and a mean body condition score of 3.7 ± 0.4 [10]. Inclusion criteria for participation are based on a comprehensive clinical examination and normal hematological and biochemical assessments. Camels with poor health, a body condition score below 3, or with lip inflammation or skin disease were excluded. Each animal was housed individually in separate pens and fed a diet of grass hay with concentration. Water was freely available, and feed was withheld for 8 h before each trial. Trials took place indoors with ambient temperatures ranging between 30 °C and 32 °C.

Study design and procedure

The camels were randomly assigned to one of three treatment groups, with six camels per group (three intact males and three non-pregnant females from each breed). Randomization was conducted using a simple randomization technique through random number selection. Each group received one of three treatments applied to the upper and lower lips in equal volumes. Treatment 1 involved the administration of 100 IU of botulinum toxin type A (BOTOX ALLERGAN® Westport, Co. Mayo, Ireland), while Treatment 2 consisted of a higher dose of 200 IU of BOTOX (Allergan). Treatment 3, serving as the control, involved the administration of 5 ml of sterile

Table 1 Study design outlining the random allocation of camels into three treatment groups, including the number of animals per group, sex distribution, treatment details, injection sites, and administration technique

Group	Number of Camels	Sex Distribution	Treatment	Injection Sites	Injection Technique
Treatment 1	6 (3 males, 3 females)	3 intact males, 3 non-pregnant females (from each breed)	100 IU Botulinum Toxin Type A (BOTOX ALLERGAN®)	Upper and lower lips	Multiple sites, 2 cm apart, using a 21-gauge, 5-cm hypodermic needle
Treatment 2	6 (3 males, 3 females)	3 intact males, 3 non-pregnant females (from each breed)	200 IU Botulinum Toxin Type A (BOTOX ALLERGAN®)	Upper and lower lips	Multiple sites, 2 cm apart, using a 21-gauge, 5-cm hypodermic needle
Control (Treatment 3)	6 (3 males, 3 females)	3 intact males, 3 non-pregnant females (from each breed)	5 ml sterile normal saline (0.9% sodium chloride)	Upper and lower lips	Multiple sites, 2 cm apart, using a 21-gauge, 5-cm hypodermic needle

normal saline (0.9% sodium chloride, Pharmaceutical Solution Industries, Al-Khobar, KSA). treatments were diluted in sterile 0.9% normal saline to a total volume of 5 ml. Injections were administered at multiple sites along the upper and lower lips, spaced 2 cm apart, using a 21-gauge, 5-cm hypodermic needle. Each camel was restrained in a sternal position, and the head was stabilized using rope halter. The study design is illustrated in Table 1.

The inner lip area was cleaned with water and scrubbed with diluted povidone–iodine before injection. Neither local nor systemic anesthesia was used for the injection. One investigator prepared all treatments, color-coded them, and assigned them to be administered by another investigator who was blinded to the treatment allocations. Evaluation and assessment were conducted by additional investigators who were also blinded to the treatments. Injection of treatments is illustrated in (Fig. 1-a).

Evaluation and assessments

Morphological assessments

Morphological evaluations of the lips were conducted weekly over two months, assessing changes in shape, inflammation, tissue reactions at injection sites, and lip thickness, all measured using a scoring scale. The changes in the shape of camel lips were assessed using a four-point scale ranging from 0 to 3. At the same time, the inflammation and tissue reactions at injection sites were evaluated using a four-point scale ranging from 0 to 3 (Table 2).

Lastly, lip thickness was measured in millimeters by using skinfold caliper and scored according to the following scale: 0 for normal thickness, 1 for mild thickening (1–2 mm increase), 2 for moderate thickening (2–4 mm increase), and 3 for severe thickening (greater than 4 mm increase). Other criteria included dropping, widening, of the lower lip (Elsabal) and post injection complications of the lips. These scoring criteria facilitate objective evaluation of the morphological changes in the lips post-injection. Morphological assessment is illustrated in (Fig. 1-b).

