IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 1 Ver. I (Jan. – Feb. 2018), PP 82-90
www.iosrjournals.org

The Possible Ameliorative Role of *Anethum Graveolens* Against Tramadol-Induced Testicular Damage in Male Albino Rat

[1]Fawzy M. lashin and Ibrahim M Abo laila[2]
[1-]*Hormone evaluation Dep.*
[2-]*Molecular drug evaluation Dep. National Organization for Drug Control and Research (NODCAR), Giza, Egypt*
Corresponding author: Fawzy M. lashin

Abstract: *Nowadays, tramadol hydrochloride (TM) is becoming abused more popular among teens in most countries worldwide; especially between males. The aim of present study was to investigate the biochemical and histopathological profiles of subchronic model of TM toxicity on testicular functions. Experimental study, Rats was divided into three groups. Group one received saline, group two received oral doses of TM (100 mg / kg b.w), group three received anethum graveolens (AG) two hours before tramadol (NG+TM). The experiment lasted for a one month. The results showed that a significantly decrease in serum testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and cortisol, but elevated prolactin (PRL) and estradiol (E2) in male albino rats after 30 days of TM group. In the same concern 8-hydroxydeoxyguanosine (8-OHdG), protein carbonyl (PC) and MDA levels, as indicators of oxidative stress, were significantly ($P<0.05$) increased in group TM in comparing with control group. Moreover, TM group displayed an increase of tumor necrosis factor-α (TNF-α), and nitric oxide (NO) in the testes tissues and decreased in glutathione-S-transferase (GST) activity and glutathione (GSH). Also, showed a significant increase in acid phosphatase, N-acetyl-β-D-glucosaminidase and β-galactosidase in testes rat, meanwhile Anethum graveolens (AG) administration caused significantly ameliorative in all parameters in comparing to TM group.*

Keywords: *Tramadol, Testis, oxidative damage, Anethum graveolens, Histopathology, rats.*

Date of Submission: 24-12-2017 Date of acceptance: 06-01-2018

I. Introduction

Tramadol (TM) is a centrally acting synthetic opioid analgesic agent, used orally for the treatment of moderate to severe pain. The mechanism of its analgesic action is complex. Most reports suggest that the analgesic activity and other clinical effects of tramadol are a result of opioid and non-opioid mechanisms. Tramadol binds to the μ-opioid receptor, although much more weakly than morphine. It also inhibits the neuronal reuptake of norepinephrine and serotonin as do the antidepressant drugs such as amitriptyline and desimpramine **(Raffa *et al.*, 1992; Raffa, 1996; Dayer *et al.*, 1997; Grond and Sablotzki, 2004; Gillman, 2005).**

Recently it has been revealed that tramadol decreased lipid peroxidation and regulates noradrenalin uptake; therefore, these therapeutic properties are used for the management of ischemia **(Ashrafzadeh Takhtfooladi *et al.*, 2015 b).** It is reported ROS levels decreased after administration of tramadol in myocardial infarction in isolated rat hearts. However, the effects of tramadol on remote testicular injury caused by skeletal muscle ischemia/reperfusion are not clear **(Bilir *et al.*, 2007).**

Tramadol is one of the most important drug that may induce reproductive disorders in the male testicular cell **(Wei *et al.*, 2011),** a common urologic emergency that occur with cuts off the blood supply to the testis. Ischemia -reperfusion (IR) injury is one of the main pathophysiologic conditions, which happens during testicular torsion of the testis **(Wei *et al.*, 2011).** If testicular torsion is not treated within 4 to 6 h, infarction will occur and surgical distortion is currently the only treatment and allows blood reperfusion. However, even in men who have undergone surgical distortion within 4 to 6 h, the ipsilateral testes often become permanently dysfunctional **(Wei *et al.*, 2011).** It seems, the main pathophysiology of testicular distortion is ischemia - reperfusion injury of the testis **(Wei *et al.*, 2011).**

The physiological levels of ROS antioxidants include superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPx) and total antioxidant status (TAS) are essential for proper function of male reproductive organ .On the other hand because of polyunsaturated fatty acids (PUAFAs) content of the spermatozoa, it is vulnerable to the attack of ROS **(Chi *et al.*, 2008).**

