



## Evaluation of the Role of Botulinum Toxin an Injection in the Prevention of Femoral Artery Post Anastomotic Thrombosis in Male Albino Rats

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### ABSTRACT

**Background:** The botulinum toxin A potential role in preventing femoral artery post-anastomotic thrombosis lies in its ability to reduce smooth muscle spasm. By inhibiting acetylcholine release, it may decrease vasoconstriction at the anastomosis site, thus improving blood flow. The present work aimed to assess the effect of botulinum toxin type A injection in prevention of post anastomotic thrombosis in femoral arteries in male albino rats.

**Methods:** We conducted a study with twenty male albino rats, each weighing between 200 and 350 grams. These rats were carefully divided into two groups. In Group A, we precisely administered 10 units of botulinum toxin type A, diluted in a small amount (0.5 cc) of sterile saline solution (0.9% sodium chloride), around the femoral artery in their right leg. This targeted injection was performed within the perivascular space, the area immediately surrounding the artery. The contralateral left side femoral arteries were injected with normal saline in the perivascular space and considered as the control group (group B). When comparing between the femoral arteries diameters on both sides 7 days after botulinum toxin type A injection and before the start of anastomosis.

**Results:** The post-anastomotic diameter of the femoral arteries showed a statistically significant difference ( $p=0.002$ ) between the groups that were investigated. A statistically significant difference was revealed between diameter of femoral artery before and after microvascular anastomosis in group B ( $p=0.025$ ). A statistically significant difference was revealed between the studied groups according to the occurrence of thrombosis in anastomosed femoral arteries 1 hour after exposure to ice packs cold challenge ( $p=0.001$ ). One hour following cold challenge with ice packs, there was a statistically significant difference in the diameter of the anastomosed femoral arteries across the groups that were evaluated. ( $p=0.033$ ).

**Conclusion:** Botulinum toxin A had a significant role in enhancing vascular outcomes. The findings suggest that BTX-A injections can effectively reduce the incidence of thrombosis by promoting vasodilation and improving blood flow in the femoral arteries.

**Keywords:** Botulinum toxin A, Femoral artery, Anastomotic thrombosis, Male albino rats.

### INTRODUCTION

Free tissue transfer is a valuable reconstructive technique that is used to repair complex tissue defects throughout the body. While highly

successful, the most challenging complication remains the potential for blood clots to form within the transferred tissue's blood vessels (vascular thrombosis). It is believed that the significant

contributing factor of the clotting is vasospasm, particularly at the delicate connection points of the tiny vessels (microvessel anastomosis). To minimize this risk, meticulous care should be done to handle the vessels gently during surgery. Techniques such as carefully removing the outer layer of the vessels (serosal stripping) to reduce sympathetic nerve fibers, and avoiding medications that constrict blood vessels (vasopressors), are strategies that are employed to help prevent vasospasm and ensure the best possible outcome for patients. [1]. Botulinum toxin type A has shown promise in effectively treating the vasospasm associated with Raynaud's phenomenon, offering relief for patients experiencing this challenging condition [2].

Botulinum toxin type A may work to inhibit vasospasm through a couple of key mechanisms. It's thought to interrupt the cold-triggered vasoconstriction signals normally transmitted by the sympathetic nervous system. Additionally, it appears to prevent the activation of alpha-2 receptors in the smooth muscle of blood vessels, further reducing the potential for constriction. These potential actions make it a promising treatment option in certain cases [3].

Encouragingly, further research has demonstrated improvements in blood flow, muscle perfusion (blood supply within the muscle), and glucose uptake following botulinum toxin administration. The effects of Botulinum toxin type A typically become apparent within 3 to 5 days and can persist for several weeks, and in some cases, even months, offering sustained benefits [4].

At present, we lack an effective animal model to study vasospasm at the site of surgical vessel connections (anastomotic vasospasm). Developing such a model would be invaluable in exploring potential preventative measures, such as pre-treating the recipient site of a free tissue transfer with a neuromuscular blocking agent, we aimed at this study to assess the effect of botulinum toxin type A injection in prevention of post anastomotic thrombosis in femoral arteries in male albino rats at Zagazig University Hand and Microscopic Centre (ZUHMC).

