ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Effect of Botulinum Toxins Injection on Liver Enzyme and Histochange of Liver and Kidney of Female Rats

Sameera Jameel, Azhar Imran Majeed Alawadi, Rana Talib Al-Muswie*

Dentistry College, Thi-Qar University, Iraq.

***Corresponding Author: Rana Talib Al-Muswie**

Abstract

The present work aimed to study the effect of Botulinum toxins on liver enzyme ,Urea ,Creatinine and histological change of liver and kidney .Two doses 0.1 and 0.2 ml of Botulinum toxins were used and the animals were injected Intramuscular administration of botulinum toxin for 1 day as two dose only. The alanine aminotransferase (ALT) level in the rats of the control group (A) is significantly lower ($P<0.05$) than both the rats group (B) treated with Botulinum toxins and group(C). The aspartate aminotransaminase (AST) level in the rats of the control group is significantly lower ($P<0.05$) than both the rats treated with Botulinum toxins. Also, The results showed a significant increase in the level of urea and Creatinine of the female rats treated with Botulinum toxins compared with control group, In the histologicaly, the results showed vaculation in hepatocytes, and infiltration of inflammatory cells in liver of group (B), while in group (C), there was inflammatory cells, detachment of some hepatocytes, and congestion in area of Portia,. Kidney of group B showed congestion of blood cells and increase number of nuclei. In kidney of group C there was an increase in the number of nuclei, edematous glomerulus, and glomerular lobulation.

Keywords: *Botulinum toxins; Liver Enzyme, Urea, Creatinine, Liver, Kidney.*

Introduction

Botulinum toxins now play a very significant role in the management of a wide variety of medical conditions, especially focal dystonias and strabismus, hemifacial spasm, headaches [1], and various spastic movement disorders, hyperhidrosis [2], hypersalivation [3], and some chronic conditions that respond only partially to medical treatment. Botulinum toxin, one of the most poisonous biological substances known, is a neurotoxin produced by the bacterium *Clostridium botulinum* [4].Currently it is used in almost every sub-specialty of medicine .The use of Botox (Botulinum toxin-A) for the cosmetic.

BTX use was mainly confined to correct muscles of facial expression over the upper one-third of the face. Botulinum toxin induces weakness of striated muscles by inhibiting transmission of alpha motor neurones at the neuromuscular junction [5].Several studies have demonstrated that BTX-A enhanced the blood flow and improved ischemia in animal models [6]. Botulinum toxins act at four different sites in the body: Junction of the neuromuscular, postganglionic parasympathetic nerve endings, autonomic ganglia and postganglionic sympathetic nerve endings that release acetylcholine [7].Sometimes it can be used as an alternative to surgical intervention [8]. It is generally believed that the adverse effects of BoNT are directly related to dosage [9].Capillary uptake is more likely when using larger doses of toxin and/or larger volumes [10]. In this study, we examined the side effect for BTX-A on liver Enzyme, creatinne and urea .Also histochange in liver and kidney when used two different doses of Botox.

Materials and Methods

Experimental Design

Female rats (190--200 g), were obtained from the animal house of University of Thi-Qar, Iraq, College of Science, Biology Department. The animals were held in an air conditioned room (22 ± 3°C) with 55 ±5% humidity and a 12-hour light/dark cycle.

The animals were housed in a well ventilated 12 hrs light and 12 hrs dark cycles. They were fed with a standard diet and had free access to water. All animals were treated in accordance to the principles of laboratory animal care. Thereafter, the rat were randomly divided into three groups, control (n=10) and experimental (n=20) groups. The control group (G1) just received 8mldistilled water daily. However, the experimental groups split into three groups each included ten rats:

- The first group (control group) was injected with distilled water daily.
- The second group was injected with (0.1ml/animal/day) of Botulinum toxins.
- The third group was injected with (0.2ml / animal/day) of Botulinum toxins.

Blood Collection

The rats were sacrificed and put in chloroform and the blood samples collected by cardiac puncture and placed in appropriately labeled bottles for the various assays for aspartate aminotransferase test and alanine aminotransferase test. Creatinine was measured according to the method of [11] the used reagents were supplied by (Biolabo france). Liver and Kidney, were excised then fixed in buffered neutral formalin for 48 hours. Following the fixation procedure the tissues were dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax, (5-6) µm thick sections were obtained by arotary microtome. These sections were stained with Harris hematoxylin and eosin [12].