Anethum graveolens L. (dill) is used both as a medicine and an aromatic herb and is shown to have therapeutic properties (**Bahramikia and Yazdanparast (2009)** it is used in medicine as an anti-hypercholesterolaemic plant **(Bahramikia, and Yazdanparast (2008)** It has traditionally been used for

gastrointestinal ailments, such as flatulence, indigestion, stomachache colic, and to tract intestinal gas **(Radulescu *et al.* 2010).**

Phytochemical analysis of *Anethum graveolens L*. (dill) plant revealed the presence of alkaloid, carbohydrate, resin, terpenoids, flavonosides, saponin, steroid, tannin, flavanoid and absence of reducing sugar, glycosides, anthraquinone, phlobatanins **(Dahiya and Purkayastha 2012; Pathak *et al*. 2014)**

The leaves of dill are rich in minerals like phosphorus, potassium and magnesium and used in salads and tea. Dill seeds are commonly used for bladder inflammation, liver diseases and insomnia **(Kaur and Arora, 2010).** Furthermore, the dill essential oil has hypolipidemic activity and could be used as a cardio-protective agent in decreasing blood cholesterol **(Hajhashemi and Abbasi, 2008)**. In Europe dill was mentioned as brain tonic in 17th century **(Stannard, 1982).** Dill can also be used as galactagogue and for the treatment of vomiting **(Stavri and Gibbons, 2005; Zargari, 1991)**. Moreover, it is used as an antispasmodic agent, anticonvulsant, anti-emetic and anti-cramp (in children) remedy and also recommended topically as a wound healer **(Naseri *et al*, 2012).**

II. Materials and Methods

Materials

Drug: Tramal (Tramadol HCl), 225 mg capsules, was obtained from Mina-Pharm, Egypt. ***Anethum graveolens L. (dill)*** was obtained from Sigma Chemical Company, USA. All other chemicals used in the present investigation were of bio-analytical grade.

III. Experimental animals

Adult male albino rats (30 animals) weighing between 180 and 200 g were used in the present study. The rats were obtained from the animal house of the National Organization for Drug Control and Research Egypt (NODCAR). The animals were kept under standard laboratory conditions of light/dark cycle (12/12 h) and temperature (20 -25°C) for one week. They were provided with a nutritionally adequate basal laboratory diet. The basal diet consists of casein 10%, cotton seed oil 4%, salt mixture 4%, and vitamin mixture 1%, carbohydrates (sucrose and starch 1:1) 80.8% and choline chloride 0.2% **(American Institute of Nutrition, 1980)**

IV. Experimental design

The first groups, was comprised 10 rats served as control and administered I ml distilled water o.p for a month. **Group II**: The second groups, comprised 10 rats, were administered oral dose of Tramadol HCl suspended in equal to 100 mg /Kg b.wt. /day for a month .**Group III:** The third groups, comprised 10 rats, were administered oral dose of 200 mg/ kg *Anethum graveolens* before received Tramadol HCl two hour. Doses calculated for animals according to **Paget and Barnes, (1964) equation.**

Handling of tissue samples

Rats were sacrificed by cervical dislocation to obtain testes then washed with cooled saline (0.9 %). Organs under investigation were homogenized in ice-cold 1.15% KCl–0.01 M sodium, potassium phosphate buffer (pH 7.4) with a Potter-Elvehjem glass homogenizer to prepare 10 % w/v homogenate. The homogenates were centrifuged at 10,000×g for 20 min at 4°C (**Sigma-3K30, Germany**). The resultant supernatant was used for different enzyme assays.

V. Biochemical Analyses

Methods

Lipid peroxides formation was determined in serum and testes as thiobarbituric acid reactive substances (TBARS). It was determined according to the method of **(Uchiyama and Mihara 1978)**. Glutathione-S-Transferases (GST), the activity of GST was calculated according to **Habig *et al.* (1974).** Determination of Tumor Necrosis Factor-α and 8-Hydroxy Deoxyguanosine (8OHdG)**.** Levels of TNF-α and 8-OHdG were quantified using ELISA kits (AssayPro) and 8-OHdG level was determined by ELISA kit (SunLong Biotech Co,Ltd., China) according to the manufacturer's instructions and guidelines. Measurement of Nitric Oxide (NO) was estimated according to **Wang *et al.* (2002)** using Griess reaction. Briefly, to an aliquot of supernatants, Griess reagent was added and the colored product formed was read at 540 nm. NO was quantified using a standard curve. Protein carbonyls (PC), Levels of PC were determined according to **Levine *et al.* (1990**). Determination of lysosomal enzymes activities: The activity of three lysosomal acid hydrolases, acid phosphatase (ACP), N-acetyl-β-glucosaminidase (β-NAG) and β-galactosidase (β-GAL) have been measured in kidney and testes according to the method described by **Van Hoof and Hers (1968). Luteinizing hormone (LH),** follicle stimulating hormone (FSH), prolactin (PRL), testosterone (Tes.), estradiol (E2) and cortisol (Cor.) were determined using ELISA kits according to manufacture structure. Determination of Blood glucose: Serum glucose concentration was determined using Sentinel kits based on the method of **Trinder,**