## METHODS

This experimental study was conducted on 20 Sprague-Dawley young adult male rats with an average weight of 200-350 grams that were all subjected to the experiment, during the study period between August 2023 to January 2024; the Institutional Animal Care and Use Committee of the Faculty of Medicine, Zagazig University, approved

the study (ZU-IACUC/3/F/124/2023). Following the recommendations of the Declarations of Helsinki as well as the European Community's rules for the use of experimental animals, the experiment was carried out.

## Methods:

All the maneuvers carried on in this experiment concerning the rats were highly ethical and merciful. The subjects of the experiment were 20 Sprague-Dawley young adult white male albino rats with an average weight of 200-350 grams with intact femoral arteries bilaterally. We excluded all female rats that weighed below 200 gm or above 350 gm or previously injured femoral arteries on either side.

## Study grouping:

For Group A, we administered a precise dose of 10 units of botulinum toxin type A (Botox, Allergan Inc.) diluted in a small volume (0.5 cc) of sterile saline solution (0.9% sodium chloride). This was carefully injected into the perivascular space surrounding the right femoral artery in each rat. The contralateral left side femoral arteries were injected with normal saline in the perivascular space and considered as the control group (group B).

The rats were placed in supine position on a rodent operating board. All rats received proper anesthesia "ketamine Intra-peritoneal (0.005 mg/gm body weight) during all surgical procedures". Hair of the abdomen was shaved using electric clippers. The skin was then disinfected by povidone-iodine solution.

**Under a microscope magnification:** A skin incision was done in both right and left femoral regions. Dissection and identification of targeted femoral arteries on both sides was done.

**Using the crack width ruler,** the diameter of both femoral arteries in each rat was measured and recorded. Then both femoral arteries were cut and standard microsurgical anastomosis using 9-0 nylon sutures on a round needle was done. The diameter of both vessels was measured and recorded and this is considered as **time 0**. Any difficulties in anastomosis was recorded, Then the diameter of the repaired femoral arteries on both sides was measured with the crack width ruler and recorded.

After that the wound was closed using 5/0 polyproline sutures. After the surgical suturing was complete, we induced a localized cold challenge by applying ice packs (16°C) to the lower extremity for 5 minutes. To assess the impact, we reopened the incision sites after one hour and carefully examined the vessels under a microscope. We meticulously measured the diameter of each vessel and

documented any clot formation (thrombus) both immediately after the procedure (time 0) and one hour after the cold challenge. We also observed and recorded any changes in the extremity's color.

Vessel's diameter and any thrombus formation post anastomosis was determined by reopening the incision sites at 1 hour after exposure to ice packs cold challenge test and visual inspection under a microscope was performed. All vessels' diameters were measured and any thrombus formation was detected by visualization under microscope magnification and both recorded both at time 0 and 1 hour after the cold challenge as shown in (Fig 1).

Statistical Analysis:

To do the statistical analysis, we used SPSS version 28 (IBM Co., Armonk, NY, USA). To show quantitative parametric data, we utilized the words range, standard deviation, and mean. A P-value of less than 0.05 was considered a statistically significant finding, and the student t-test was employed to compare the normal distributions of the groups that were analyzed. For quantitative variables with normally distributed distributions, the paired t-test (t) was employed for comparing two sets of data.

### RESULTS

The mean weight of the studied rats was 277.75 ( $\pm 49.45$  SD) with a range of (213-350) grams. In terms of the diameter of the femoral arteries before

surgery (after intervention), a statistically significant difference was seen between the two groups (Table 1)

A statistically significant difference was found between the studied groups as regards post anastomotic Diameter of femoral arteries ( $p=0.002$ ). The right-side femoral arteries anastomosis was technically easier than the left side femoral arteries, suturing was easier and faster with less effort and better vessel quality on the right-side femoral arteries when compared to the left side femoral arteries in each rat separately (Table 2).

For group A, the change in femoral artery diameter between pre- and post-microvascular anastomosis was not statistically significant. A statistically significant difference was revealed between diameter of femoral artery before and after microvascular anastomosis in group B ( $p=0.025$ ) (Table 3).

A statistically significant difference was revealed between the studied groups according to the occurrence of thrombosis in anastomosed femoral arteries 1 hour after exposure to ice packs cold challenge ( $p=0.001$ ) (Table 4).

Also, a statistically significant difference was found between the anastomosed femoral arteries diameter in the studied groups 1 hour after exposure to ice packs cold challenge. ( $p=0.033$ ) (Table 5).

