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The Role of Vitamin C against Structural Changes in Testis of Male Albino Rats Induced by Tramadol and its Withdrawal

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Abstract:

Nowadays tramadol misused and abused is wide spread mainly among young and middle-aged people, especially males as it used in management of premature ejaculation and for orgasm extension. However, these patients are susceptible to many problems after long term administration of tramadol including histological changes on testis. This study is designed to study the effect of tramadol on the histological change of testis and its withdrawal effect with the possibility of vitamin C to reverse these changes. Fifty adult male albino rats were used in this study. Animals were divided into five equal groups: Group A (control group), rats were injected with distilled water. Group B (treated with tramadol only)(TGI), rats were injected with tramadol (50mg/kg b.wt.). Group C (treated with tramadol + vitamin C) (TGII), rats were injected with vitamin C (100mg/Kg/b.wt.) half hour prior to tramadol injection in the same dose as in TGI. These animals were injected intraperitoneally (I.P.) for 4 wks. Group D (treated group B withdrawal) (TGI WD), rats treated as group B then left for two wks. as withdrawal group. Group E (treated group C withdrawal) (TGII WD), rats treated as group C then left for two wks. as withdrawal group. At the end of the experiment, the rats were killed and testis was removed and preserved in a fixative and processed to get paraffin wax block and prepared for microscopic examination. The results showed histological changes in the tramadol treated rats were irregularity and atrophy of S.T. with widening of the interstitisum along with arrest of spermatogenesis at level of spermatid in most of seminiferous tubules (S.T.) while in others it arrested at level of spermatocyte with exfoliated germinal epithelium in the lumens of most tubules. Vacuoles are present in cytoplasm of spermatocytes with detachment of degenerated germinal epithelial cells from basement membrane, the interstitisum has been shown vacuolated homogenous acidophilic material. After adding of vitamin C in Group C (TGII), the histological findings have been shown improvement in the histological structure of testis. In both withdrawal groups (Group D &E), there were signs of improvement in the S.T. mainly in group E that appeared somewhat like normal. This study was concluded that tramadol caused structural changes in testicular tissues with poor reversibility following withdrawal but adding vitamin C has role in alleviated the histological changes and accelerated the reversibility.

Key Words: Tramadol, vitamin C, Testis, withdrawal

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دور فيتامين سي ضد التغيرات الهيكلية في خصية ذكور الجرذان البيضاء التي يسببها الترامادول وسحبها

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الخلاصة

في الوقت الحاضر ينتشر إساءة استخدام الترامادول وإساءة استخدامه على نطاق واسع بشكل رئيسي بين الشباب ومتوسطي العمر، وخاصة الذكور لأنه يستخدم في إدارة سرعة القذف وإطالة النشوة الجنسية. ومع ذلك، فإن هؤلاء المرضى عرضة للعديد من المشاكل بعد تناول الترامادول على المدى الطويل بما في ذلك التغيرات النسيجية في الخصية .هذه الدراسة صممت لدراسة تأثير الترامادول على التغير النسيجي للخصية وتأثيره على الانسحاب مع إمكانية عكس هذه التغيرات لفيتامين سي. تم استخدام خمسين من ذكور الجرذان البيضاء في هذه الدراسة. قسمت الحيوانات إلى خمس مجموعات متساوية: المجموعة) Aالمجموعة الضابطة)، حقنت الفئران بالماء المقطر. المجموعة B (عولجت بالترامادول فقط (TGI)(، تم حقن الجرذان بالترامادول (50 مغ/ كغ من وزن الجسم). المجموعة C (عولجت بالترامادول + فيتامين سي (TGII) (، تم حقن الجرذان بفيتامين سي (100 مغ/ كغ من وزن الجسم) قبل نصف ساعة من حقن الترامادول بنفس الجرعة كما في المجموعة B ، وحقنت جميع الحيوانات في غشاء البريتون ولمدة 4 أسابيع.

