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Effect of Botulinum toxins on skin of laboratory rats

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Abstract:

The current study aims to know the histopathological effects of Botox on the skin of laboratory animals and the extent of its effect on weight gain and loss, as well as to predict whether there are side effects that cause harm and harm to animals. Botox consists of (7) types of neurotoxins, however only toxins A and B are used clinically. The current study was conducted on eighteen of female laboratory rats. For a month, divided into three 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 D, which was injected with a concentration of 0.1ml, and Group E, which was injected with a concentration of 0.05ml. The skin thickness was measured. 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:

Botox consists of (7) types of neurotoxins. However, only toxins A and B are used clinically (Sampaio, *et al.* 2004). Botox A is used to treat many diseases in the field of medicine, especially in dermatology for cosmetic purposes. The first type of Botox is introduced to the market as ona botulinum toxin A. In 2002, it was recommended for cosmetic treatment of forehead lines by the Food and Drug Administration (FDA). A second formulation of ona botulinum toxin A, produced in France, received a license for cosmetic use in the European Union in 2006 and was approved by the US Food and Drug Administration in 2009. Botox type A is used by the community to describe all ingredients used in cosmetic treatments (Flynn, 2012). A study has conducted in (1994) referred the effectiveness of Botox A in reducing the appearance of facial wrinkles. Since then it has been used as a cosmetic treatment Botox injections can be used to treat wrinkles (Awan, 2017).

The core neurotoxin is composed of three distinct parts (binding domain, transport domain, and catalytic domain, which are the three unique functional parts of core neurotoxins). The heavy chain is composed of binding and transport domains while the light chain has the catalytic domain (Carruthers and Carruthers 2017).

The binding domain of the primary neurotoxin is located at the C terminus of the protein, it initiates its effects on the cells to which it binds by specific binding to the neuronal cell membrane (Brunger and Rummel 2009). The C terminus initially binds to gangliosides, which are abundant on the surface of motor neurons (Rossetto and Montecucco 2007). Synaptic vesicle (SV2) is a protein found in neurotransmitters and neuropeptides that contain vesicles within nerve endings which may play a role as a receptor for botulinum toxin type A (Wan, *et al.* 2010).

In 2006, Dong, *et al*. provided a key to unlocking experimental findings on the physiology of BoNT, such as assessing the rate and magnitude of muscle weakness resulting from continuous stimulation of the nerve that stimulates these muscles. The more neurotransmitter release that occurs actively, the more SV2 is present on the cell surface (Jahn, 2006).

The duration of BoNT effects varies with several factors including serotype and site of administration (Foran, *et al*. 2003). The effects of botulinum toxin are reversible, and recovery from Botox effects can be gradual, but complete recovery of nerve and muscle function is not achieved until the damaged nerve endings regain activity (Rogozhin, *et al*. 2008). However, SNAP-25 cleavage has been observed in vivo after 80 days of BoNTA treatment in striated muscle. The effect typically persists for 26 weeks or more, while the effect of Botox on facial muscles typically lasts for 12 to 16 weeks (Herschorn*, et al*. 2011). The clinical efficacy of one BoNT product (onabotulinumtoxinA) has been shown to persist for at least six months after intravesical injection in patients with bladder muscle activity (Herschorn*, et al*. 2011) or intradermal injection in individuals with severe symptoms of primary axillary hyperhidrosis (Lowe, *et al*. 2007).

2.Materials and methods

2.1Samples collection

The current study was carried out at the college of education for pure sciences in Dhi Qar province using thirty of female laboratory rats that were acquired from the laboratory animal breeding center in Babylon Province from the beginning of January to the end of October of 2024. The rats' weights ranged from (160 to 200) grams, while their ages ranged from 11 to 13 weeks.

2.2 Preparation of animals

The entire study period in the animal house connected to the college of education for pure sciences in order to acclimate and adapt to structured and controlled laboratory conditions, including ventilation, lighting, and temperatures that reached(20-25c). the floor of the cages was covered with sawdust, which was changed once a week. The rats were housed in plastic cages made especially for breeding rats. Water and their special concentrated diet, which included corn, protein, wheat grains, and soybeans every day, were also given to them with care.