**Table 1:** Comparison between the studied rats regarding femoral artery diameter after perivascular injection of botulinum toxin type A and before anastomosis

	Group A (n = 20)	Group B (n = 20)	Test of Sig.	p
<b>Diameter (mm)</b>				
Range.	0.4 – 0.65	0.4 – 0.65	t= 2.208	0.033*
Mean $\pm$ SD.	0.54 $\pm$ 0.07	0.5 $\pm$ 0.07		
<b>Diameter of right compared to left</b>	<b>No</b>	<b>%</b>		
Wider	14	70		
Equal	6	30		

**Table 2:** Comparison between the studied groups regarding post-anastomotic measurements

	Group A (n = 20)	Group B (n = 20)	Test of Sig.	p
<b>Diameter (mm)</b>				
Range.	0.4 – 0.65	0.4 – 0.65	t= 3.322	0.002*
Mean $\pm$ SD.	0.54 $\pm$ 0.07	0.47 $\pm$ 0.06		
<b>Diameter of right compared to left</b>	<b>No</b>	<b>%</b>		
Wider	16	80		
Equal	4	20		

**Table3:** Comparison between diameter of femoral artery before and after microvascular anastomosis in groups A and B

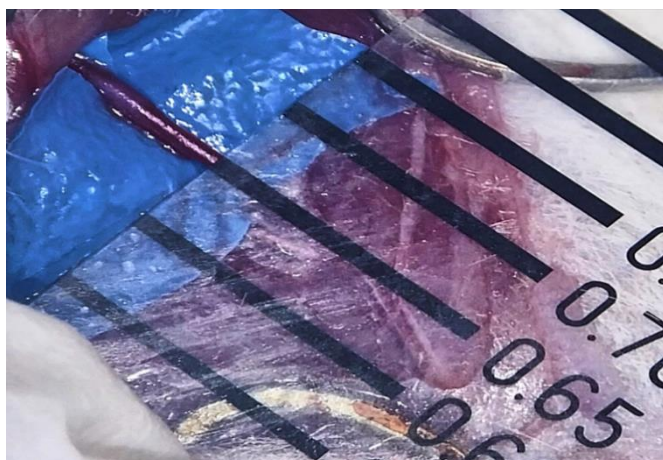
Group A				
	Pre-operation (n = 20)	Post-operation (n = 20)	Test of Sig.	p
Diameter (mm)				
Range.	0.4 – 0.65	0.4 – 0.65	t1=	1.0
Mean ± SD.	0.54 ± 0.07	0.54 ± 0.07	0.0	
Group B				
	Pre-operation (n = 20)	Post-operation (n = 20)	Test of Sig.	p
Diameter (mm)				
Range.	0.4 – 0.65	0.4 – 0.65	t1=	0.025*
Mean ± SD.	0.5 ± 0.07	0.47 ± 0.06	2.438	

**Table4 :** Comparison between the studied groups after microvascular anastomosis according to the occurrence of thrombosis 1 hour after exposure to ice packs cold challenge

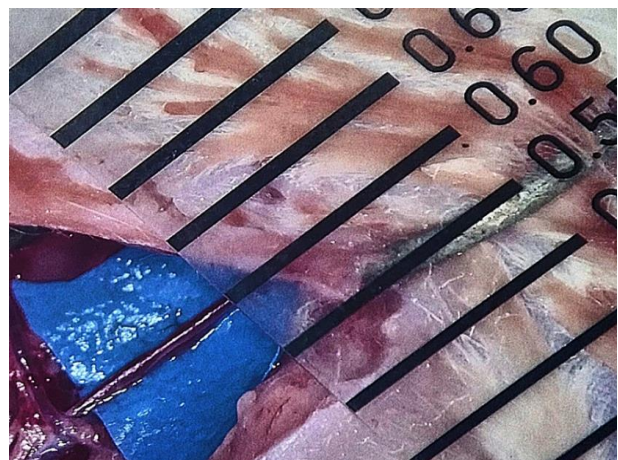
	Group A (n = 20)		Group B (n = 20)		Test of Sig.	p
<b>Thrombosis</b>	No.	%	No.	%		
No	19	95.0	9	45.0	$\chi^2=$ 11.905	0.001*
Yes	1	5.0	11	55.0		

**Table 5:** Comparison between the diameter of studied groups after microvascular anastomosis according to the diameter of femoral arteries 1 hour after exposure to ice packs cold challenge