Ultrasound examination

Ultrasound images of the lip tissue were taken before and after the injection on the day of injection (Day 0). Follow-up ultrasounds were performed weekly for two months

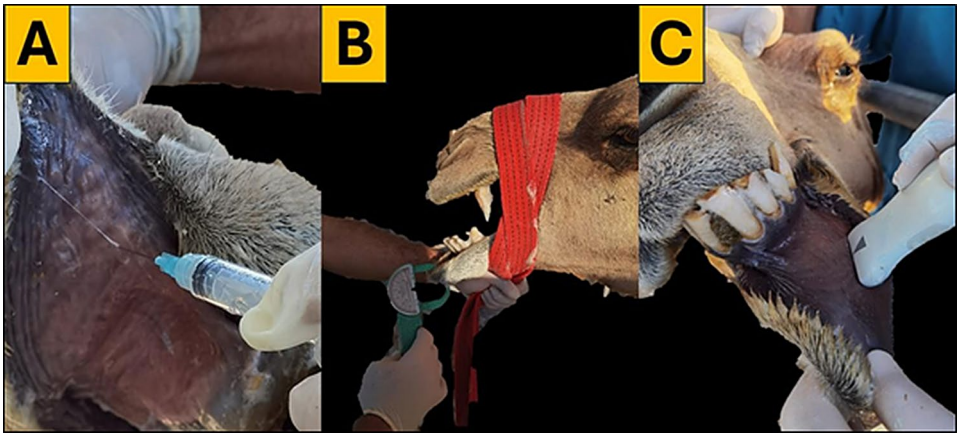

Fig. 1 **A**) Injection of treatments into the upper and lower lips. **B**) Morphological assessment and measurement of lip thickness. **C**) Ultrasonographic evaluation of the lips

Table 2 Scoring system for evaluating changes in lip shape and inflammatory reactions in camels following treatment, based on clinical observations of swelling, distortion, asymmetry, and inflammatory signs such as redness and tissue alteration

Clinical Description	Criteria	Score
Lip Shape Changes	The lips maintain their original shape without any observable alterations, no swelling, distortion, or asymmetry present.	0 No change
	Slight, barely noticeable changes, minimal swelling or faint asymmetry, but the overall structure of the lips is largely preserved.	1 Mild change
	More pronounced changes in lip shape, noticeable swelling, moderate distortion, or visible asymmetry; the altered shape is discernible but not severe.	2 Moderate change
	Drastic alterations in lip shape, significant swelling, severe distortion, marked asymmetry, dropping of lower lips, deformed or dysfunctional lips.	3 Severe changes
Inflammatory Reactions	Completely normal appearance, no visible signs of inflammation such as redness, swelling, or tissue alteration; consistent texture and color of the skin with surrounding areas.	0 No reaction
	Slight redness or minimal swelling, subtle and localized changes related to the immediate area of injection, no significant impact on surrounding tissues; minor tenderness upon touch but no discomfort otherwise.	1 Mild reaction
	Noticeable redness and swelling, pronounced inflammation contained within a defined area, moderate tenderness or discomfort, signs of involvement of surrounding tissue; no observation of necrosis or severe damage.	2 Moderate reaction
	Marked redness and significant swelling, severe signs such as necrosis, tissue hardening, or fluid accumulation; extension of the reaction beyond the immediate injection area affecting nearby tissues, functional impairment or tissue damage, severe pain or discomfort.	3 Severe reactions

after the injection. High-resolution ultrasound equipment (Esoate, My Lab Gold, Italy) with a 10–13 MHz linear probe was used for scanning. The probe was positioned on the internal surface of the lips, capturing images from the caudal to anterior and upper to lower areas. The thickness of the different tissue layers was measured from the ultrasound images taken before and after the injections. All measurements were recorded for subsequent analysis. The ultrasonographic assessment is illustrated in (Fig. 1-c).

