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Possible effects of Botox on the skin of laboratory rats

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ABSTRACT
Botulinum toxin is a polypeptide chain consisting of a heavy chain (100 kDa) and a light chain (50 kDa). Botox consists of 7 types of neurotoxins, however only toxins A and B are used clinically .The current study was conducted on (18) samples of female laboratory rats divided into five groups, with 6 rats per group, where they were injected with different concentrations of Botox. These groups include: Group A, which is the control group, Group B, which was injected with a concentration of 0.025 of Botox, Group C, which was injected with a concentration of 0.2,.The results of the statistical analysis showed a significant decrease ($p \leq 0.05$) in the average thickness of the skin layers. Botox injections affect the thickness of the skin layers without being affected by the injection dose .

Keywords: botulinum toxin, skin texture , BOTOX

1. INTRODUCTION

Over the years, Botulinum toxin has been known for its anti-wrinkle effects, and studies have also identified its anti-inflammatory effects. Botox has been used in ophthalmology since the 1970s, and in the past 20 years, its use has expanded to include various health fields, especially dermatology. (Satriyasa 2019, Han et al. 2017).
Botulinum toxin is made from a toxic substance produced by the bacterium Clostridium botulinum in large quantities. Clostridium botulinum bacteria are found everywhere in the form of spores. These spores develop in good environments to form bacteria. They are anaerobic bacteria, their shape is rod-shaped and positive for the Gram stain. These bacteria can germinate and produce a neurotoxin called botulinum toxins (Sobel et al. 2004). In 1897, Van Ermengem discovered these bacteria when Belgian patients were infected with botulism. After that, several strains of bacteria were distinguished that released distinct types of toxins, which were seven serotypes (A, B, C, D, E, F and G). In 1928, Dr. Herman Somme isolated the most potent serotype, BoNT type A (Carruthers and Carruthers 2017). Each serotype has its own distinct pharmacological properties, and only substances containing type A or type B is approved for human use. (Comella et al. 2005).
In the late 1960s and early 1970s, Dr. Alan Scott began human trials using BoNTA to treat strabismus (Lipham, 2024). The FDA approved human trials of BoNTA in volunteers in 1978; a study published later in 1980 showed that intramuscular injections of BoNTA could correct astigmatism. The manufacturer licensed BoNTA in 1989 for the nonsurgical treatment of strabismus, blepharospasm, hemifacial spasm, Meige's syndrome cervical dystonia, and spastic torticollis. As a result, Botox was created in 1992. BoNTA is used to treat frown lines. In 1994, the first trial of BoNTA to treat hyperkinetic facial lines began (Carruthers and Carruthers 2017).

1.1 Aim of the study

To know the histopathological effects of Botox on the skin of laboratory animals.

2. MATERIALS AND METHODS

2.1 Sample collection

In the College of Education for Pure Sciences, Dhi Qar Province, the current study was conducted on (18) samples of female laboratory rats that were obtained from the Laboratory Animal Breeding Center in Babylon Province during a period ranging from January 2024 to October 2024. The ages of the rats ranged between (13-11) weeks and their weights ranged between (200-160) grams.

2.2 Preparation of animals

For the purpose of adaptation and acclimatization under organized and controlled laboratory conditions in terms of ventilation, lighting and temperature which reached (20-25) degrees Celsius, all animals were left throughout the study period in the animal house affiliated to the College of Education for Pure Sciences. The rats were placed in plastic cages specially designed for raising rats, and the floor of the cages was covered with sawdust which was replaced weekly to maintain the cleanliness of the rats. Care was also taken to feed them by providing them with water and their special concentrated feed which consisted of corn, protein, wheat grains and soybeans daily.

2.3 Botulinum toxin

The treatment was obtained from a pharmacy in Baghdad Province, produced by the Spanish company Lantox, as shown in the picture. The medicine was diluted with normal saline.

2.4 Experimental design

The experiment was conducted on 18 rats divided into 3groups, with 6 rats per group, where they were injected with different concentrations of Botox for one month . These groups include: Group A, which is the control group, Group B, which was injected with a concentration of 0.025 of Botox, Group C, which was injected with a concentration of 0.2

2.5 Statistical analysis

Statistical analysis was performed using Analysis of Variance (ANOVA) in analyzing the results statistically, using Statistical Package for Social Sciences (SPSS) program, and the Least Significant Difference (LSD) test was used, and the significance between the studied samples was tested under the probability level ($P \leq 0.05$) and using the Duncan Test. The histological results were measured by Olympus microscope in the Histological Laboratory Unit of the Turkish Teaching Hospital for all samples under the magnification power of X100.