2.3 Botulinum toxin

The medication, which was made by the Spanish business Lantox, was purchased from a pharmacy in the Baghdad Governorate. Three milliliters of regular saline were used to dilute Botox (UNITS 100) (Dessy, *et al*. 2007).

2.4 Experimental design

Eighteen rats, six in each of the three groups, were used in the experiment. For a month, the rats received injections of varying quantities of Botox. These groups are as follows: Group A is the control group (non-treated with botox); Group D received an injection at 0.1ml; and Group E received an injection at 0.05ml.

2.5 Statistical Analysis

The results were analysed using Analysis of Variance (ANOVA) in the Statistical Package for Social Sciences (SPSS) program. The Least Significant Difference (LSD) test was used, and the significance between the studied samples was tested at the probability level ($P \leq 0.05$) and using the Duncan Test. The histological results of all samples

were measured using an Olympus microscope in the Turkish Teaching Hospital's Histological Laboratory Unit.

3. Results

3.1 Histological assessments of the thickness of the epidermal layer

The statistical analysis revealed a significant decrease ($p \leq 0.05$) in the average thickness of the epidermal layer of the skin when comparing group D to the control group throughout the same period of time. Group D's average thickness was (0.03±0.08). While the control group had an average dermis layer thickness of (0.05±0.17) for the same time period.

The average thickness of the skin's epidermis layer decreased significantly ($p \leq 0.05$) in group E when compared to the control group over the same time period, with an average thickness of 0.02±0.05. However, there was no significant difference ($p \leq 0.05$) in the average thickness of the epidermis layer while comparing groups D and E over the same time period, as shown in Table (1).

Table (1): Shows average thickness of epidermis layer among groups .

Groups	Epidermal thickness (mm) (M±SD)	LSD	P-Value (0.05)
Control	0.05±0.017	0.09*	0.033
Group D	0.03±0.08		
Control	0.05±0.017	0.12*	0.005
Group E	0.02±0.05		

3.2 Histological assessments of the thickness of the dermal layer

Group D appeared a substantial decrease ($p \leq 0.05$) in the average thickness of the skin's dermis layer compared to the control group, with an average thickness of (0.89±0.19).

When comparing group E to the control group, the statistical analysis revealed a substantial ($p \leq 0.05$) drop in the average thickness of the skin's epidermal layer, with group E's average thickness being 0.96±0.12. When comparing groups D and E, there was no discernible difference ($p \leq 0.05$) in the average thickness of the dermis layer, as shown in Table (2)

Table (2) Shows average thickness of dermis layer among groups .

Groups	Dermal thickness (mm) (M±SD)	LSD	P-Value (0.05)
Control	4.23±0.25	3.34*	0.000
Group D	0.89±0.19		
Control	4.23±0.25	3.27*	0.000
Group E	0.96±0.12		