Hematology and biochemical analysis

Blood samples were collected from the jugular vein puncture using a 14-gauge IV catheter (BD Biosciences; Franklin Lakes, NJ, USA) at five points: pre-treatment (T0), and 1 week (1w), 2 weeks (2w), 4 weeks (4w), and 8 weeks (8w) post-injection. Samples were collected into EDTA tubes for hematology and into non-EDTA containing tubes for serum separation. Hematological parameters were measured including Hemoglobin (Hb), Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), and differential leukocyte counts (neutrophils, eosinophils, basophils, monocytes, and lymphocytes). Biochemical analysis was performed on serum samples using an automatic biochemical analyzer (Humalyzer-3000, USA) and various parameters including glucose, total protein (TP), albumin, urea, alanine aminotransferase (ALT), triglycerides (TG), and aspartate transaminase (AST) were measured. All analyses were conducted at the biochemistry laboratory of the Department of Clinical Science, Faculty of Veterinary Medicine, King Faisal University.

Statistical analysis

All data were statistically analyzed using GraphPad prism (version 9, USA). Data was subjected to normal distribution using Shapiro-Wilk test. Continuous data, including hematological, biochemical, and ultrasound measurements, were found to be normally distributed. Therefore, they were expressed as mean ± standard deviation (SD). A two-way repeated-measures ANOVA test was performed. This allowed evaluation of the effects of treatment, time, and the interaction between treatment and time on hematological and biochemical parameters, as well as on ultrasound-measured lip thickness. Post-hoc comparisons between groups at each time point were conducted using Tukey's test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant.

Results

Morphological assessments

None of the injected camels had a recent history of illness. The visible mucosal lip lesions were observed in only two of the injected camels (Treatment 2). The swelling of the upper and/or lower lips was identified through visual inspection and manual palpation in all treated camels. The drooping and swinging of the injected lips were noted across all injected camels, but no hardness was detected or felt upon palpation. Additionally, corrugated lip skin was not observed in any of the camels. None of these abnormalities were reported in the control group.

Morphological changes in the lips varied significantly among the treatment groups ($p = 0.001$). Camels treated with 200 IU of BOTOX showed the most pronounced changes, with a mean shape change score of 3.0 ± 0.0 (range: 3–3). The 100 IU BOTOX group exhibited moderate changes, with a mean score of 1.75 ± 0.87 (range: 0–2). In contrast, the control group (normal saline) showed minimal or no changes, with a mean score of 0.4 ± 0.0, but individual scores ranged from 0 to 4. The effect of time was also significant ($p = 0.01$), and the time × treatment interaction was statistically significant ($p = 0.01$).

Inflammation at the injection sites was observed in both BOTOX-treated groups ($p = 0.04$), with treatment 2 obviously showing higher inflammation scores (1.4 ± 1.0) than treatment 1 (0.86 ± 0.6). The control group exhibited no signs of inflammation (mean score: 0). Tissue reactions followed a similar pattern ($p = 0.041$), with moderate to severe redness and swelling detected in the BOTOX groups, while the control group demonstrated no adverse tissue reactions (mean score: 0). The effect of time on tissue reaction was also significant ($p = 0.013$).

The lip thickness also increased significantly in the BOTOX-treated groups ($p = 0.01$), with treatment 2 showing the greatest thickening (2.4 ± 0.54 mm) compared to treatment 1 (1.8 ± 0.44 mm). Mild thickening was occasionally observed in the control group but was statistically insignificant (0.4 ± 0.2 mm). The effect of time on lip thickness was significant ($p = 0.01$), and the time × treatment interaction was also significant ($p = 0.01$).

Other complications, such as drooping or widening of the lower lip (Elsabal), were noted predominantly in the higher-dose group. Changes in the shape of the lip, lip thickness, and tissue reaction after different treatment regimens are illustrated in Figs. 2A, B, and C.