المجموعة) D انسحاب المجموعة B المعالجة)، تم معاملة الجرذان كمجموعة B ثم تركت لمدة أسبوعين كمجموعة انسحاب وسميت (TGI WD).المجموعة) E انسحاب المجموعة C المعالجة)، تمت معاملة الجرذان كمجموعة C ثم تركت لمدة أسبوعين كمجموعة انسحاب وسميت (TGII WD) . وفي نهاية التجربة، تم قتل الفئران وإزالة الخصية وحفظها في مثبت ومعالجتها للحصول على كتلة شمع البارافين وتجهيزها للفحص المجهري.

أظهرت النتائج تغيرات نسيجية في الفئران المعالجة بالترامادول مثل عدم انتظام وضمور في الانابيب الناقلة للمني مع اتساع النسيج الخلوي و توقف تكوين النطف عند مستوى الخلية النطفية السبرماتيد في معظم الأنابيب المنوية ، بينما في حالات أخرى توقف عند مستوى الخلايا المنوية السبرماتوسايت مع ظهارة جرثومية مقشرة في تجويف معظم الأنابيب. توجد فجوات في السيتوبلازم للخلايا المنوية مع انفصال الخلايا الظهارية الجرثومية المتحللة من الغشاء القاعدي وظهور مادة حامضية متجانسة محتوية على فجوات في النسيج الخلالي . بعد إضافة فيتامين سي في المجموعةTGII) C) ، أظهرت النتائج النسيجية تحسنًا في البنية النسيجية للخصية. في كلتا مجموعتي الانسحاب) مجموعتي D و (E بعد أسبوعين من انسحاب الترامادول كانت هناك علامات تحسن في الانابيب الناقلة للنطف وخصوصا في مجموعة E وظهر يشبه الشكل الطبيعي إلى حد ما. استخلصت هذه الدراسة ان الترامادول تسبب في حدوث تغيرات هيكلية في أنسجة الخصية مع ضعف الانعكاس بعد الانسحاب ولكن إضافة فيتامين سي له دور في التخفيف من التغيرات النسيجية وتسريع الانعكاس.

الكلمات المفتاحية : ترامادول ، فيتامين سي ، خصية ، انسحاب.

Introduction:

Tramadol is a centrally acting synthetic opioid analgesic that is used broadly for combating moderate to severe pain (Nossaman et al., 2010). Previous studies suggested that its analgesic effects result from opioid and non-opioid mechanisms as its bind to μ opioid receptor and also inhibition of reuptake of noradrenaline and serotonin so its act as antidepressant for example: desimpramine (Gillman, 2005). Tramadol absorbed rapidly orally, that occurs in upper part of small intestine and has extensive tissue distribution. Tramadol bioavailability is 70% and rise to 100% in repeated or multiple doses (Zhang et al., 2014). The metabolized of tramadol done in the liver via cytochrome p450 enzyme system and its bio transformed products are expelled by the kidneys by urine accordingly (Adikwu et al., 2018). Tramadol misuse, abuse and dependence have been progressively reported particularly in young adult's male, in recent times young addicts naturally substituted tramadol for heroin (Lee et al., 2011).

In such patient's recurrent tramadol administration might result in accumulation of toxic metabolites in their bodies, thus increase the risk for pharmacokinetics interactions and or decrease the clearance of tramadol leading to increase its potential for toxicity (Tjaderborn M, 2013).

The physiological level of reactive oxygen species ROS and antioxidants as glutathione peroxidase (GPx), superoxide dismutase (SOD) and malonaldehyde (MDA) are vital for proper function of male reproductive organs as spermatozoa are contained polyunsaturated fatty acid in their wall so, it is vulnerable to the attack by ROS (Chi et al., 2008).

Antioxidants, which enriches the endogenous antioxidant defense systems inside cells (Vigueras et al.,2011), can defend against the injurious effect of ROS on testes and may be of medical value in supported conception procedures (Yousef et al., 2003).

One of these antioxidants is vitamin C which is water-soluble antioxidant, similarly is indirectly defends the cell membranes and lipid-based structures (Padayatty et al., 2003).

for this reason, the present study concentrated on the effect of tramadol on the histological change of testis and its withdrawal effect with the possibility of vitamin C to fight the oxidative stress induced by tramadol.