Statistical Analysis

Standard analysis of the data of different studied groups was performed using the computerized statistical program: The SPSS program (Statistical Program for Social Sciences).The results were expressed as mean± S.E. Analysis of variance (ANOVA) was used to compare the results of different groups. The differences are considered to be significant at the level 0.05($P \leq 0.05$).

Results

The results showed the alanine aminotransferase (ALT) level in the rats of the control group(A) is significantly lower ($P<0.05$) than both the rats group(B) treated with Botulinum toxins at dose 0.1 ml and group(C) dose 0.2 ml. The aspartate aminotransaminase (AST) level in the rats of the control group is significantly lower ($P<0.05$) than both the rats treated with Botulinum toxins. (Table1).

Also, The results showed a significant increase ($p<0.05$) in the level of urea and Creatinine of the female rats treated with Botulinum toxins **(**BOTOX) at dose 0.1ml and dose 0.2 ml (Table2).

Table 1: Effect of Botulinum toxins on liver enzyme in female rats

Animal groups	ALT(UL)	AST(UL)
First group	32.12± 0.34 ^{c}	63.65± 2.76 ^{c}
Second group	48.65± 0.65^{b}	87.21± 1.24^{b}
Third group	58.65± 0.543^{a}	98.64± 1.64 ^{a}
LSD	6	8

Values are means ± S.E
Different letters refer to significant differences at ($p<0.05$)
Same letters refer to no significant differences at ($p<0.05$)

Table 2: Effect of Botulinum toxins on Urea and Creatinine levels of female rats

Animal groups	Urea (mg/dL)	Creatinine (mg/dL)
First group	38.37± 0.54^{b}	0.81± 0.06 ^{c}
Second group	40.60± 0.30^{a}	0.90± 0.02^{b}
Third group	41.36± 0.41^{a}	0.96± 0.05^{a}
LSD	1.2	0.05

- Values are means ± S.E.
- Different letters refer to significant differences ($p<0.05$).
- Same letters refer to no significant differences ($p<0.05$).

Histological Change

In control group (A), the liver cellularity was within the normal limits (Fig. 1). In the animals which treated with low dose 0.1ml of toxin of butulinum, there was vaculation in hepatocytes, and infiltration of inflammatory cells in liver of group (B) (Fig. 2)., while in group (C) The animals treated with high dose 0.2 ml of toxin of butulinum also revealed congestion in the area of Portia, detachment of some hepatocytes and inflammatory cells, (Fig.3). (Figure 4) shows section for normal

kidney (especially cortical tubules and glomeruli) with normal limits from the control animal group (A). Kidney of group (B) dose 0.1ml toxin of butulinum showed congestion of blood cells and increase number of nuclei (Fig.5).

But after administrated dose0.2 ml of toxin of butulinum, the lesions were showed more hyalinization of some renal tubules, patches of hemorrhage, and inflammatory cells mainly mononuclear cells (Fig. 6).