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Ultrasonographic findings

Ultrasound imaging revealed clear structural changes in the lip tissue layers following BOTOX injection. On Day 7 post-injection, both BOTOX-treated groups showed increased hypoechoic regions in the subcutaneous tissue, indicative of edema or tissue reaction ($p = 0.02$). These changes were more pronounced in Treatment 2 and persisted through Day 14 ($p = 0.01$), gradually diminishing by Day 28.

Measurements of tissue thickness from ultrasound images aligned with morphological findings. The highest increase in tissue thickness was recorded on Day 7 for Treatment 2 (23.5 ± 6.6 mm, $p = 0.003$) compared to Treatment 1 (18.6 ± 4.6 mm, $p = 0.01$) and the control group (14.8 ± 4.2 mm, $p = 0.09$). By Day 54, tissue thickness in both BOTOX groups began to normalize but remained slightly elevated compared to baseline values ($p = 0.04$), while the control group showed no significant variation across all time points ($p = 0.07$). The ultrasonographic findings, alongside the remarkable morphological changes, are illustrated in Figs. 3, 4, 5, and 6.

Discussion

The application of BOTOX in veterinary medicine, particularly in Adult Arabian Dromedary Camels, remains an underexplored research area. While extensively studied in humans, its use in camels is limited, and the assessment of its efficacy using non-invasive imaging techniques lacks sufficient literature. This study provides a novel evaluation of the efficacy of ultrasound in detecting and localizing botulinum toxin type A (BOTOX) injections in the lips of adult Arabian dromedary camels, focusing on the morphological and structural changes induced by different doses (100 IU and 200 IU).

Botulinum toxin-A (BOTOX), a heat-labile neurotoxin produced by *Clostridium botulinum*. However, in its purified form, BOTOX is widely and safely used for therapeutic and cosmetic applications, often with off-label

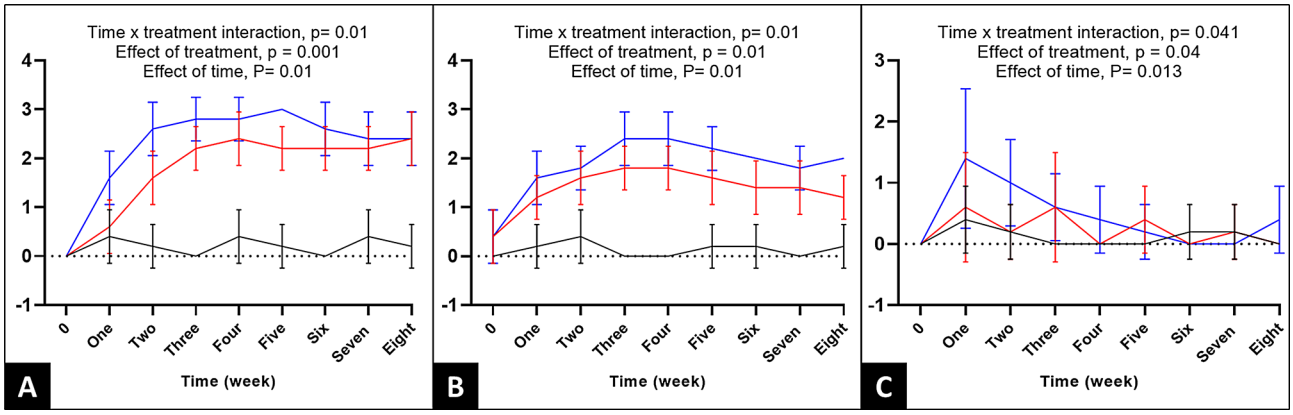

Fig. 2 Changes in (**A**) lip shape, (**B**) lip thickness, and (**C**) tissue reaction after treatment with BOTOX 100 and 200 in camels. (X-axis: score value; Y-axis: time in weeks)