Material and methods: Fifty adult male Wister albino rats weighing about (220-250) gm. and aged three months were used in this study which done in animal's house in veterinary college / Mosul University from April 2018 to January 2019. Animals were kept at controlled room temperature

(23-25Cº) with a 12 hours' light/ dark cycle and placed in plastic cages using homogenized wood shaving as bedding for acclimatization one week before the experiment. Tramadol and vitamin C ampoules were used in the current study. The animals were divided into five equal groups as following: Group A (control group), rats were injected with distilled water intraperitoneally (I.P.) daily for 4 wks. Group B (treated with tramadol only)(TGI), rats were injected with tramadol (50mg/kg b.wt.) I.P. daily for 4 wks. Group C (treated with tramadol + vitamin C) (TGII), rats were injected with vitamin C (100mg/Kg/b.wt.) half hour prior to tramadol injection in the same dose as in TGI for 4 wks. Group D(treated group B withdrawal) (TGI WD), rats treated as group B then left for two wks. as withdrawal group. Group E (treated group C withdrawal) (TGII WD), rats treated as group C then left for two wks. as withdrawal group. Toward the end of study, the animals were killed and testis were detached and put in bouin's solution for 24 hours then kept in 10% neutral buffered formalin as fixator and processed to get paraffin sections of 5µm thickness, that stained with Hematoxylin and Eosin to be examined by light microscopic.

Histopathological result:

Group A:(control group): Under microscope this group showed normal structural features of rats testis, that be made up of many rounded to oval seminiferous tubules (S.T.) separating interstitisum containing blood vessels together with clumps of Leydig cells, spermatogenic and Sertoli cells forming the lining of each tubule that is surrounded by a basal lamina **(Fig.1).**The cells of spermatogenic linage consist of spermatogonia that appeared as small rounded cells resting on a basement membrane, followed by primary spermatocytes with spherical dark nuclei and early rounded and late elongated spermatids along with sperms that fills the lumen of the tubules in addition to in between the germ cells there was Sertoli cells with triangle nuclei **(Fig.2).**

Group B (treated with tramadol only) (TGI): Microscopically, this group was showed irregularity and atrophy of S.T. with widening of the interstitisum **(Fig. 3)** along with the arrest of spermatogenesis at level of spermatid in most of S.T. with halo appearance of the early spermatid, and exfoliated germinal epithelium in the lumens of S.T. **(Fig.4).** Other tubules the spermatogenesis arrested at level of spermatocytes and vacuoles are present within the cytoplasm of them and dark looking nucleus of spermatogonia along with detachment of degenerated germinal epithelial cells from basement membrane **(Fig.5).** The interstitisum was showed homogenous acidophilic material and vacuoles **(Fig.6).**

Group C (treated with tramadol + vitamin C) (TGII): Histological findings of this group was showed improvement in the structure of testis in which most of S.T. were looked normal without atrophy and less acidophilic homogenous material deposition in interstitisum with normal blood vessels and leydig cells **(Fig.7).** Improved spermatogenic lineage cells and a sperms full their lumen **(Fig.8).**

Group D (treated group B withdrawal) (TGI WD): In this group treated as group B then left for 2wks. as a recovery group, there were signs of mild improvement in the S.T. with increased layers of spermatogenic cell layers and sperms concentration in the lumen of recovered S.T.**(Fig.9).** and the remaining still affected in form of absence of sperms from the lumen with presence of some vacuoles between the spermatogenic cells in addition to, deposition of vacuolated eosinophilic materials in the interstitisum **(Fig. 10).**

Group E (treated group C withdrawal) (TGII WD): In this group treated as group B then left for 2wks. as a recovery group, there was improvement in structural changes when compared to those in TGII and also better than TGI WD as most of S.T. appeared as normal with improvement in spermatogenesis and most of tubules were filled with sperms **(Fig.11)** with normal looking interstitisum **(Fig.12).**