(1969). Sperm quality analysis**,** Sperm quality was determined by three parameters: Sperm concentration, motility and vitality. Sperm concentration was analyzed using the haemocytometer. Sperm suspensions from the caudal epididymis were diluted 1:200 with fixative solution (sodium acid carbonate-formaldehyde solution) and counted according to the procedure indicated in the WHO laboratory manual. The diluted samples were put into the counting chamber and the number of sperm was counted using a haemocytometer with improved doubles Neubauer ruling under a light microscope. The sperm concentration was expressed as $\times 10^6$/ml. Sperm motility was analyzed and averaged by counting the motile and non-motile spermatozoa and expressed as the percent motility. To analysis of sperm motility one drop of sperm suspension were placed on the slide and covered with a cover slip and examined under the microscope using ×40 objective. **World Health Organization (1999). Histopathological study.** A testis was fixed in 10% neutral-buffered formalin. The fixed specimens were washed, dehydrated, and embedded in paraffin wax. **Bancroft and Gamble (2002)**

VI. Statistical analysis

The values were expressed as the mean6 SEM for the 10 rats in each group. Differences between groups were assessed by one way analysis of variance using the statistical package for social sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test according to the method outlined by **Snedecor and Cochran (1980).** Significance at P values <0.05 has been given respective symbols in the tables and figures.

VII. Results

The results in **Figure 1** showed, Tramadol (TM) administration (100 mg/ kg) on MDA and TNF-α levels caused a significant increase (*p<0.05*) compare with control group of rats and significant reduced in GST level. While the group of rats received *anethum graveolens* (AG) two hours before Tramadol (200 AG+100TM) showed that ameliorative effect in MDA, GST and TNF-α, compared to group treated TM alone. Also, there were after five ten day's recovery of stopping treatment by tramadol showed that remained significantly different compared to control group of rats. **(Figure1).**

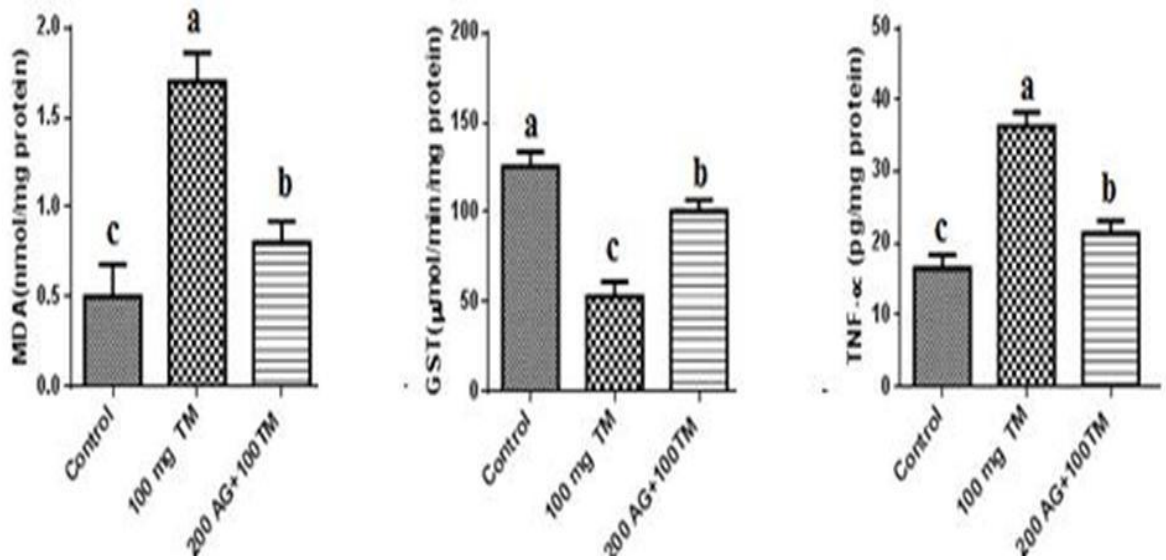


Figure 1: Effect of *anethum graveolens* (AG) (200 mg/kg) on oxidative markers (MDA GST and TNF-α, activity) of rat testes tissue administration of tramadol. Values with the same letters are not significant at (p<0.05) Anethum graveolens L = (AG) Tramadol= (TM).