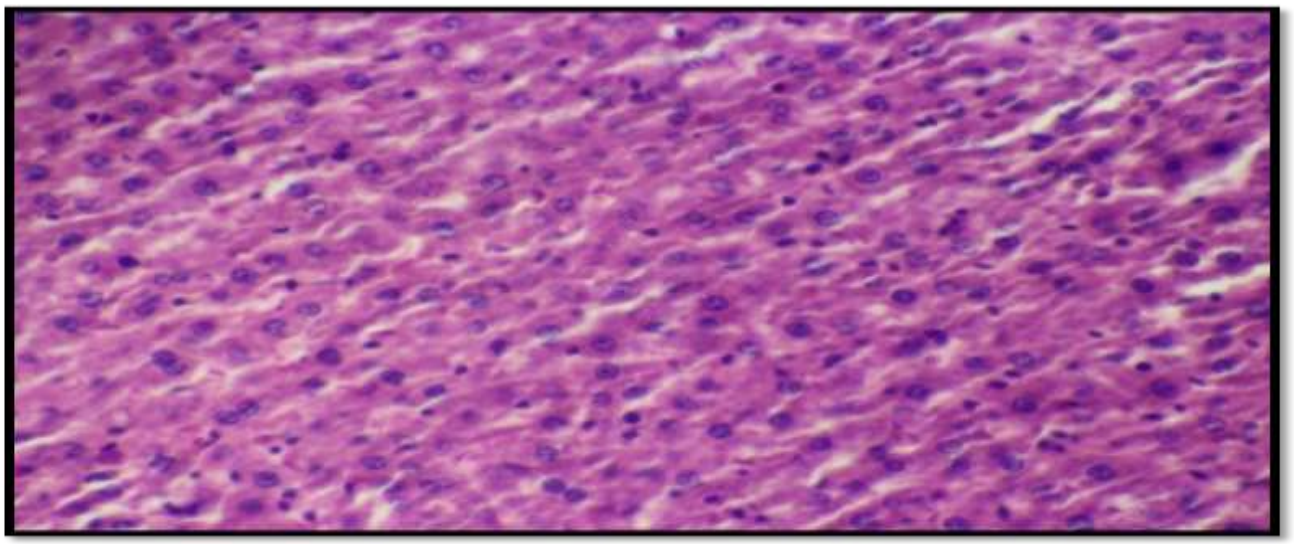

Fig. 1: Histological sections of liver stained with (H & E), examined under microscope (x200). Liver untreated normal section considered as control group

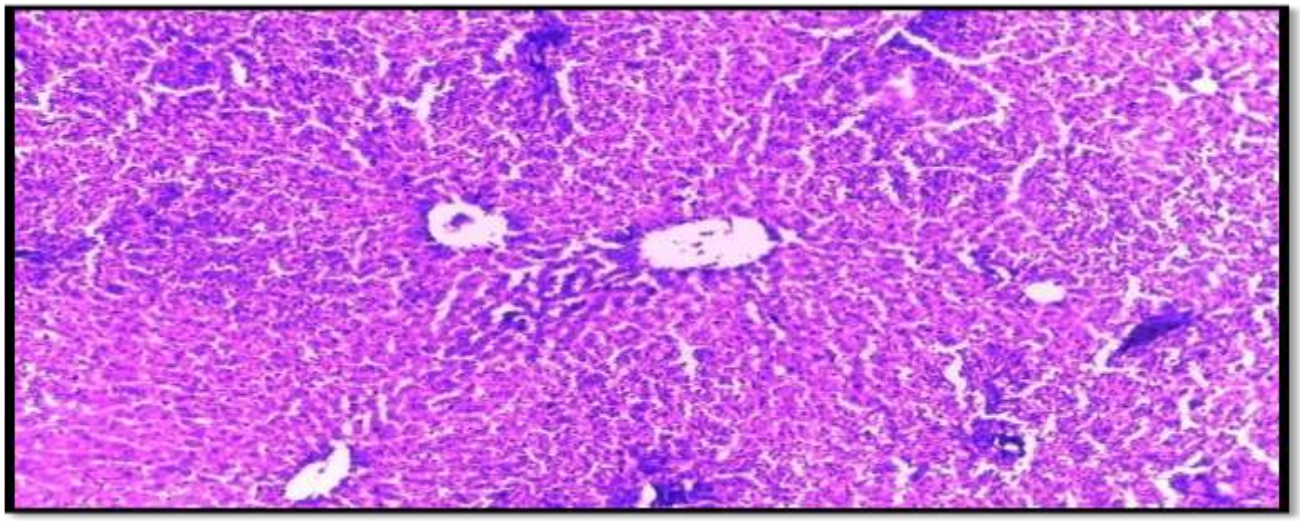

Fig.2: Section of the liver from the animals in group treated with 0.1ml toxin of butulinum. Vaculation in hepatocytes and infltration of inflammatory cells (H&E) 200 x