	Group A (n = 20)	Group B (n = 20)	Test of Sig.	p
<b>Diameter (mm)</b>				
Range.	0.4 – 0.65	0.4 – 0.65	t= 2.208	0.033*
Mean ± SD.	0.54 ± 0.07	0.5 ± 0.07		
<b>Diameter of right compared to left</b>	No	%		
Wider	14	70		



(A)



(B)





(C)



(D)



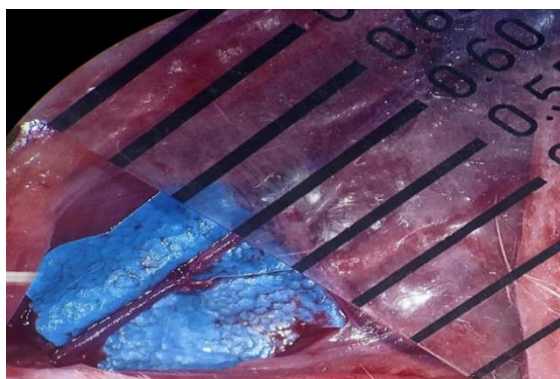
(E)



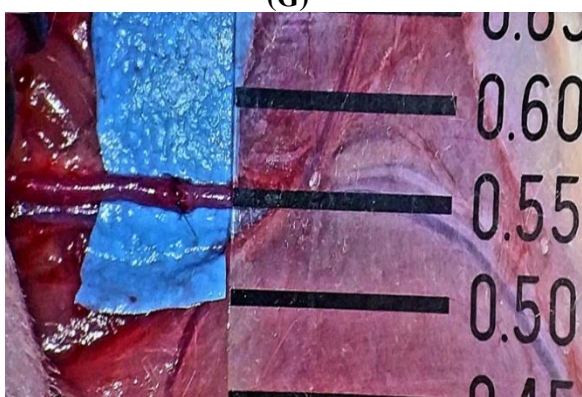
(F)



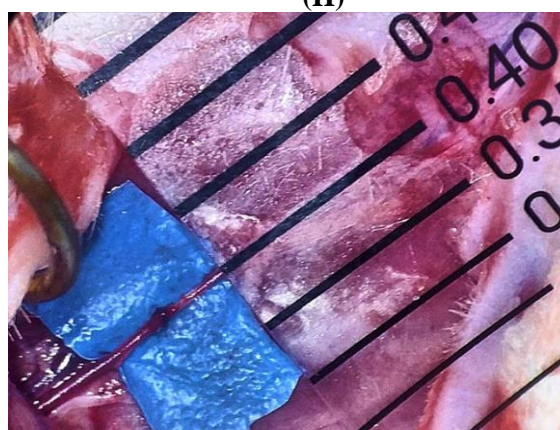
(G)



(H)



(I)

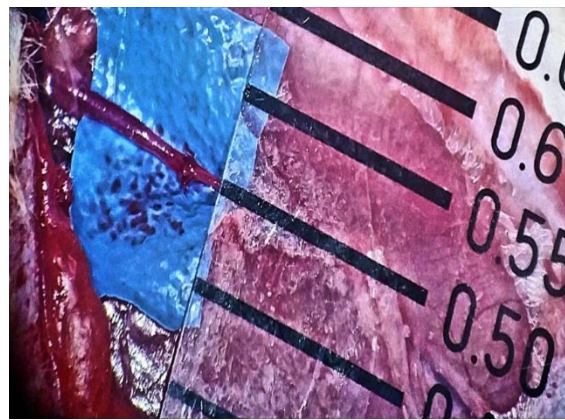


(J)



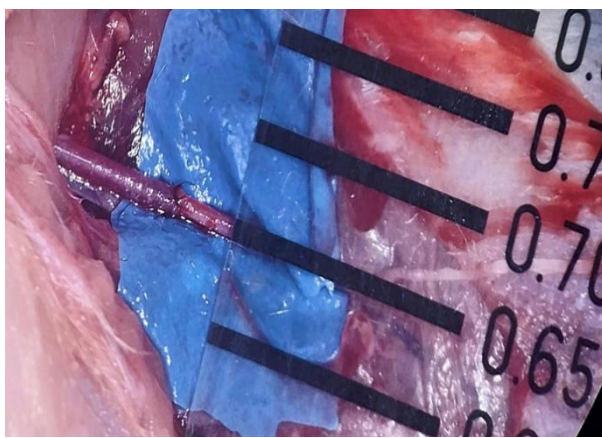


(K)