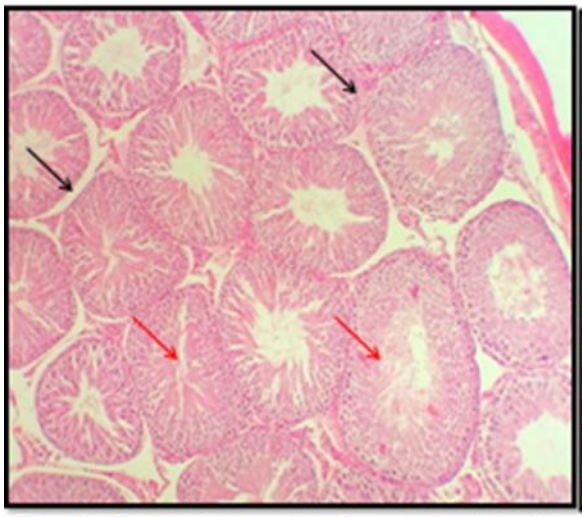

Fig. 1: Photomicrograph of control rat testis group showing well defined S.T. with normal stage of spermatogensis (black arrows),and sperms fill the (red arrows) (H&EX100).

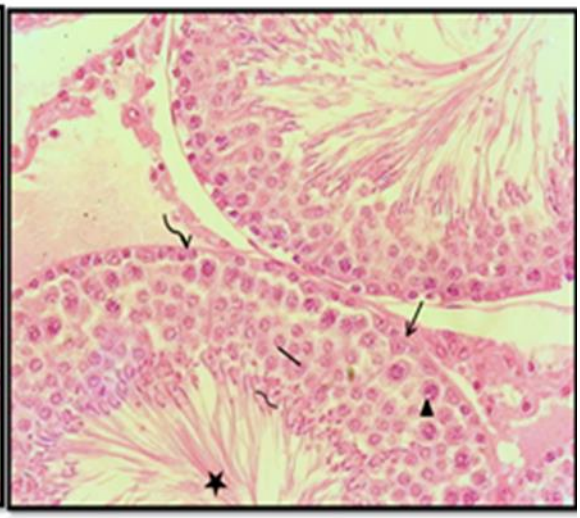

Fig.2: Photomicrograph of control rat testis group showing normal lining germinal epithelial s(blackarrow),spermatogenic cells(curvedarrow), spermatocyte(arrow head), early spermatid(black line),late spermatid(curved line) and spermatozoa (asterisk) (H&EX400).

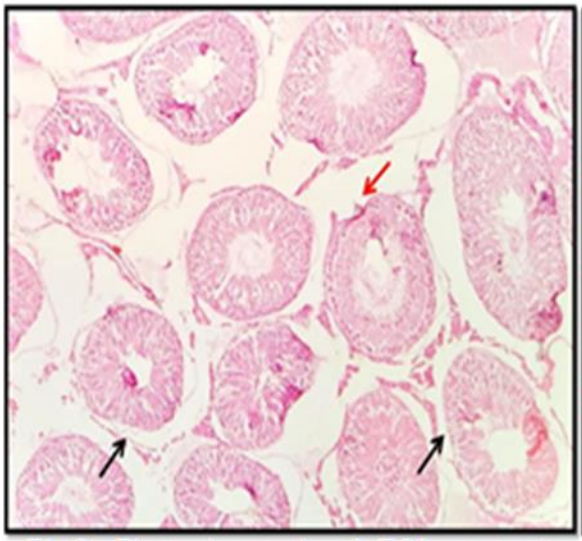

Fig.3: Photomicrograph of TGI rat testis group showing atrophy and disorganization of S.T.(black arrows),and widening of interstitisum(red arrow)(H&EX100).

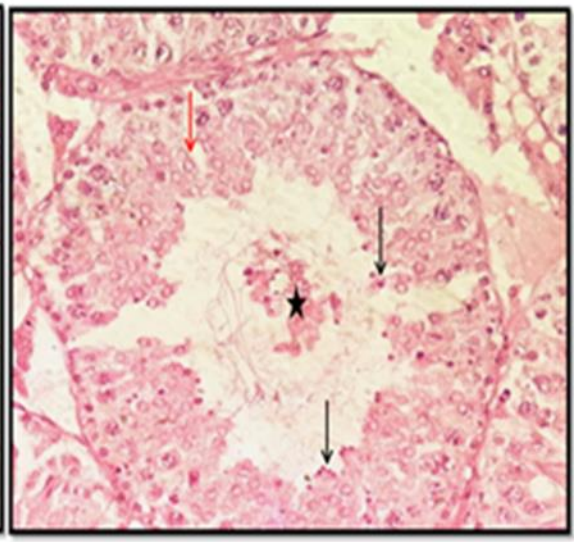

Fig. 4: Photomicrograph of TGI rats testis group showing arrest of spermatogenesis at level of spermatid (black arrow) with halo appearance of early spermatid (red arrow) and exfoliated germinal epithelial cells in the center of tubule (asterisk) (H&EX400).