3. RESULTS

3.1 Histological measurements of epidermal layer thickness

The results of the statistical analysis of the current study in female laboratory rats showed a significant decrease ($p \leq 0.05$) in the average thickness of the epidermal layer of the skin when comparing group B treated for one month with the control group, as the average thickness of the epidermal layer in group B was (0.02±0.07), while the average thickness of the epidermal layer in the control group for the same period of time was (0.05±0.17).

The results of the statistical analysis also showed a significant decrease ($p \leq 0.05$) in the average thickness of the epidermal layer of the skin when comparing group C with the control group during the same time period, as the average thickness of the epidermal layer in group C was ((0.03±0.08).

while there was no significant difference ($p \leq 0.05$) in the average thickness of the epidermis layer when comparing groups B, C during the same period of time, as shown in Table (1).

Table 1: shows the significant differences at the probability level ($p \leq 0.05$) between the average thickness of the epidermis layer for both the control group and the other study groups.

Groups	**Epidermal thickness (mm) M±SD))**	**LSD**	**P-Value 0.05))**
Control	0.05±0.17	0.10*	0.018
B Groups	0.02±0.07		
Control	0.05±0.017	0.09*	0.033
C Groups	0.03±0.08		

3.2 Histological measurements of dermal layer thickness

The results of the statistical analysis of the current study in female laboratory rats showed a significant decrease ($p \leq 0.05$) in the average thickness of the dermis layer of the skin when comparing group B treated for one month with the control group, as the average thickness of the dermis layer in group B was (0.97±0.38), while the average thickness of the dermis layer in the control group for the same period of time was (4.23±0.25).

The results of the statistical analysis also showed a significant decrease ($p \leq 0.05$) in the average thickness of the dermis layer of the skin when comparing group C with the control group during the same period of time, as the average thickness of the dermis layer in group C was (0.94±0.15).While there was no significant difference ($p \leq 0.05$) in the average thickness of the dermis layer when comparing groups B, C during the same period of time, as shown in Table (2).

Table 2: shows the significant differences at the probability level (p≤0.05) between the average thickness of the dermis for both the control group and the other study groups

Groups	Dermal thickness (mm) M±SD))	LSD	P-Value 0.05))
Control	4.23±0.25	3.27*	0.000
B Groups	0.97±0.38		
Control	4.23±0.25	3.30*	0.000
C Groups	0.94±0.15		

3.3 Histological measurements of subcutaneous layer thickness

The results of the statistical analysis of the current study in female laboratory rats showed a significant decrease (p≤0.05) in the average thickness of the subcutaneous layer when comparing group B treated for one month with the control group, as the average thickness of the subcutaneous layer in group B was (0.67±0.46), while the average thickness of the subcutaneous layer in the control group for the same period of time was (1.67±0.21).

The results of the statistical analysis also showed a significant decrease (p≤0.05) in the average thickness of the subcutaneous layer when comparing group C with the control group during the same period of time, as the average thickness of the subcutaneous layer in group C was (0.36±0.19).While there was no significant difference (p≤0.05) in the average thickness of the subcutaneous layer when comparing groups B, C during the same period of time, as shown in Table (3).

Table 3: shows the significant differences at the probability level (p≤0.05) between the average thickness of the subcutaneous layer for both the control group and the other study groups.

Groups	Hypodermal thickness (mm) M±SD))	LSD	P-Value 0.05))
Control	1.67±0.21	0.99*	0.006
Groups B	0.67±0.46		
Control	1.67±0.21	1.31*	0.001
Groups C	0.36±0.19		