The data obtained from figure 2 revealed the effect of 100 mg/kg TM induced oxidative stress in testes. Obtained results indicated that TM increased protein carbonyl (PC), 8-hydroxydeoxy guanosine (8-OHdG) and Nitric oxide (NO) compare to control non-treated group. On the other hand group received *anethum graveolens* (AG) two hours before tramadol (200 AG +100TM) showed that ameliorated effect in PC, 8-OHdG and NO compared to group received TM alone. Also, there were after five ten day's recovery of stopping treatment by tramadol indicated that mild significant decrease compared to control group. **(Figure2).**

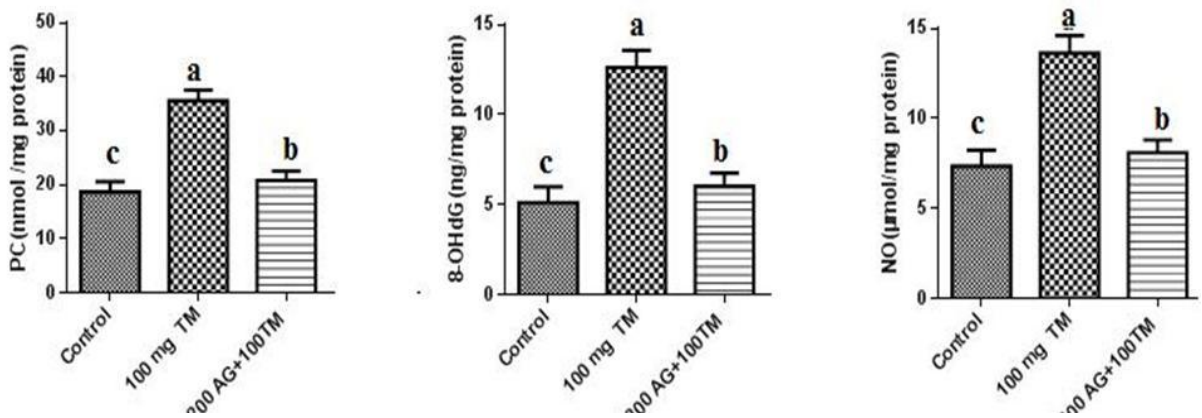


Figure 2: Effect of *anethum graveolens* (AG) (200 mg/kg) on oxidative markers (PC, 8-OHdG and NO activity) of rat testes tissue administration of tramadol. Values are mean±SE of 10 rats. Values with the same letters are not significant at ($p<0.05$) *Anethum graveolens L* = (AG) Tramadol= (TM).

The testes tissue lysozyme activity was measured after treated 30 days and recovery period 15 days. The statistical analysis of the group administered TM (100 mg/kg body weight) showed a significant increase in acid phosphatase, N-acetyl-β-D-glucosaminidase and β-galactosidase, in testes homogenate of male albino rats compare to control groups. While the administration of *anethum graveolens* (AG) two hours before Tramadol (TM) showed that, ameliorative effect in acid phosphatase, N-acetyl-β-D-glucosaminidase and β-galactosidase compare with group of rats received tramadol alone. Also, there were after five ten day's recovery of stopping treatment by tramadol showed that remained significantly different compared to control group of rats.**Figure (3)**