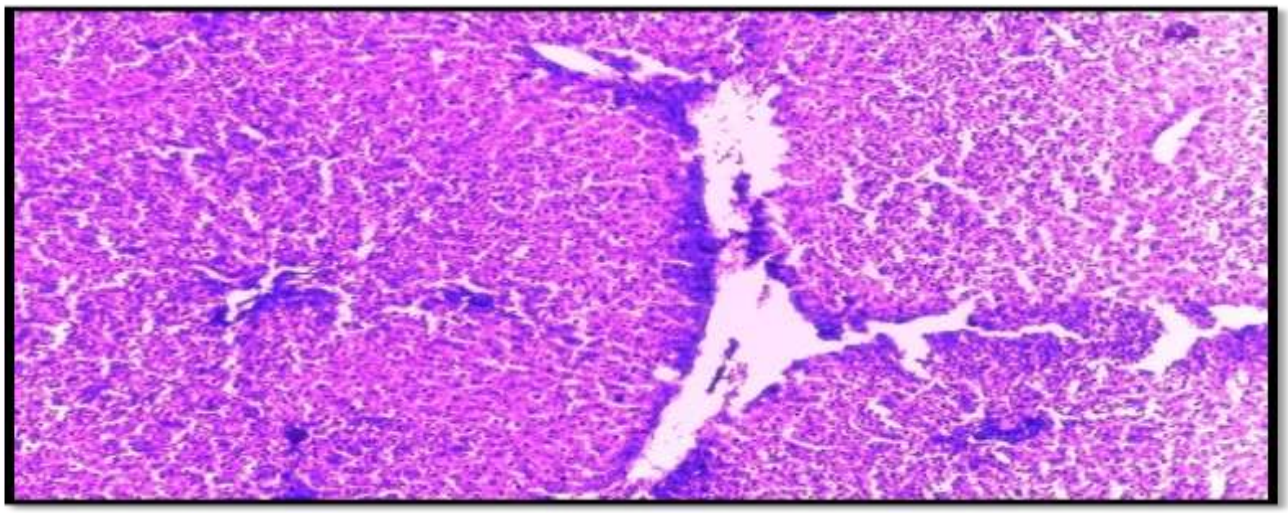

Fig.3: Section of the liver from the animals in group treated with 0.2 ml 0.1ml toxin of butulinum shows congestion in area of Portia, detachment of some hepatocytes and infltration of inflammatory cells and vaculation in hepatocytes. (H&E) 200x

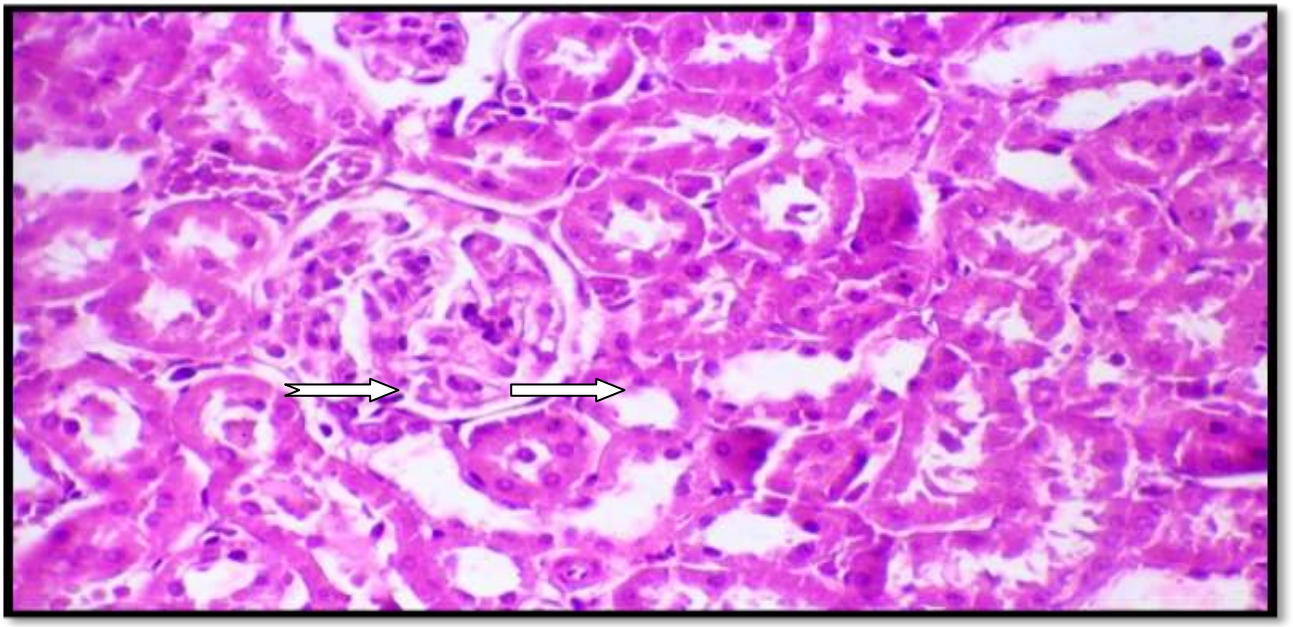

Fig.4: Section on kidney from control rats showed the glomnerulus ⇨ cortical tubules ⇨ within Normal limits (H&E) 800x