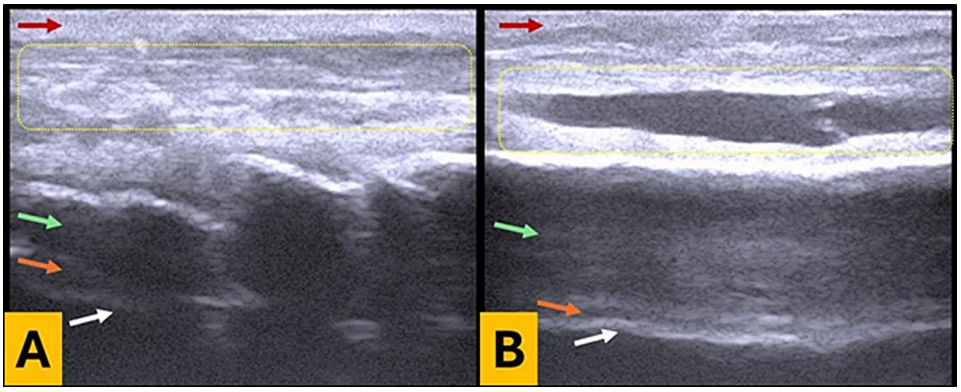

Fig. 3 **A**) Pre-injection ultrasound examination of the lip. **B**) Immediate post-injection ultrasound image of the lip following BOTOX administration. The red arrow indicates the mucosa, the yellow dotted rectangle marks the submucosa, showing BOTOX distribution within it (**B**). The green arrow denotes the muscularis, the orange arrow indicates the dermis, and the white arrow marks the epidermis

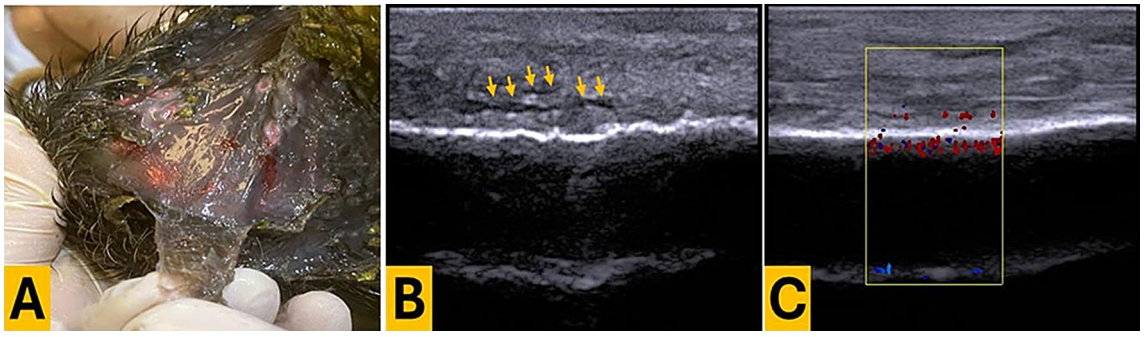

Fig. 4 Morphological and ultrasonographic assessment of the lips at day 7 post-injection of 200 IU of BOTOX. **A**) Observations of diffuse inflammatory mucosal diphtheritic reactions. **B**) Visualization of the diffusion of the BOTOX solution within the submucosal layer. **C**) Color Doppler imaging revealed inflammatory responses, with a regular arrangement of the orbicularis oris muscle