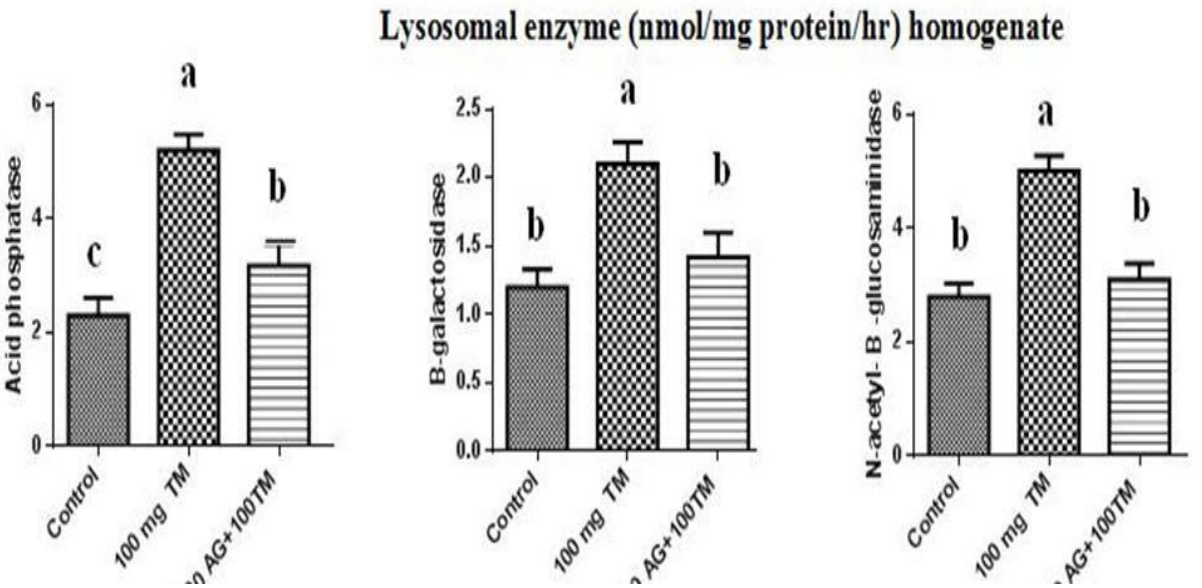


Figure3: Effect of anethum graveolens (AG) (200 mg/kg) on lysosomal enzymes (acid phosphatase, N-acetyl- β -glucosaminidase and β -galoctosidase) in testes tissue administration of tramadol. Values with the same letters are not significant at ($p<0.05$) *Anethum graveolens* L = (AG) Tramadol= (TM).

Data in Table (1) showed that administration of 100 mg tramadol /Kg b. wt./day significantly decreased serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, cortisol and Glucose (Glu) and there were higher significant increase in prolactin (PRL) and estradiol (E2) in male albino rats compared to control group non-treated. Meanwhile, the administration of *anethum graveolens* (AG) two hours before tramadol in group (200 AG +100TM) showed that ameliorative the luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, cortisol, prolactin (PRL), Glucose (Glu) and estradiol (E2) compared with the group of rat received tramadol alone. Also, there were after five ten day's recovery of stopping treatment by tramadol indicated that mild significant decrease in all parameter compared to control group non-treated.

Table (1): Effect of *anethum graveolens* (AG) (200 mg/kg) administration against tramadol (TM) (100 mg/kg) on sex hormones in serum of male albino rats (Mean ±SE)

Parameters	Treatment (1 month)		
	Control	100 mg Tram	200 AG +100TM
LH µIU/ml	1.85 ± 0.15[a]	0.92 ± 0.12[d]	1.62 ± 1.85[c]
FSH µIU/ml	2.32 ± 0.13[a]	0.82 ± 0.09 [d]	1.32 ± 1.19 [c]
Tes total (ng/ml)	3.70 ± 0.40[a]	1.43 ± 0.27 [d]	3.32 ± 2.17[c]
Cortisol (ng/ml)	168.0 ± 2.0[a]	134 ± 3.71[d]	151.0 ± 2.66[c]
PRL(ng/ml)	5.92 ± 1.0[a]	14.3 ± 2.15[d]	8.92 ± 1.32[c]
E2 (pg/ml)	46.8 ± 1.2[a]	73.55 ± 3.18[d]	51.8 ± 2.27[c]
Glu.(mg/dl)	191 ± 1.23[a]	155 ±1.0[d]	131± 0.92[c]

Values are mean± SE of 10 rats. Values with the same letters are not significant at ($p<0.05$) *Anethum graveolens L* = (AG) Tramadol= (TM).