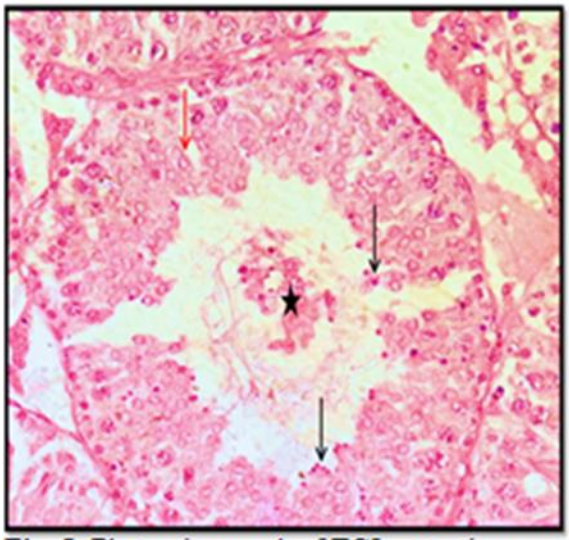

Fig. 5: Photomicrograph of TGI rat testis group showing arrest of spermatogenesis at level of spermatocyte (black arrow) with intracellular space(curved arrow),spermatogonia cells with dark nucleus(red arrow) and degenerated with separation of spermatogenic cells from baseent membrane (asterisk) (H&EX400).

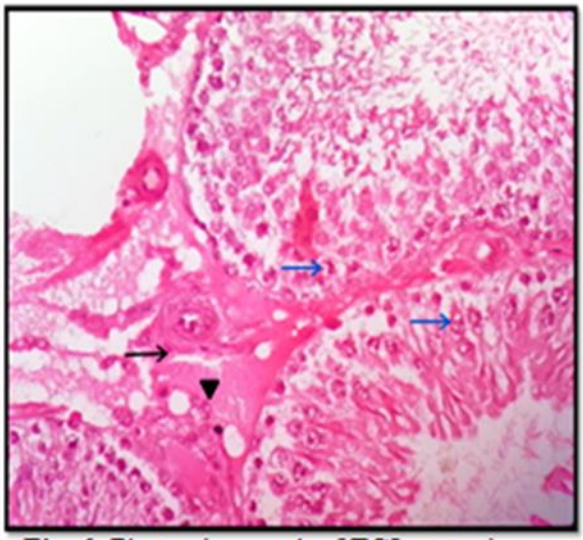

Fig. 6: Photomicrograph of TGI rat testis group showing interstitisum with homogenous acidophilic material (black arrow) ,ledyig cells(arrow head) and disorganization of within germinal epithelial cells intercellular space (red arrow) (H&EX400).

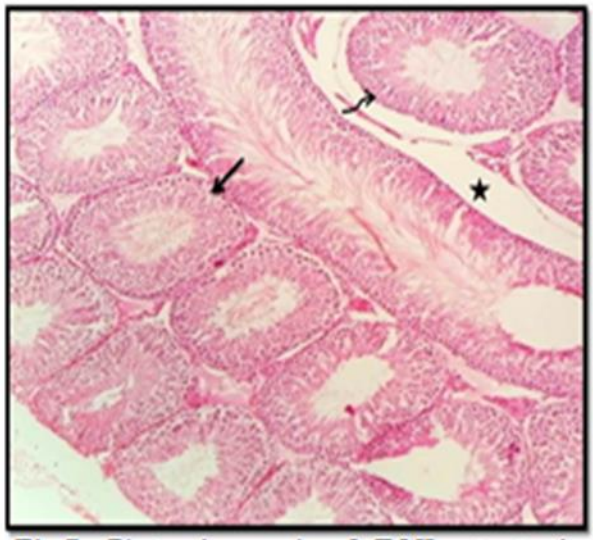

Fig.7: Photomicrograph of TGII rats testis group showing most of S.T. looked with normal spermatogsis (black arrows),some tubule still strophied (curved arrow), with less widening interstitium (asterisk)(H&EX100).