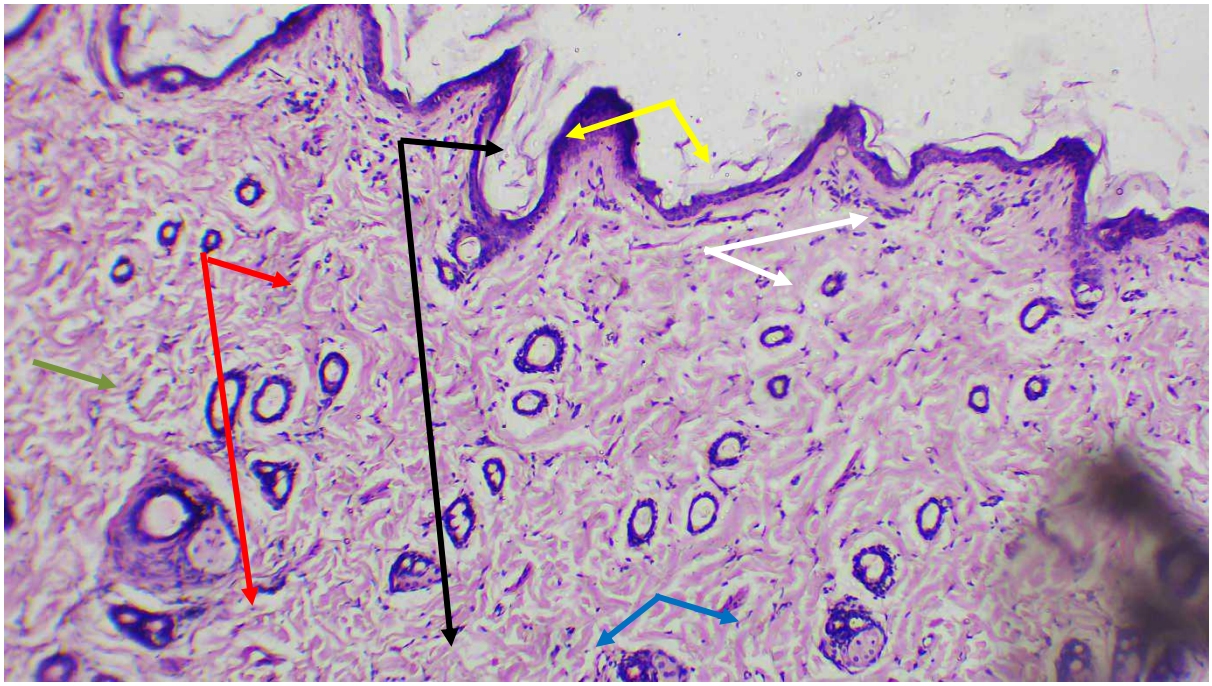
3.3 Histological assessments of the thickness of the subcutaneous layer

When comparing the average thickness of the subcutaneous layer in group D to the control group during the same time period, the results revealed a significant decrease ($p \leq 0.05$), with the average thickness in group D being (0.27±0.22). While the control group had an average dermis layer thickness of (0.21±1.67) for the same time period

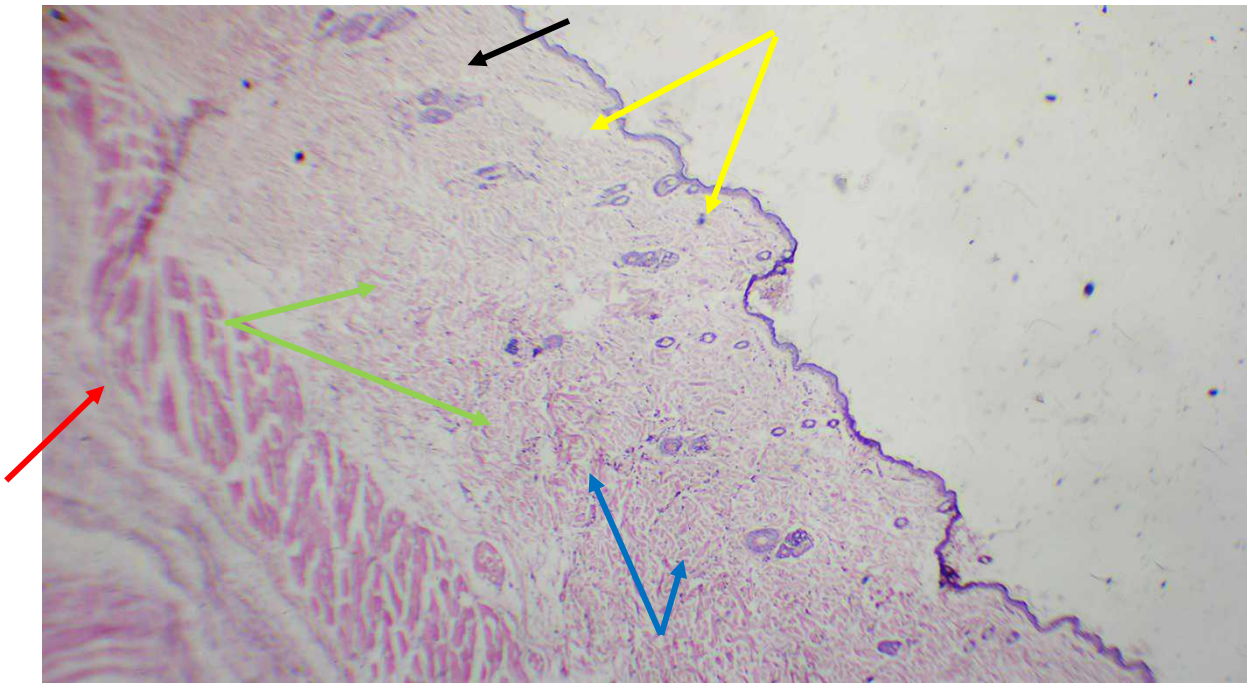
The statistical analysis revealed a significant decrease ($p \leq 0.05$) in the average thickness of the subcutaneous layer in group E compared to the control group over the same time period. Group E had an average thickness of (0.67±0.04). The average thickness of the subcutaneous layer did not differ significantly ($p \leq 0.05$) between groups D and E over the same time period, as shown in Table (3)