Our study in table (2) showed that administration of 100 mg tramadol /Kg b. wt./day significantly decreased in sperm count, viability and motility compare to control group. Meanwhile, the administration of *anethum graveolens* (AG) two hours before tramadol in group (200 AG +100TM) showed that ameliorative effect in sperm count, viability and motility compare to group treated TM alone. Also, there were after five ten day's recovery of stopping treatment by tramadol showed that remained significantly different compared to control group of rats.

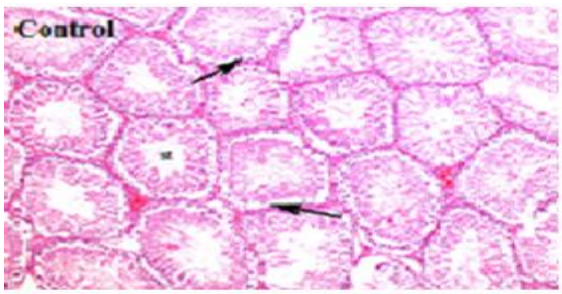
Table (2): Effect of *anethum graveolens* (AG) (200 mg/kg) administration against tramadol (TM) (100 mg/kg) on sperm motility, sperm vitality and sperm count in semen of male albino rats (Mean ±SE)

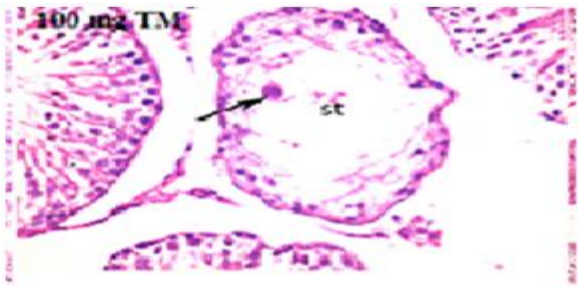
Parameters	Treatment (1 month)		
	Control	100 mg TM	200 AG+100TM
Sperm count (Million/cc)	3.85 ± 0.27	1.8 ± 0.41	2.65 ± 0.17
Viability (%)	82 ± 0.31	45 ± 0.51	65 ± 0.23
Motility (%)			
Progressive:	75 ± 0.48	48 ± 0.61	65 ± 0.44
Non-progressive:	25 ± 0.29	52 ± 0.39	35 ± 029

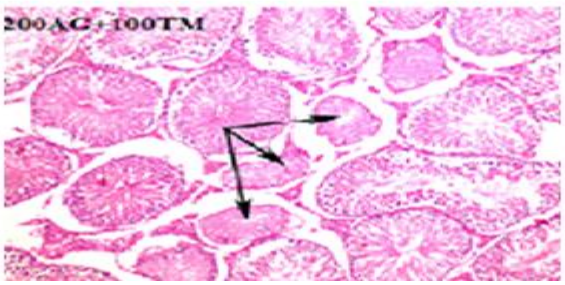
Values are mean± SE of 6 rats. Values with the same letters are not significant at ($p<0.05$*) Anethum graveolens L* = (AG) Tramadol= (TM).

VIII. Histopathological observation

Figure Control: Photomicrograph of cross section of testicular tissue of group control showing normal seminiferous tubules (ST) lining spermatocytes (S) and spermatogenesis, (H&E, X: 200).Figure100 mg TM: Photomicrograph of cross section of testicular tissue, showing focal testicular degeneration (TD) with single or multiple layer of vacuolated spermatocytes (VS) with a massive destruction in spermatogenesis, (H&E, X: 200). Figure 200 AG+100TM: Photomicrograph of cross section of testicular tissue, showing mild atrophy of seminiferous tubules with interstitial calcification, (H&E, X: 200).







IX. Discussion

The present study focuses on the effect of tramadol on testis cells activities and antioxidant/ inflammatory stress biomarkers, as well as MDA, PC, 8-OHdG, NO and TNF-α level in rat were observed a significant increase and was observed a significant decrease in GST in TM-treated rats when compared with the control rats. The activity of this enzyme has been used to assess oxidative stress in cells. Many studies have shown that tramadol has high affinity for SH groups in several enzymes such as GST , thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes. These results are similar to **Andrade *et al.,* (2011); and Bianchi *et al,* (2007).** Who reported that, Tramadol induced a significant increase in brain TNF-α level in rats, this effect of tramadol may be attributed to decreased level of serotonin since an inverse relationship has been demonstrated between TNF-α and serotonin levels **(Kubera *et al.,* 2005).** The association of stress-related depression with the activation of inflammatory signaling pathways has been previously reported **(Bierhaus *et al.,* 2003).** Nitric oxide and pro-inflammatory cytokines such as TNF-α are known to be important mediators in inflammation and brain injury (**Meda *et al.,* 1995).**