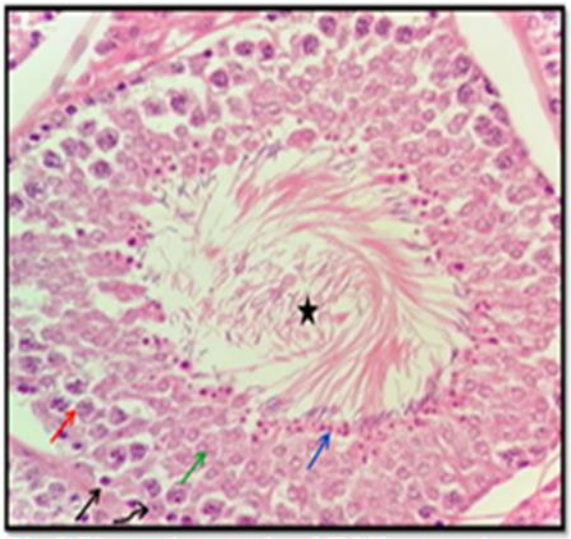

Fig.8: Photomicrograph of TGII rat testis group showing restoring of normal spermatogenic cells epithelium, spermatogonia (black arrow), spermatocyte(red arrow),early spermatid(green arrow),latespermatid(bluearrow),sperms(asteris) and sertoli cell (curved arrow) (H&EX400).

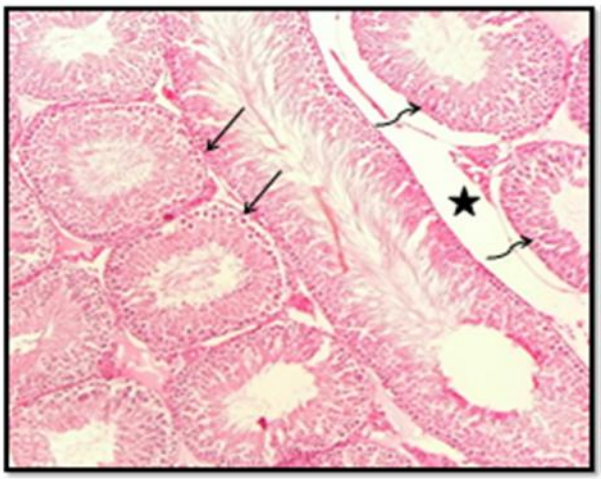

Fig. 9: Photomicrograph of TGIWD rat testis grou pshowing some of S.T. still atrophied(curved arrows) with widening interstitisum (asterisk),others S.T. return normal (black arrows)(H&E X 100).

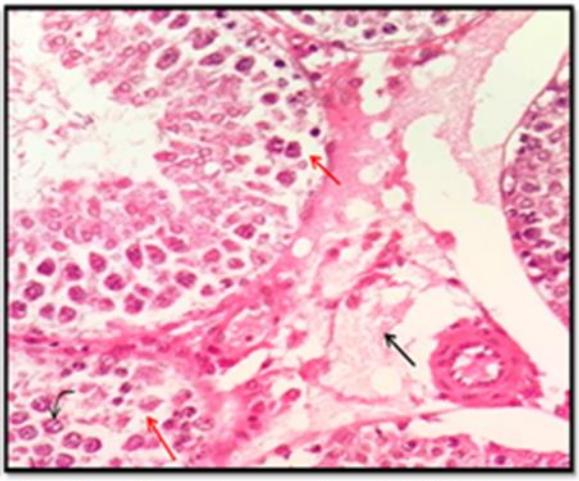

Fig.10: Photomicrograph of TGIWD rat testis group showing wide interstitisum containing vacuolated homogenous acidophilic material (black arrow), some of S.T. still has spaces among germinal cells (red arrow) and vacuolated spermatocyte (curved arrow) (H&E X 400).