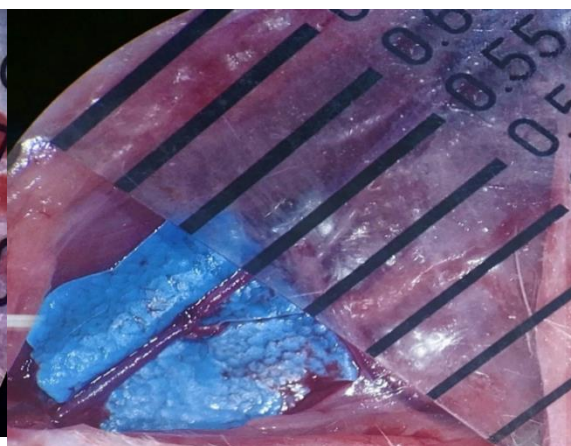


(L)

**Fig. 1:** (A): Diameter of right side femoral artery in 350 gm male albino rat measuring 0.65 mm , 7 days after the injection of botulinum toxin type A, (B): Diameter of left side femoral artery in 350 gm male albino rat measuring. 0.60 mm , 7 days after injection of normal saline, (C): Diameter of right side femoral artery in 250 gm male albino rat measuring 0.55 mm , 7 days after injection of botulinum toxin type A, (D): Diameter of left side femoral artery in 250 gm male albino rat measuring 0.45 mm , 7 days after injection of normal saline, (E): Diameter of right side femoral artery in 300 gm male albino rat measuring 0.60 mm , 7 days after injection of botulinum toxin type A, (F): Diameter of left side femoral artery in 300 gm male albino rat measuring 0.55 mm , 7 days after injection of normal saline, (G): post anastomotic diameter of right side femoral artery in 350 gm male albino rat measuring 0.65 mm ( 7 days after injection of botulinum toxin type A) .. better vessel quality, wider lumen and easier anastomosis, (H): Post anastomotic diameter of left side femoral artery in 350 gm male albino rat measuring 0.60 mm (7 days after injection of normal saline). narrower lumen, poor vessel quality and more difficult anastomosis, (I): Post anastomotic diameter of right-side femoral artery in 250 gm male albino rat measuring 0.55 mm (7 days after injection of botulinum toxin type A). better vessel quality, wider lumen and easier anastomosis, (J): Post anastomotic diameter of left side femoral artery in 250 gm male albino rat measuring 0.40 mm (7 days after injection of normal saline). narrower lumen, poor vessel quality and more difficult anastomosis, (K): Post anastomotic diameter of right-side femoral artery in 300 gm male albino rat (7 days after injection of botulinum toxin type A). better vessel quality, wider lumen and easier anastomosis, (L): post anastomotic diameter of left side femoral artery in 300 gm male albino rat measuring 0.50 mm (7 days after injection of normal saline). narrower lumen, poor vessel quality and more difficult anastomosis.