On the other hand, the administration of *A. graveolens (AG)* two hours before tramadol displayed a mitigated effect on MDA, PC, 8-OHdG, NO and TNF-α, level and GST compared with TM challenged group. This effect of *anethum graveolens* (AG) on tramadol might be due to its antioxidant process. Our findings, which are consistent with previous reports **(Li *et al.,* 2000 and Tanwar *et al.,* 2010),** confirm the dill leaves and basil seeds extracts were very effective in controlling radiation induced alterations antioxidant enzyme and accelerated recovery to normal control. The restoration of the antioxidant enzyme levels can partly explain the observed protection against lipid peroxidation and prevents further generation of free radicals like peroxynitrite and hydroxyl radicals.

Our interest in the toxicity of TM developed from lysozyme enzyme observations increases in acid phosphatase, N-acetyl-β-D-glucosaminidase, β-galactosidase in testes. This result is in similar with that reported by **Dehpour *et al.,* (1996).** Who reported that, marker lysozyme enzymes sensitively reflect the significance of the testes damage due to the alternations in the permeability of the membrane tissue, the enzymes are leaked and it results in an increase in proximal and distal tubules which are released after testes tubular damage. This may be due to membrane damage of lysosomes accumulating myeloid body formation and subsequent release of hydrolytic lysosomal enzymes such as NAG, β- D-glusosaminidase and cathepsin-D into cytosol which may lead to testes tubular cell necrosis **Kojima *et al.,* (1987).**

Meanwhile, the group of rats received *Anethum graveolens (AG)* two hours before tramadol proved improvement on acid phosphatase, N-acetyl-β-D-glucosaminidase, β-galactosidase in testes against the undue effect of Tramadol. *AG* has antioxidant ability as it scavenge free radical and suppress the oxidation process these results are similar to **Heim *et al.,* (2002); and Wolfe and Liu, (2008).** They reported that, the flavonoids ability to act as antioxidants depends on their molecular structure; characterized by two or more aromatic rings with at least one or more hydroxyl groups apiece and conjugated electrons giving also metal chelating properties.

In the present study, observed the effect of Tramadol (TM) administration (100 mg/ kg) caused a significant reduce in serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and cortisol and there were higher significant increase in prolactin (PRL) estradiol (E2) and glucose in male albino rats. These results are in the same line to those obtained by **Chowdhury. (1987)** who reported decreased levels of serum LH and testosterone with increased PRL secretion after morphine and methadone administration. **Christensen *et al.* (1989)** reported reduction of cortisol levels after treatment with antidepressants and increased prolactin (PRL) after sertraline administration **(Broadbear *et al,*. 2004).** Also, **Hezog *et al.,* (2004)** reported reduced testosterone and elevated E_2 levels while **Daoud *et al.* (2004)** reported significant reduction in serum testosterone and FSH levels in antiepileptic treated patients and in rats. **El-Gaafarawi *et al.(*2005)** have been observed in an independent investigation, the reduction of serum levels of LH, FSH, testosterone and the induction of PRL secretion after paroxetine treatment.

Meanwhile the administration of *anethum graveolens* (AG) two hours before tramadol mitigation the toxic effect of tramadol and restores the serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, cortisol and Glucose (Glu) level to be near the control level when compared to the group rats received TM alone. The ameliorative effect of *anethum graveolens* (AG) on tramadol might be due to its antioxidant activity. There is at present, growing interest in the scientific research for aromatic and medicinal plants because of their antioxidant properties. These properties are due to many active phytochemical including flavonides, terpenoids, carotenoids, coumarins, curcumines and so on **(Koleva *et al.,* 2001).** Herbs and spices are one of the most important targets to search for natural antioxidants. So far, many investigations on antioxidant properties of spices, volatile oils and extracts have been carried out. Extracts of herbs (rich source of antioxidants) are being used for more pronounced and marked effects than their crude forms. Dill has also been reported as antioxidant (**Singh *et al.,* 2005; Bahramikia and yazdanparast, 2009).** *A. graveolens* has some pharmacological effects, such as anti-microbial, anti-spasmodic, anti-secretary, and mucosal protective effects **(Radulescu., *et al* 2010).** Flavonoids are naturally polyphenolic