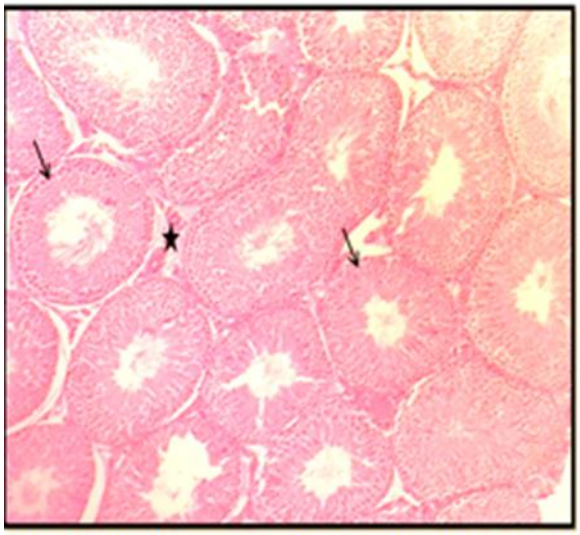

Fig.11: Photomicrograph of TGIIWD rat testis group showing normal arrangement of spermatogenic cells (black arrows) with narrow interstitisum still containing slight acidophilic homogenous materials

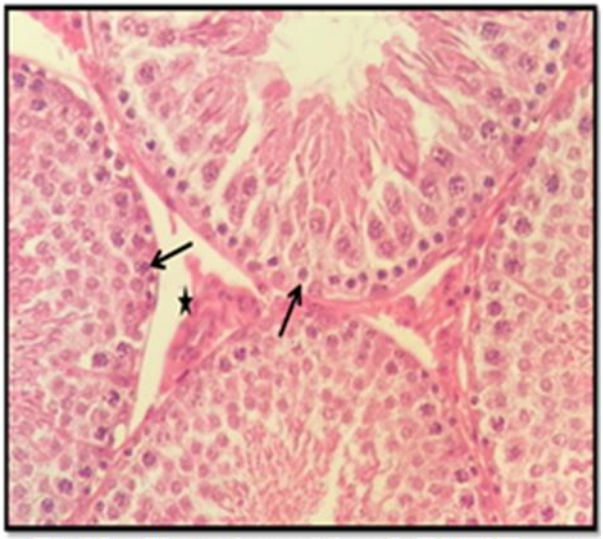

Fig.12: Photomicrograph of TGIIWD rat testis group showing normal interstitisum (asterisk) with normal germinal epithelium of the surrounding tubules(black arrows) (H&EX400).

Discussion:

The wide spread misuse of tramadol among young people, especially males, was strengthened the necessity to study the histopathological changes of long-standing use of tramadol on testicular tissue in adult male albino rats (Youssef & Zidan, 2016).

Treated groups:

Group B (treated with tramadol only) (TGI): Rat's testis that treated with tramadol shown irregularity and atrophy of S.T. and arrest of spermatogenesis along with decrease sperms population and exfoliated degenerated cells in the lumens of S.T. and vacuoles are present in cytoplasm of spermatocytes with dark looking nucleus of spermatogonia.

A similar finding was reported by many researchers (Azari et al., 2014; El−Ghawet, 2015; El Sawy & Abdel Malak, 2015; Soliman et al.,2017) and such histological changes might be explained by increasing lipid peroxidation and accumulation of ROS which leading to cell damage (Ragab & Mohamed, 2017).

Furthermore, Youssef and her colleague were they attributed these findings to oxidative stress and harmful effect of free radicals as the testicular cells and sperms have abundant polyunsaturated fatty acids in their plasma membranes and lipid peroxidation made by tramadol can finally end result in dysfunction and structural damage of cells (Youssef & Zidan, 2016). While Abou El Fatoh et al., (2014) explained the toxicity of tramadol due to lowered sex hormones level in male rats and such decrease in FSH, LH and testosterone might be responsible for toxic effects of tramadol on testis.

The current study showed wide intercellular spaces between the spermatogenic cells. Thus run in agreement with many authors (Azari et al., 2014; El Sawy & Abdel Malak, 2015; Abou Elnaga et al., 2018) which was explained as exposure of spermatogenic cells to ROS causing disturbance of the blood–testis barrier subsequently resulting in passage of toxic agents between the cells and widening of intercellular spaces (Mohamed et al., 2014).