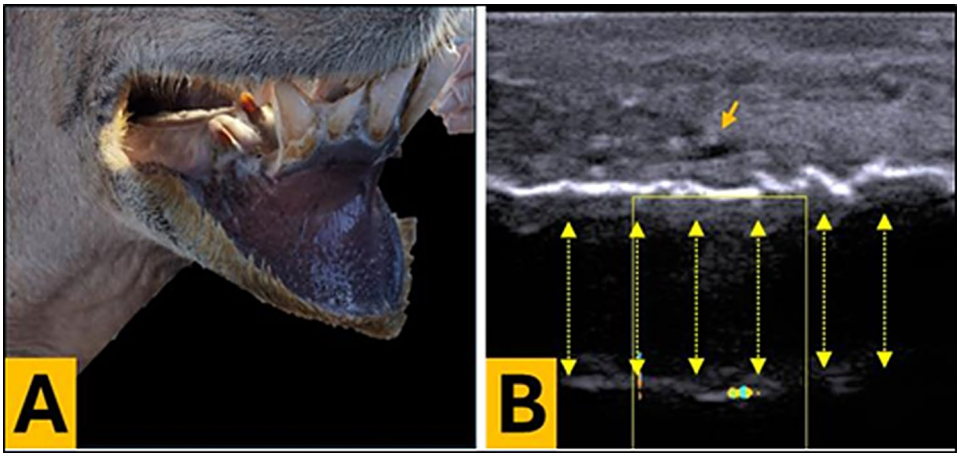

Fig. 5 Morphological and ultrasonographic images at 14 days post-injection. (**A**) Drooping of the lower lip, indicating paralysis of the orbicularis oris muscle. (**B**) Resolution of inflammation, with a restored, regular muscle contour (yellow arrows)

indications [11]. Botulinum toxin (BOTOX) inhibits acetylcholine release at the neuromuscular junction, leading to temporary muscle paralysis. In the lips, this results in muscle relaxation, altered contour, potential drooping, and minor vascular effects [12, 13].

In human studies, filler complications are classified by onset and severity, with serious complications such as vasculitis and vascular compression being reported [14, 15]. Our findings align partially with these reports, as visible mucosal lip lesions were observed in only two camels receiving the higher BOTOX dose. Swelling of the upper

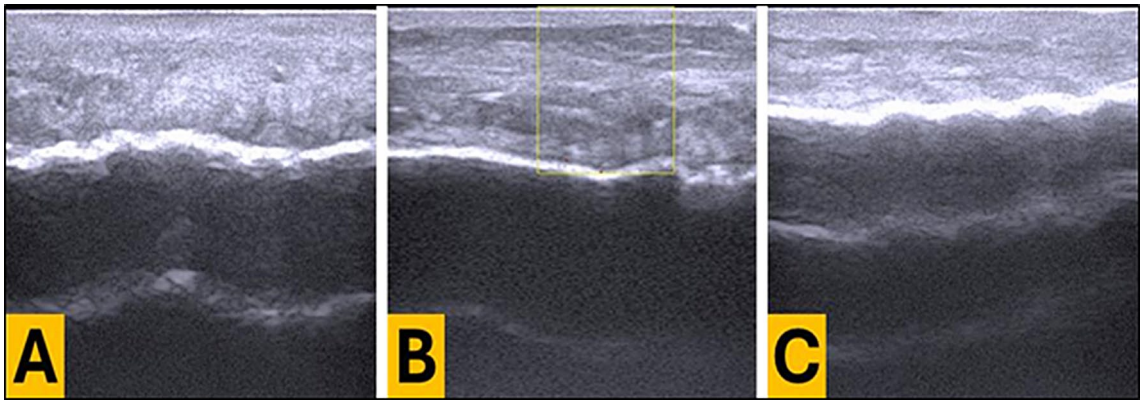

Fig. 6 Ultrasonographic images of BOTOX-injected lips at (**A**) 28 days, (**B**) 35 days, and (**C**) 54 days post-injection, showing no significant changes

and lower lips was consistently identified in all BOTOX -treated camels through visual inspection and palpation, but not in the control group, paralleling results seen in humans and animals [16, 17].