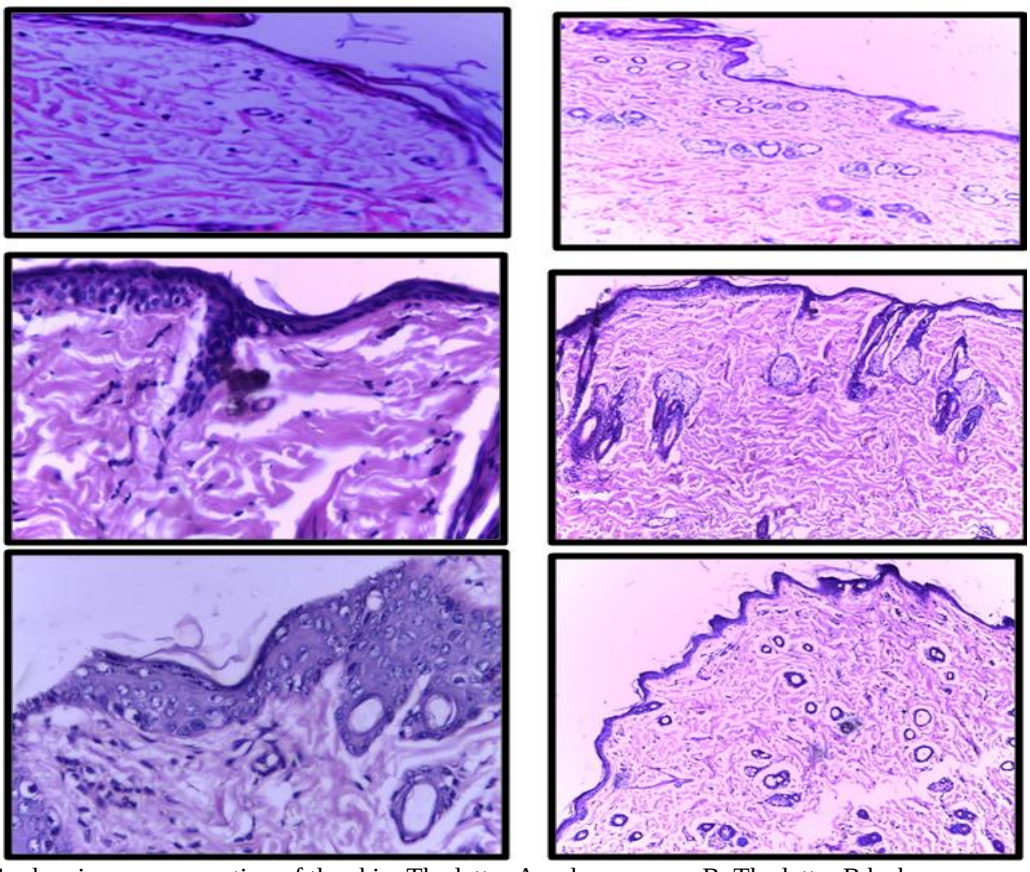

Figure 1: showing a cross section of the skin. The letter A,a shows group B. The letter B,b shows group C. The letter C,c shows the control group. The capital letter is 400X. The lowercase letter is 100X.

4. DISCUSSION

The results of the current study indicated that injecting female laboratory rats with Botox at different concentrations led to a decrease in the thickness of the epidermis layer when comparing the injected groups with the control groups during the same time period. As for the dermis layer, it showed a decrease in thickness during the period treated with Botox. While the thickness of the subcutaneous layer was thinner in all groups injected with Botox when compared with the control for the same time period. No significant differences were observed in the thickness of the skin layers between all groups injected for the same time period, as Botox works to improve skin elasticity, reduce wrinkles, and rejuvenate the skin, as it works by preventing nerve signals to the muscles, which makes the muscles relax and lose their ability to contract temporarily, which helps remove wrinkles (Zhu and Chandran 2023).

The current study is consistent with the study of (Han et al. 2017) which showed that when laboratory animals were injected with TNCB, it led to a significant increase in skin thickness in mice. When this group was injected with Botox, TNCB-sensitive mice exposed to intradermal injections of BoToX showed significantly thinner skin. Botox injections can affect surrounding tissues including adipose tissue and continuous irritation or injury to tissues can lead to loss of subcutaneous fat, which results in decreased skin thickness, consistent with what was indicated by (Shah 2008). When intradermally injected with BoNTA, there was a decrease in sebum production and subsequent improvements in oily skin and pore size, possibly by preventing the release of acetylcholine from neurons near the sebaceous glands. Botox helps reduce the activity of sweat and sebaceous glands. When Botox is injected subcutaneously, it works to disrupt the nerve signals that stimulate the sweat glands to secrete sweat and the sebaceous glands to secrete oil, also consistent with what was indicated by (Min et al. 2015). When the anterior muscle was injected with BTX-A at an amount of 35-40 units in 10 different sites after one month of treatment, an 80% decrease in sebum secretion was shown.