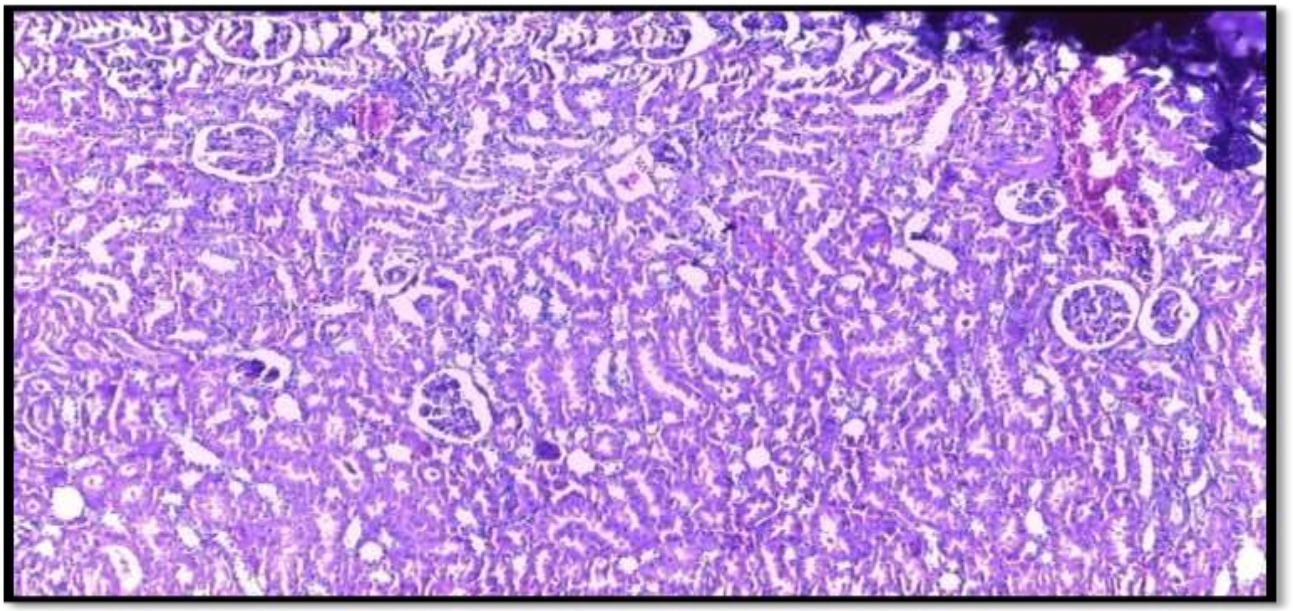

Fig.5: Section of the kidney from the animals in group B treated with 0.1ml toxin of butulinum. (H & E 200X) (EG = edematous glomerulus, LG = glomerular Lobulation, and *N* = Increased numbers of nuclei)