The current study provides long term investigation into the efficacy use of ultrasound for the detection and localization of botulinum toxin type A injections in the lips of adult Arabian dromedary camels. The primary objective was to assess the efficacy of ultrasound as a diagnostic tool while administering two different doses (100 IU and 200 IU). In this study, they were administered as subcutaneous injections. A report of the non-significant difference between subcutaneous and intramuscular injections was reported [18]. A long-term or high-dose administration of BOTOX may lead to atrophy of the injected muscle [19].

Further long term study is to be conducted to evaluate the atrophy and dimensional decrease of the injection sites as reported in previous studies [20]. No change in food intake was recorded in all camels unlike reported in experimental animals [21]. The onset of action was determined 4 days after injection of the lower dose and 2 days after the higher dose. The mean onset of action in experimental animals was 24 h post-injection [20]. Although histological and immunohistochemical studies were not conducted in this study and the objective focused on the morphological effects, previous reports on rats declared significant changes [22].

The anti-inflammatory effects of Botulinum toxin type A remain uncertain. Some reports suggest its potential to reduce inflammation [23], while others have found no such effect [24]. Our results here showed that post-injection inflammation was significant and related. Similar results were reported [25]. Severe inflammation and vasculitis were reported in human patients after administration of cosmetic dose [26, 27], although inflammatory reactions were determined morphologically and ultrasonographic, vasculitis was not evidenced in all participated camels.

The ultrasonographic findings reported here align with findings from studies on humans [28]. Irregular muscle fiber arrangements were observed on the first week post injection. Ultrasonography was valuable to detect fibrosis of the muscles due to repetitive injections of botulinum toxin A [29].

Interestingly, all BOTOX treated camels exhibited lip drooping and swinging, which are significant findings given the use of BOTOX for aesthetic enhancement in camel beauty contests [3]. However, no hardness or corrugated changes in the dermal side of the lips were detected, distinguishing these responses from those commonly observed in humans.

Camels treated with the higher dose exhibited the most pronounced changes, while the control group showed minimal effects. This highlights the risks of higher doses and underscores ultrasound's value in non-invasive tissue assessment.

Hematobiochemical changes in camels have been reported to be essential under various conditions to detect blood parasites [1], metabolic disorders related to intestinal obstruction [30], effect of chemotherapy after tumor removal [31], and infectious pulmonary diseases [32]. Herin our hematobiochemical results revealed no significant differences between the BOTOX-treated camels and the control group, suggesting that systemic effects were minimal.

Such findings are critical in the context of detecting illegal aesthetic modifications in camels, highlighting the utility of ultrasound in identifying subtle tissue changes that may not be apparent through physical examination alone. Morphological assessments indicated a dose-dependent effect of BOTOX on lip structure.

Conclusions

The results demonstrate the effects of BOTOX on lip morphology and tissue characteristics in camels. Higher doses (200 IU) resulted in more significant and prolonged changes, as evidenced by both clinical assessments and

ultrasound imaging. These findings confirm the utility of both morphological scoring and ultrasonographic evaluation in detecting and monitoring the effects of BOTOX injections in camel lips. Considering this perspective, this study represents an initial basic science assessment of veterinary application research.

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Author contributions
M.M., M.A., S.A., A.A., A.I.A., and M.S. conceived the project, M.M. A.A.A., A.I.A., and M.S. executed the experiment, M.M., M.E. J.H., and H.E. analyzed and evaluated results, M.A., M.E., and S.S. evaluated procedures, M.M., and M.E. wrote the manuscript, M.M., and M.E., reviewed the final manuscript text, M.M. secured funding.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics and consent to participate
This study received approval from the Research Ethics Committee (REC) of King Faisal University, Saudi Arabia (Approval No. KFU-REC/2022-MAR-EA000539). All procedures were carried out in accordance with applicable guidelines and regulations and are reported following the ARRIVE guidelines. The camels used in this study were sourced from the herd owned by the Camel Research Center, King Faisal University, Al-Ahsa, Saudi Arabia, for research purposes. As the animals belonged to the institution, consent for participation was not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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