Table (3) Shows average thickness of subcutaneous layer among groups .

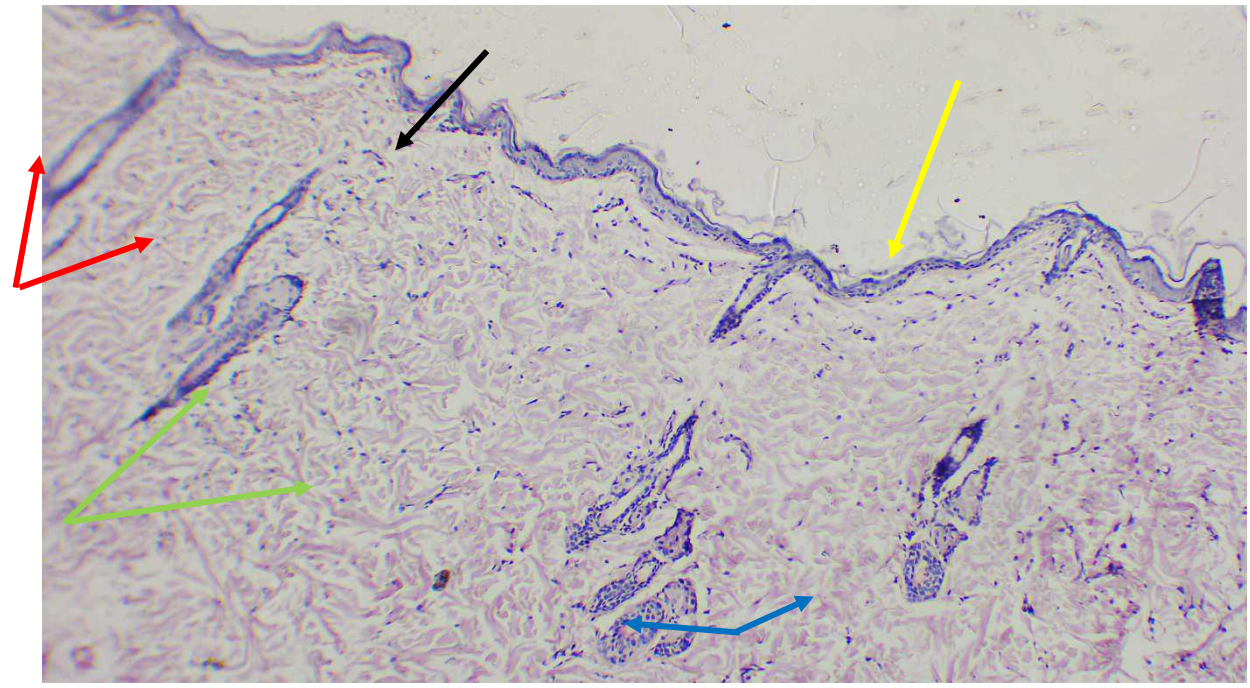
Groups	Hypodermal thickness (mm) (M±SD)	LSD	P-Value (0.05)
Control	1.67±0.21	1.40*	0.000
Group D	0.27±0.22		
Control	1.67±0.21	1.00*	0.006
Group E	0.67±0.04		