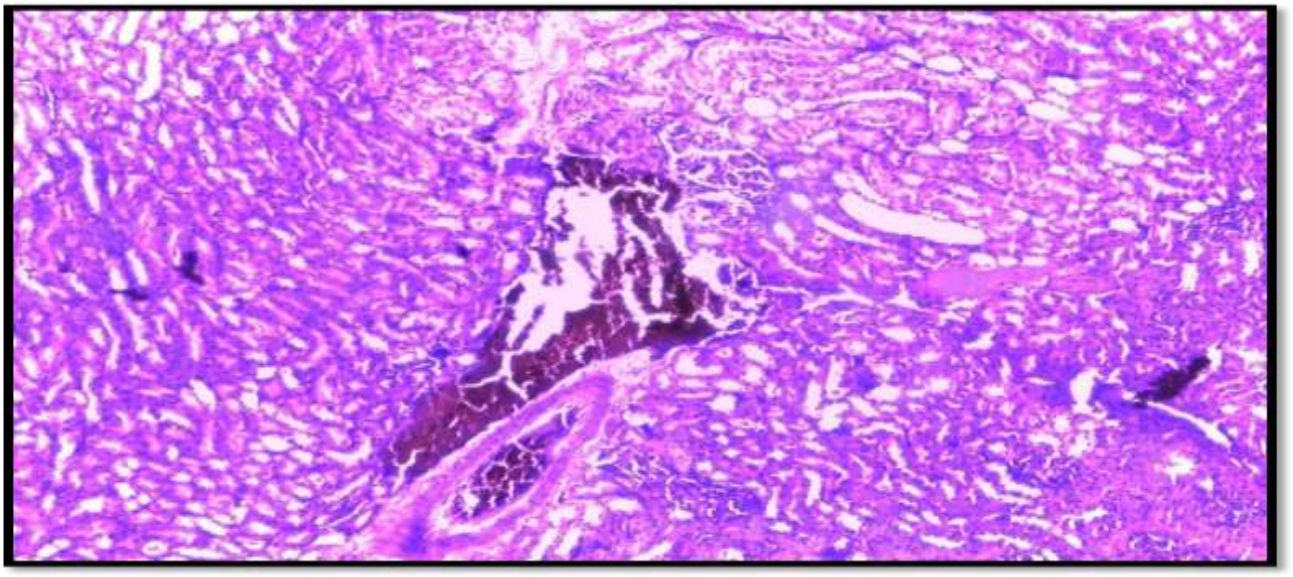

Fig.6: Section of the kidney from the animals in group C treated with 0.2 ml toxin of butulinum). (H & E 200X (G = Swollen Glomerulus, *N* = Increased numbers of nuclei, Haemorrhage

Discussion

The results of this study regarding the use of high dose of Botulinum toxins showed an effect on the liver enzyme and functions of kidney also effect of the histological level of both liver and kidney, we found increased in ALT and AST in the third group compared with second group and control group .Injection with botulinum toxin are generally well tolerated and side effects are few. In our study we had been used high dose from Botox and observed the results at animals groups, the side effect showed in liver enzyme and kidney function .In another study for researchers have been found, development of neutralizing antibodies has also been described [13].

To minimize the risk of antibody formation, use of the lowest dose at the longest feasible interval is advised. The critical factors resulting in antibody formation are unknown, but there may be an increased risk of antibody formation with higher and/or more frequent doses. If antibodies to BTX A develop, BTX could be tried, as there is no cross-reactivity between the 2 toxin types [13, 14]. And more dilute concentrations may decrease the incidence of adverse effects in this patient population.

Careful attention to drug dose, dilution, site of injection is required for optimal outcome. Adverse events associated with the use of BoNT are significantly higher in patients with an underlying systemic disease [15]. Migration of the toxin from CSF to blood through the arachnoid villi causing systemic toxicity was suggested as a possible mechanism. In the literature, there is only one study which evaluated in vivo central toxicity of BoNT in mice by injecting it into cerebrospinal fluid (CSF) [16].Toxicity of botulinum neurotoxins in central nervous system of mice. However, the literature on BoNT-induced muscle atrophy as an adverse reaction of chronic, repeated injections is sparse Some animal studies have focused on the atrophy-inducing effects of BoNTs [17,18].

Another researcher group also conducted an animal study of male albino rats, received BoNT-A [19],the findings of the study suggested that BoNT-A induced relaxation of the surrounding smooth muscles as evidenced by the difference in resting sinusoidal diameter between the treatment and control groups.

In present study, the change of structure of histological for liver and kidney tissue this change the function of organs as founding in our research agree with [17, 18], the results showed a statistically significant larger mean resting sinusoidal diameter in the two treatment when however, only a few case reports or case series in humans are available describing muscle atrophy as either a wanted or untoward effect .Protective effect of botulinum toxin A after cutaneous ischemia-reperfusion injury.

Although commercially available preparations of BoNT have an excellent safety profile, especially for cosmetic purposes [20].In the present study, the side effect in results for effect high doses of botulinum toxins injection. The mechanism of these adverse effects is not well understood, but it may represent an immunologically-mediated reaction to the foreign proteins in BoNT preparations [21].