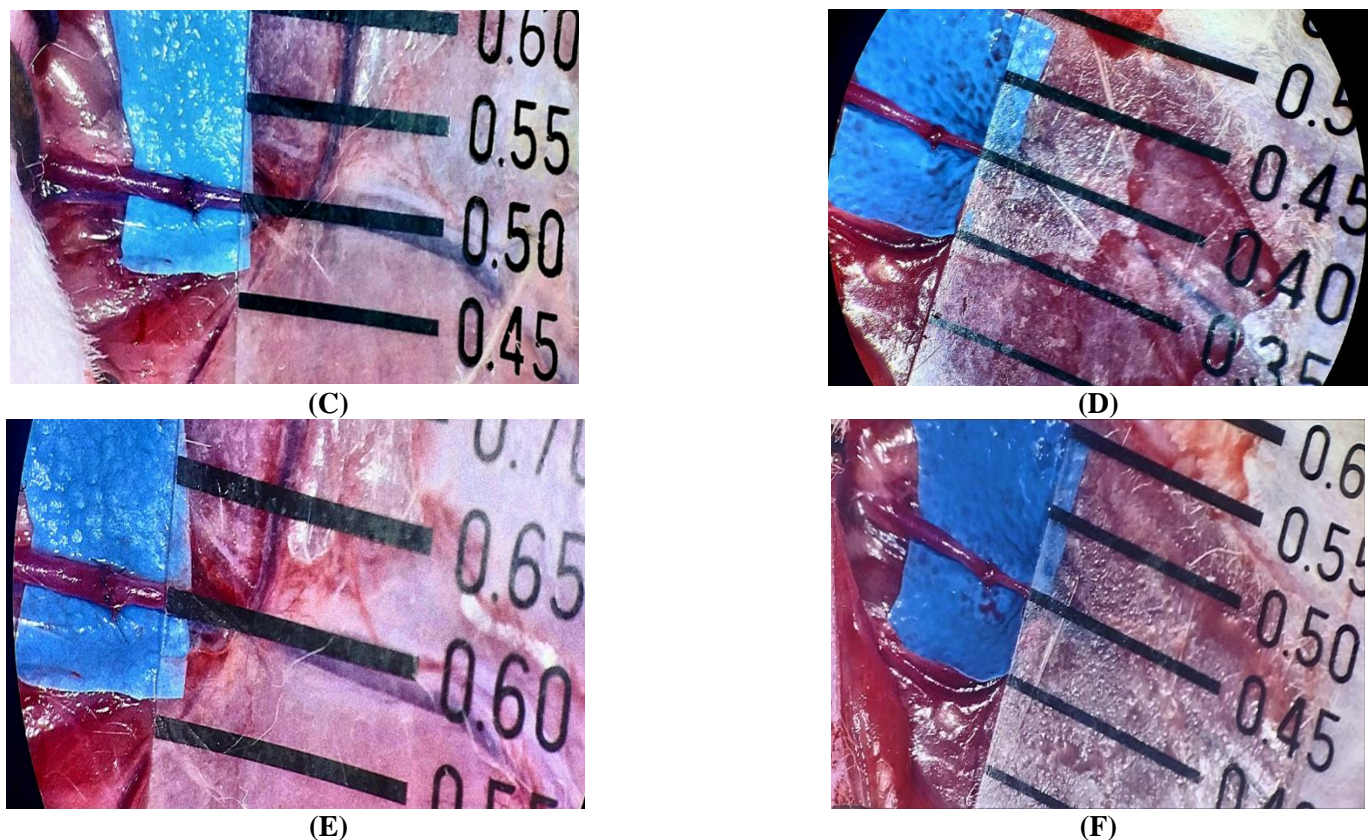


(A)



(B)





**Fig. 2:** (A): Diameter of right side femoral artery in 350 gm male albino rat measuring 0.65 mm ( 1 hr after exposure to ice pack cold challenge test ), (B): Diameter of left side femoral artery in 350 gm male albino rat measuring 0.55 mm (1 hr after exposure to ice pack cold challenge test ), (C): Diameter of right side femoral artery in 250 gm male albino rat measuring 0.50 mm ( 1 hr after exposure to ice pack cold challenge test ), (D) Diameter of left side femoral artery on 250 gm male albino rat measuring 0.40 mm ( 1 hr after exposure to ice pack cold challenge test ), (E): Diameter of right side femoral artery in 300 gm male albino rat measuring 0.60 mm ( 1 hr after exposure to ice pack cold challenge test ), (F): Diameter of left side femoral artery in 300 gm male albino rat measuring 0.45 mm ( 1hr after exposure to ice pack cold challenge test ).

### DISCUSSION

A common technique for reconstructing difficult defects across the body is free tissue transfer. Vascular thrombosis continues to be the most frequent reason for free flap failure. Vasospasm is thought to be one of the key elements in the development of thrombosis, particularly in microvessel anastomosis [5].

Limiting vascular manipulation during surgery, avoiding vasopressor use, and serosal excision of sympathetic fibers from vessels are all ways that have been suggested to prevent vasospasm occurrences [6].

Both the inhibition of sympathetically mediated cold-induced vasoconstriction and the prevention of alpha 2 receptor activation in vascular smooth muscle have led to the suggestion that botulinum toxin type A may be an inhibitor of vasospasm. At the neuromuscular junction, botulinum toxin type A

blocks acetylcholine release. Reduced norepinephrine release from presynaptic terminals is another effect [7].

Research conducted after botulinum toxin injections has shown improvements in blood flow, muscle perfusion, and glucose absorption. Botulinum toxin type A begins working in three to five days and lasts for weeks or months [8]. The purpose of the research was to determine whether injecting male albino rats with botulinum toxin type A prevented vasospasm and post-anastomotic thrombosis in the femoral arteries.