The current study is in agreement with (Shuo et al. 2019) who showed that intradermal injection of BoNT-A resulted in a decrease in sebum production and pore size and no major side effects were observed as BoNT-A effectively reduces sebum production and secretion.

The study did not agree with the study of (El-Domyati et al. 2015) which showed skin changes after BTX injection, as there was an increase in the width of wrinkles and the thickness of the granular layer while other histological measures including the thickness of the epidermis ($P = 0.6$), the thickness of the subcutaneous skin ($P = 0.5$) and the depth of wrinkles ($P = 0.08$) did not show any significant differences regarding the dermal changes after 3 months of BTX injection - the collagen bundles which were disorganized became more organized and cohesive around the wrinkles at the same time there was no significant increase in the density of collagen I ($P = 0.2$) and III ($P = 0.4$) while elastin did not decrease significantly after BTX-A injection ($P = 0.1$).

Bonaparte and Ellis (2014) found that injection of onabotulinum toxin A increases skin elasticity. Muscle paralysis caused by botulinum toxin may reduce recurrent skin wrinkles and future wrinkle sites over time. Elastin and collagen are weakened at these sites. Onabotulinum toxin may have a direct effect on the skin at the tissue level to help identify potential changes in facial skin after onabotulinum toxin treatment. This is consistent with our study, as Botox injections cause irritation or injury to tissues at the injection site. This irritation can alert the body to an inflammatory response that leads to tissue destruction and reduced collagen production.

A study by Wu (2015) showed that treatment with diluted BoNTA can significantly reduce superficial skin texture including moderate skin roughness after BoNTA treatment on the forehead. This reduction in roughness may explain the smoother and brighter appearance of the skin after treatment. This finding has had a significant impact on clinical trials where intradermal injections of diluted BoNTA are increasingly being used on a large area of the face and neck to enhance the skin, resulting in a tightening effect attributed to improved skin texture and radiance. Botulinum toxin type A (BoNT-A) can directly remodel the skin or cause loss of its natural shape. Intradermal injections produce a skin tightening effect. Different commercial preparations of BoNT-A toxins cause different changes in fibroblasts in vitro. The choice of product and dilution used may affect the clinical outcomes of intradermal injections of BoNT-A (Wanitphakdeedecha et al. 2019).

Park et al. (2018) indicated that when Botox was injected into the masseter muscle and the thickness of the muscle and the thickness of the subcutaneous layer were measured before and after BoNT injection, and the differences between the experimental and control groups were analyzed, the thickness of the subcutaneous layer did not differ significantly between the experimental and control groups, and it was concluded that BoNT only affects the muscles. This is not consistent with our current study, as Botox injection affects the subcutaneous layer. When Botox is injected, the muscles under the skin relax over time. The lack of muscle activity can reduce the stimulation that the surrounding tissues, including skin cells, need to produce collagen, which leads to thinning of the layer. Botox injection may also affect the small blood vessels in the area, and the lack of blood flow may hinder the availability of oxygen and nutrients to the adipose tissue, which contributes to its loss.

The researcher (Lee et al. 2009) conducted a study in which two wounds were made on the back of 15 mice. One of the wounds was injected with Botox and the other wound was used as a control. There were statistically significant differences in wound size at the third and fourth week between the Botox and control groups ($P<0.05$). The Botox group showed less infiltration of inflammatory cells compared to the control group at the

second week (P<0.05). The Botox group showed fewer fibroblasts and less fibrosis than the control group at the fourth week (P<0.05). The Botox group showed a very strong collagen density compared to the control group at the eighth week (P<0.05). The wounds in the Botox-treated group showed larger wound size, less inflammatory cell infiltration, less fibrosis, and significantly more collagen than the control group. Botox can be used to reduce wound fibrosis. When Botox is injected close to the wound, it blocks nerve signals that cause muscle contraction. This muscle relaxation reduces tension in the skin and tissue surrounding the wound, allowing the wound to heal better and faster. Reducing tension helps reduce friction or overstretching that can trigger inflammation.

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