Fig(1): Normal epidermis of group (A) (yellow arrow). Dermis layer (black arrows). Fibroblasts (white arrows). Collagen fibres (red arrows). Hair follicle (green arrow). Sweat gland (blue arrows) (100x, H&E) (without treatment).



Fig(2): Epidermis thinness of group () (yellow arrow). Dermis layer (black arrow). Muscular tissue (red arrows). Collagen fibers (green arrows). Sweat gland (blue arrow) (40x, H&E) (with .05 of Botox).



Fig(3): Epidermis thinness of group () (yellow arrow). Dermis layer (black arrow). Hair follicle (red arrows). Collagen fibers (green arrows). Sweat gland (blue arrow) (100x, H&E) (with .05 of Botox).

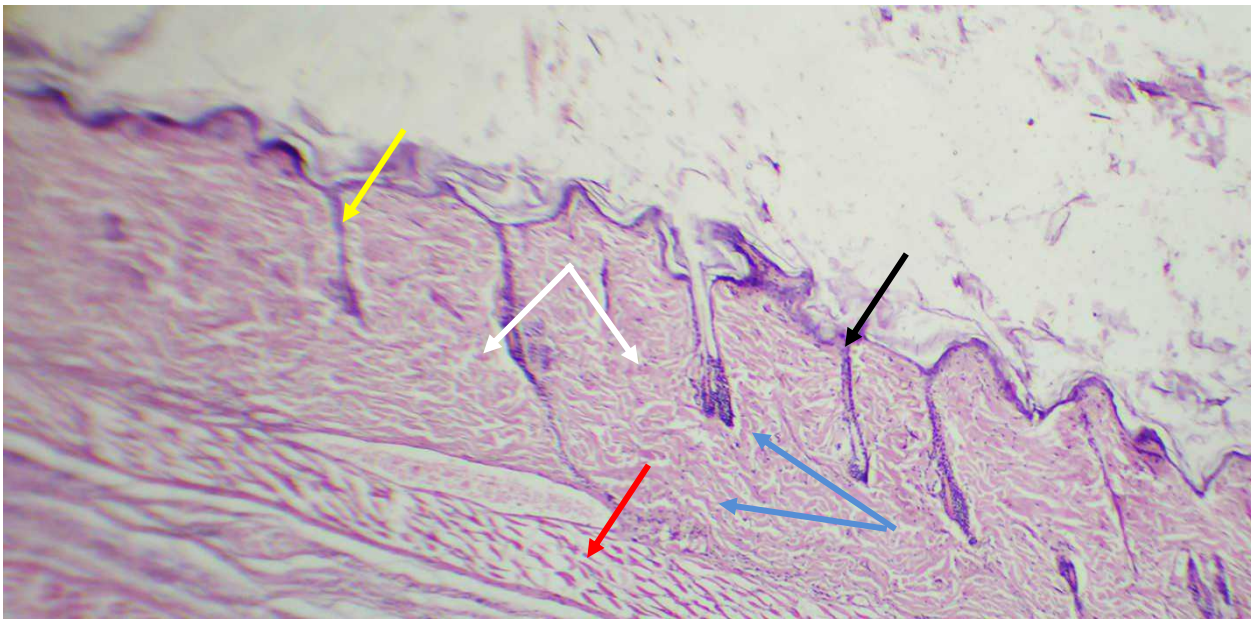


Fig (4): Epidermis thinness (black arrow). Hair follicle (white arrows). Dermis layer (yellow arrow). Collagen fibers (blue arrows). Relax of muscular tissue (40x, H&E) (with .1 of Botox).