The mean weight of the studied rats was 275.2 ( $\pm 51.34$  SD) with range (213-342) grams. This agrees with all the studies and reports working on femoral artery anastomosis in the rats.

**In the present study,** the diameter of the pretreated femoral artery with botulinum toxin type A was significantly wider than the contralateral group

(pretreated with normal saline). This agrees with, Roh et al. [9] who found that Every location that was treatment with botulinum toxin type A had a consistently increased vessel diameter. When compared to the 0.53 mm average diameter of the control group's saline-treated arteries, the 0.68 mm diameter of the ones treated with botulinum toxin type A was noticeably bigger. The average diameter of the veins treated with botulinum toxin type A was 0.95 mm, which was noticeably greater than the 0.73 mm average diameter of the corresponding control group's saline veins.

**In the present study**, the diameter after microvascular anastomosis of the pretreated femoral arteries with botulinum toxin type A was significantly wider than the post anastomotic diameter in the contralateral group pretreated with normal saline.

Also, Yan et al. [10] indicated that BTX-A, when administered to blood vessels prior to microvascular anastomosis, enlarges the arteries and inhibits thrombosis in experimental animals. It was believed that BTX-A pretreatment could avoid vascular anastomosis failure. Furthermore, patients whose vascularity had not improved after conservative methods to induce vasodilation had their pain reduced after receiving BTX-A. In order to block pathways that cause chronic neuropathic pain, the toxin innervates blood vessels indirectly.

Janz et al. [11] reported that pre-treatment with Botulinum Toxin Type A (BTX-A) may offer a promising new approach to preventing thrombosis before microvascular procedures, particularly in cases like crush injuries where limb salvage is critical. A controlled clinical trial is necessary to definitively determine whether BTX-A can improve the success rate of saving severely injured and acutely ischemic digits. Patients at higher risk of vasospasm or thrombosis, such as those who smoke heavily, have diabetes, or experience impaired kidney function, would be ideal candidates for evaluating the potential benefits of BTX-A injection in this context.

Our findings align with previous research, such as the work of Schweizer et al., which demonstrated a substantial increase in blood flow within 24 hours of administering botulinum toxin type A to dorsally based pedicled flaps. Their study showed an impressive improvement, with blood flow increasing up to 187% above baseline measurements by day 5, as measured by laser Doppler. This further supports the potential benefits of botulinum toxin in enhancing blood flow in these delicate procedures.

**In the present study**, the incidence of thrombosis in anastomosed femoral arteries one hour after ice packs cold challenge test was significantly lower in the femoral arteries pretreated with botulinum toxin type A when compared to the contralateral femoral arteries pretreated with normal saline. Also, the anastomosed femoral arteries pretreated with botulinum toxin type A showed significantly wider diameter when compared to the contralateral anastomosed femoral arteries pretreated with normal saline one hour after ice packs cold challenge test. In a rat model, Park et al. [13] also found reduced rates of micro anastomotic vasospasm and thrombosis, which is consistent with our findings. The results of Schweizer et al. [12] have been corroborated by studies conducted by Roh et al. [9] and Park et al. [13]. Their rat micro anastomosis models showed an increase in peak mean blood flow velocity and anastomotic vasodilation.

In agreement with our study Hassanpour et al. [14] stated that Pulsatile bleeding without thrombosis was observed in all arteries that were injected with BTX-A. With the exception of one with bleeding, thrombosis without bleeding occurred in arteries injected with normal saline. The findings demonstrated the effectiveness of BTX-A and suggested its possible application in microvascular trauma.

Several vasodilatory mediators, including VEGF, CD31, and inducible nitric oxide synthase, are thought to be related with an increase in expression following botulinum toxin type A treatment, according to research by Kim et al. [15].

The beneficial effects of BTX-A on flap viability were achieved with a low dose of ethanol, according to Bas et al. [5]. Compared to BTX-A, a low concentration of ethanol improves blood flow, angiogenesis, and flap viability more in the first week after surgery, suggesting it may be an alternate agent to consider for perioperative use.

The BTX may have improved distal vascular flow and decreased the likelihood of thrombosis by increasing microcirculatory volume and decreasing peripheral resistance, according to Schweizer et al. [12].