Vacuoles appeared in the cytoplasm of most of spermatogenic cells and Sertoli cells in the current study run in consistent with other studies (Youssef & Zidan, 2016, Abou Elnaga et al., 2018) and such vacuoles were most possibly resulted from mitochondrial swelling and dilatation with vesiculation of the endoplasmic reticulum (Mohamed et al., 2014).

A wide interstitisum between the S.T. with deposition of vacuolated homogenous acidophilic material in the current study was noticed also by other studies (El−Ghawet, 2015; Abdellatief et al., 2015). An explanation for this change is due to excess lymphatic exudate exuding from degenerated lymphatic vessels adding to rise in vascular permeability that resulted from accretion of free radicals and ROS (Salama et al., 2003).

Group C (treated with tramadol and vitamin C) (TGII): After adding vitamin C prior to tramadol injection showed amelioration in the histological changes seen in tramadol treated group. Such improvement in the S.T. structure, includes improved spermatogenesis, increase sperms count within the Lumina of S.T. and decrease deposition acidophilic homogenous material in interstitisum, this improvement were due to fact that vitamin C had a protective effect against tramadol toxicity and such protection were due to antioxidant properties of vitamin C as described by (Muthu & Krishnamoorthy, 2012) who proved vitamin C has protective property against mercuric chloride−induced testicular damage.

Soliman and her colleges were in consistence with the present result as they found administration of rats with (500 mg/kg/day) of vitamin C prior to (50 mg/kg/day) of tramadol orally for 4 wks. has ameliorating role against testicular damage (Soliman et al., 2017). Also, the protective effect of vitamin C against oxidative stress induced by sulfasalazine in rat testicular tissue was reported (Keshavars et al., 2012).

This useful effect of vitamin C might be attributed to the fact that it's a very effective antioxidant, and a good hunter for oxygen free radicals (Valko et al., 2006). Moreover, it's vital in keeping the physiological integrity of testis, epididymis and accessory glands (Yousef et al., 2003).

Withdrawal groups:

Group D (treated group B withdrawal) (TGI WD): This group was treated as TGI then left for two weeks as a recovery period. The current group when compared histologically with TGI group, it was showed improvement and this pointed to oxidative stress induced by tramadol injection were mostly reduced although testicular tissues was not returned completely to normal tissues, but associated with good future of tramadol consumers especially regarding fertility (Ghoneim et al., 2014). Whereas a significant obvious recovery in the function and structure of testis was reported by Azari et al. after treating mice with tramadol for 12 wks. then keeping without treatment (Azari et al., 2014).

In accordance with the current result those reported by Abou Elnaga who reported in rats treated with (40mg/kg b. wt.) of tramadol for 4 and 6 weeks and then left for another 4 weeks without treatment, there was a minimal change in the recovered groups in comparison to treated groups but however, a complete recovery was not obtained (Abou Elnaga et al., 2018; Nna et al., 2017; Ibrahim & Salah-Eldin, 2019).

Group E (treated group C withdrawal) (TGII WD): This group was treated as TGII then left for two weeks as a recovery period. There was improvement in structural changes when compared to those in TGII and also better than TGI WD as most of S.T. appeared like normal with improvement in spermatogenesis and most of the tubules were filled with sperms and normal looking interstitisum, such improvement were due to benefit of using vitamin C which facilitated rapid rescue of the structural tissue and bring them to normal state as vitamin C is a water-soluble antioxidant therefore it be present inside the cells and in the extracellular matrix and hunts free radicals that may damage to existing structures in these locations (Catani et al., 2005). This is supported by Angulo who reported that supplementations of rats with ascorbic acid might resulted in decrease the lipid peroxidation level in the testicular tissue and significantly increased in plasma testosterone along with rise epididymis sperm concentration (Angulo et al., 2011).

Conclusion:

Even though tramadol was stated as very effective in pain reliving, but it has adverse effects on testicular tissue should be kept in attention as proved by current study. Although tramadol long term use, produce toxic effects on testicular tissue with poor reversibility after withdrawal of tramadol but such toxicity and reversibility could be accelerated by the use of vitamin C.

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