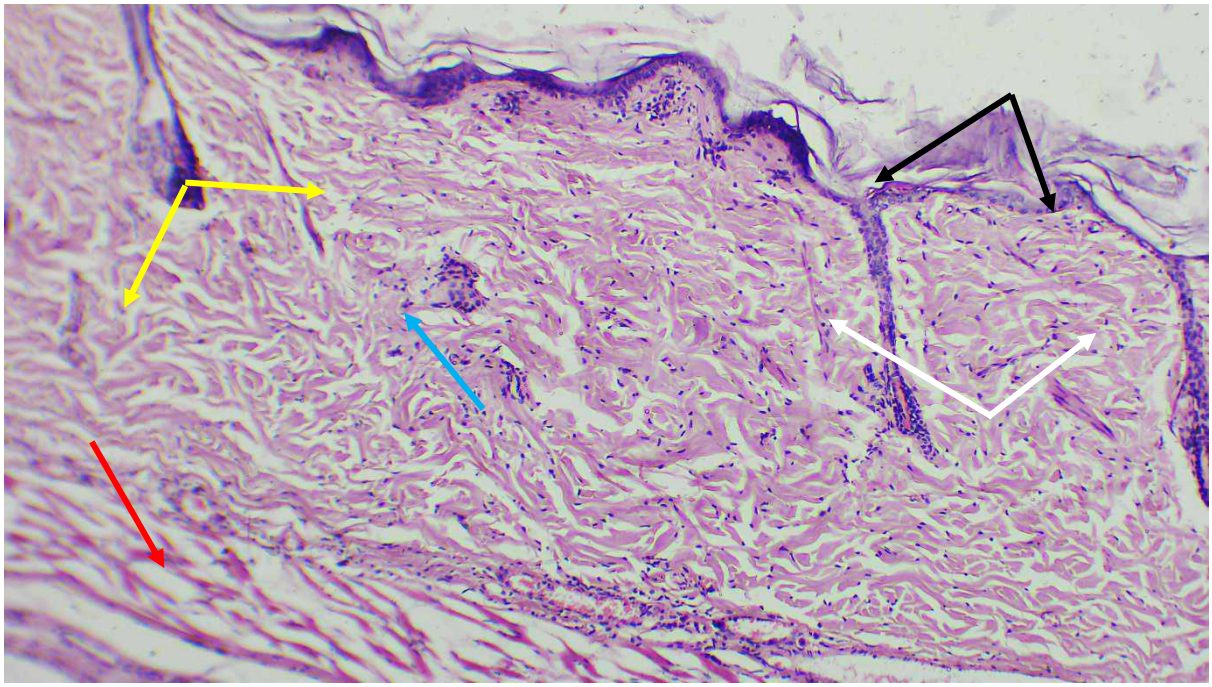


Fig (5cs): Epidermis thinness (black arrow). Hair follicle (white arrows). Collagen fibers (yellow arrow). Collagen fibers (yellow arrows). Sweat gland (blue arrow). Relax of muscular tissue (red arrow) (100x, H&E) (with .1 of Botox).

4. Discussion

The current study showed that treating female laboratory rats with Botox at various dosages reduced the thickness of the epidermal layer when compared to the control groups throughout the same time period. The dermis layer thinned during the Botox treatment. While the thickness of the subcutaneous layer was smaller in all Botox-treated groups than in the control group during the same time period. There were no significant changes in the thickness of the skin layers between all groups injected throughout the same time period. Botox acts to increase skin elasticity,

decrease wrinkles, and rejuvenate the skin by blocking nerve signals to the muscles, causing the muscles to relax and temporarily lose its capacity to contract, hence removing wrinkles (Zhu and Chandran 2023).