A larger number of arterioles, venules, and capillaries were observed in the treated region following BTX-A injection, according to Roh et al. [9]. Blood vascular endothelial growth factor (VEGF) is a potent inducer of this process. So, there are a number of ways in which the BTX-A flap might help boost flap survival rates. A dependable method for decreasing the rate of vascular problems and



enhancing survival in both pedicled and free flaps was demonstrated by BTX-A injection. Overall, the flap survival rate improved by 24.1%. It was found that no complications occurred.

Future studies should assess the use of free tissue transfer has increased considerably in the field of complex defect reconstruction. After having a significant result about effect of botulinum toxin type A injection in prevention of post anastomotic thrombosis in femoral arteries we suggest performing it on human being to detect its efficacy.

### CONCLUSION

The BTX-A has a significant role in enhancing vascular outcomes. The findings suggest that BTX-A injections can effectively reduce the incidence of thrombosis by promoting vasodilation and improving blood flow in the femoral arteries. This effect is attributed to the selective suppression of sympathetic neurons, which helps in maintaining better perfusion and reducing thrombotic events. Overall, the study highlights the potential of BTX-A as a therapeutic intervention for preventing vascular complications post-surgery, paving the way for further research and potential clinical applications in humans.

**Conflict of Interest or financial disclosure:** No potential conflict of interest to be reported by the authors.

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#### Figure legend

Fig. (1): (A): Diameter of right side femoral artery in 350 gm male albino rat measuring 0.65 mm , 7 days after the injection of botulinum toxin type A, (B): Diameter of left side femoral artery in 350 gm male albino rat measuring 0.60 mm , 7 days after injection of normal saline, (C): Diameter of right side femoral artery in 250 gm male albino rat measuring 0.55 mm , 7 days after injection of botulinum toxin type A, (D): Diameter of left side femoral artery in 250 gm male albino rat measuring 0.45 mm , 7 days after injection of normal saline, (E): Diameter of right side

femoral artery in 300 gm male albino rat measuring 0.60 mm , 7 days after injection of botulinum toxin type A, (F): Diameter of left side femoral artery in 300 gm male albino rat measuring 0.55 mm , 7 days after injection of normal saline, (G): post anastomotic diameter of right side femoral artery in 350 gm male albino rat measuring 0.65 mm ( 7 days after injection of botulinum toxin type A ) .. better vessel quality , wider lumen and easier anastomosis, (H): Post anastomotic diameter of left side femoral artery in 350 gm male albino rat measuring 0.60 mm ( 7 days after injection of normal saline ) .. narrower lumen , poor vessel quality and more difficult anastomosis, (I): Post anastomotic diameter of right side femoral artery in 250 gm male albino rat measuring 0.55 mm ( 7 days after injection of botulinum toxin type A ) .. better vessel quality , wider lumen and easier anastomosis, (J): Post anastomotic diameter of left side femoral artery in 250 gm male albino rat measuring 0.40 mm ( 7 days after injection of normal saline ) .. narrower lumen , poor vessel quality and more difficult anastomosis, (K): Post anastomotic diameter of right side femoral artery in 300 gm male albino rat ( 7 days after

injection of botulinum toxin type A ) .. better vessel quality , wider lumen and easier anastomosis, (L): post anastomotic diameter of left side femoral artery in 300 gm male albino rat measuring 0.50 mm ( 7 days after injection of normal saline ) .. narrower lumen , poor vessel quality and more difficult anastomosis.

Fig. (2): (A): Diameter of right side femoral artery in 350 gm male albino rat measuring 0.65 mm ( 1 hr after exposure to ice pack cold challenge test ), (B): Diameter of left side femoral artery in 350 gm male albino rat measuring 0.55 mm ( 1 hr after exposure to ice pack cold challenge test ), (C): Diameter of right side femoral artery in 250 gm male albino rat measuring 0.50 mm ( 1 hr after exposure to ice pack cold challenge test ), (D) Diameter of left side femoral artery on 250 gm male albino rat measuring 0.40 mm ( 1 hr after exposure to ice pack cold challenge test ), (E): Diameter of right side femoral artery in 300 gm male albino rat measuring 0.60 mm ( 1 hr after exposure to ice pack cold challenge test ), (F): Diameter of left side femoral artery in 300 gm male albino rat measuring 0.45 mm ( 1hr after exposure to ice pack cold challenge test ).

### Citation

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