References

1. Relja MA, Korsic M (1999) Treatment of Tension type headache by injection of botulinum toxin type A: Double blind placebo-controlled study. Neurology, 52: 203.
2. Shelley WB, Talanin NY, Shelley ED (1998) Botulinum toxin therapy for palmar hyperhidrosis. J. Am Acad. Dermatol., 38: 227-9.
3. Bhatia KP, Münchau A, Brown P (1999) Botulinum toxin is a useful treatment in extensive drooling of saliva. J. Neurol. Neurosurg. Psychiatry, 67: 697-9.
4. P K Nigam, A Nigam (2010) Botulinum toxin. Indian J. Dermatol., 55(1): 8-14.
5. Priori A, Berardelli A, Mercuri B, Manfredi M (1995) Physiological effects produced by botulinum toxin treatment of upper limb dystonia: Changes in reciprocal inhibition between forearm muscles. Brain, 118: 801-7.

6. Akihiko Uchiyama, Kazuya Yamada, Buddhini Perera, Sachiko Ogino, Yoko Yokoyama, Yuko Takeuchi, Osamu Ishikawa, and Sei-ichiro Motegi (2015) Protective effect of botulinum toxin A after cutaneous ischemia-reperfusion injury. Sci. Rep., 5: 90-72.
7. Sellin LC (1985) The pharmacological mechanism of botulism. Trends Pharmacol. Sci., 6: 80-2.
8. Boyd R, Graham HK (1997) Botulinum toxin A in the management of children with cerebral palsy: Indications and outcome. Eur. J. Neurol., 4: 15-S22.
9. Bakheit AM, Ward CD, McLellan DL. (1997) Generalised botulism-like syndrome after intramuscular injections of botulinum toxin type A: a report of two cases. J. Neurol. Neurosurg. Psychiatry, 62(2):198.
10. Crowner BE, Torres-Russotto D, Carter AR, Racette BA. (2010) Systemic weakness after therapeutic injections of botulinum toxin a: a case series and review of the literature. Clin Neuropharmacol., 33(5):243-7.
11. Tietz NW (1999) Text book of clincle chemistry.3 Ed. C. A. Buurtis,. R. Ashwood, W.B. Saundersp. 1239-1241.
12. Fischer AH, Jacobson KA, Rose J, Zeller R (2008) Hematoxylin and eosin staining of tissues and cell sections. CSH Protoc., 10: 2008.
13. Tampa, Fla: (2007) Gold Standard, Inc. Clinical Pharmacology [database online] Available at: http:// www. Clinical pharmacology.com. Updated November 2007.
14. Montvale NJ (2007) 61st ed. Thomson PDR;. Physicians' Desk Reference.
15. Tremaine AM, McCullough JL (2010) Botulinum toxin type A for the management of glabellar rhytids. Clin Cosmet Investig. Dermatol., 3: 15-23.
16. Luvisetto S, Rossetto O, Montecucco C, Pavone F (2003) Toxicity of botulinum neurotoxins in central nervous system of mice. Toxicon., 41(4):475-81.
17. Chen C, Stott NS, Smith HK, Stott NS (2002) Heather, K. Effects of botulinum toxin A injection and exercise on the growth of juvenile rat gastrocnemius muscle. J. Appl. Physiol., 93: 1437-1447.
18. Han K, Joo Y, Moon S, Kim K (2006) Botulinum toxin A treatment for contouring of the lower leg. J. Dermatol. Treat., 17: 250-254.
19. Metz HS, Dickey CF (1991) Treatment of unilateral acute sixth-nerve palsy with botulinum toxin. Am J. Ophthalmol., 112: 381-384.
20. Yashar Ilkhchoui, Ramsis F, Ghaly N, Nick Knezevic, Kenneth D Candido (2013) Central Nervous System Toxicity after Botulinum Neurotoxin Injection. Anesth Pain Med. Summer, 3(1): 223-225.
21. Baizabal-Carvallo JF, Jankovic J, Pappert E (2011) Flu-like symptoms following botulinum toxin therapy. Toxicon., 58(1): 1-7. doi: 10.1016/j.toxicon.2011.04.019.