The current study is congruent with that of Han *et al.* (2017), who found that injecting laboratory animals with TNCB resulted in a considerable increase in skin thickness in mice. TNCB- (Trinitro chlorobenzene) sensitive animals given intradermal Botox injections had considerably thinner skin.

Botox injections can impact surrounding tissues, including adipose tissue, and persistent tissue irritation or damage might cause subcutaneous fat to be lost, resulting in thinner skin, as suggested by Shah (2008). By blocking the release of acetylcholine from neurons close to the sebaceous glands, BoNTA administered intradermally reduced the production of sebum and improved greasy skin and pore size. Botox aids in lowering sebaceous gland and perspiration activity. In line with what was suggested by Min, *et al.* 2015, Botox subcutaneously acts by interfering with the nerve signals that cause the sweat glands to release perspiration and the sebaceous glands to release oil. After a month of treatment, an 80% reduction in sebum secretion was observed when BTX-A was injected into the anterior muscle at a dose of 35–40 units in 10 distinct locations.

Since BoNT-A efficiently reduces sebum production and secretion, the current study is consistent with (Shuo, *et al.* 2019), who demonstrated that intradermal injection of BoNT-A resulted in a decrease in sebum production and pore size with no significant side effects.

The study is disagreed with that of El-Domyati, *et al*. (2015), who found that following BTX injection, the width of wrinkles and the thickness of the granular layer increased, but other histological measurements, such as 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 reveal any significant differences in the dermal changes: after three months of BTX injection, the disorganized collagen bundles became more cohesive and organized around the wrinkles, but the density of collagen I ($P = 0.2$) and III ($P = 0.4$) did not significantly increase, while elastin did not decrease significantly after BTX-A injection ($P = 0.1$).

According to Bonaparte and Ellis (2014), onabotulinum toxin A injections improve skin elasticity. Over time, botulinum toxin-induced muscle paralysis may lessen future wrinkle locations and recurring skin wrinkles. At these locations, collagen and elastin are degraded. In order to detect possible alterations in the skin of the face following therapy, onabotulinum toxin may directly affect the skin at the tissue level. Given that Botox injections irritate or harm the tissues where they are administered, this is in line with our research. An inflammatory reaction that results in tissue damage and decreased collagen synthesis may be triggered by this discomfort.

According to a study of Wu (2015), after applying diluted BoNTA to the forehead, superficial skin texture, including mild skin roughness, can be considerably reduced. The smoother and more radiant appearance of the skin following treatment could be explained by this decrease in roughness. Due to the tightening effect caused by enhanced skin texture and radiance, this discovery has had a major influence on clinical trials where diluted BoNTA is being injected intradermally to improve the skin on a wide portion of the face and neck. The skin may be immediately remodeled or lose its natural contour as a result of botulinum toxin type A (BoNT-A). Injections administered intradermally tighten the skin. In vitro, fibroblasts are altered differently by several commercial formulations of BoNT-A toxins. The clinical results of intradermal injections of BoNT-A may be impacted by the product and dilution selection (Wanitphakdeedecha, *et al*. 2019).

According to Park, *et al*. (2018), when Botox was injected into the masseter muscle, measurements were made of the muscle's thickness and the thickness of the subcutaneous layer before and after the BoNT injection. The thickness of the subcutaneous layer did not significantly differ between the experimental and control groups, and it was determined that BoNT only affected the muscles. This contradicts our existing research because Botox injections alter the subcutaneous layer. The muscles beneath the skin gradually relax after receiving a Botox injection. The layer may thin as a result of decreased muscle activity, which lessens the stimulation required for the production of collagen by the surrounding tissues, including skin cells. A lack of blood flow may make it more difficult for the adipose tissue to receive nutrients and oxygen, which leads to its depletion. Botox injections may also have an impact on the area's tiny blood capillaries.

5. Conclusion

The current study concluded that injecting Botox into the skin of laboratory animals leads to a decrease in the thickness of the skin layers.

Conflicts of Interest

The authors declare no conflict of interest.

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