compounds which represent the main constituent s of fruits, vegetables, plant derived beverages (tea) and chocolate **(Youdim *et al.* 2002**). Flavonoids play an important role in plants, mainly protecting them against external pathogens. Flavonoids are responsible for the red, purple, and blue color of fruit s and flowers, and play a role in pollination by attracting insects. In human beings, these compounds, like other antioxidants, can cure or prevent oxidative stress by chelating active by terminating the free radical chain reaction **(Terao 2009).** The flavonoids ability to act as antioxidants depends on their molecular structure; characterize d by two or more aromatic rings with at least one or more hydroxyl groups apiece and conjugated electrons giving also metal chelating properties **(Heim *et al.* 2002; Wolfe and Liu 2008).**

The testicular histopathological results of group treated tramadol current study was supported by sex hormonal dysfunction evident by a highly significant decrease in LH, FSH, testosterone hormones and a highly significant increase in the mean value of estradiol and prolactin levels when compared with control group. The abnormalities observed in the testicular structures in the present study including atrophy of semeniferous tubules with interstitial calcification in group treated TM and focal testicular degeneration with single or multiple layers of vacuolated spermatocytes with a little evidence of spermatogenesis. This effect might be attributed to the oxidative damaging effect of free radicals since the testicular cells and sperms contain abundant polyunsaturated fatty acids in their plasma membranes. Lipid peroxidation induced by tramadol can eventually result in dysfunction and structural damage of cells **(Alvarez and Storey., 1995).**

On the other hand, the group of rats received *anethum graveolens* (AG) two hours before tramadol proved the protective effect of *anethum graveolens* (AG) against the undue effect of Tramadol. However, administration of *anethum graveolens* (AG) two hours after tramadol administration displayed a mitigated effect on testes cells. **Rekha *et al.*, (2010)** reported that, *Anethum graveolens* (AG) contents on antioxidant activity of aqueous and methanolic extracts of soup formulated with dill leaves was investigated by measuring total phenolic content, reducing power ability and free radical-scavenging activity. The phenolic acid profile contained tannic acid, protocatechuic, gentisic, vanillic acid and syringic acids.

X. Conclusion

The use of tramadol, one of the centrally acting analgesics, causes serious changes in the biochemical parameters and testicular tissue that could threaten the reproductive functions. so exaggerated of tramadol induced testicular toxicity. *Anethum graveolens (AG)* has an appreciable ability to prevent testicular toxicity caused by tramadol, possibly via its chemical constituents which has a free radical scavenging properties.

References

[1] **Alvarez JG and Storey BT (1995).** Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. Mol Reprod Dev 42: 334-346.

[2] **American Institute of Nutrition (1980).** Report of The American Institute of Nutrition. Ad Hoc Committee., J Nutrition. 110:1340-1348.

[3] **Andrade P, Visser-Vandewalle V, Hoffmann C, Steinbusch HW, Daemen MA and Hoogland G (2011).** Role of TNF-alpha during central sensitization in preclinical studies. Neurol Sci. 32(5):757–71.

[4] **Ashrafzadeh Takhtfooladi M, Haghighi Khiabanian Asl A, Shahzamani M, Ashrafzadeh Takhtfooladi M, Allahverdi A and Khansari M (2015b)**. Tramadol alleviates myocardial injury induced by acute hindlimb ischemia reperfusion in rats. Arq Bras Cardiol, 105:151-159.

[5] **Bahramikia S. and Yazdanparast R. (2009).** Efficacy of different fractions of Anethum graveolens leaves on serum lipoproteins and serum and liver oxidative status in experimentally induced hypercholesterolaemic rat models. Am. J. Chinese Med. 37:685-699.

[6] **Bahramikia, S. and Yazdanparast, R. (2009)** Efficacy of different fractions of Anethum graveolens leaves on serum lipoproteins and serum and liver oxidative status in experimentally induced hypercholesterolaemicrat models American Journal of Chinese Medicine. 37: 685-699.

[7] **Bancroft JD and Gamble M (2002)** Theory and practice of histological technique. (5th edition), Churchill Livingstone, Edinburg, London.

[8] **Bianchi M, Martucci C, Ferrario P, Franchi S, Sacerdote P 2007.**Increased tumor necrosis factor- α and prostaglandin E2 concentrations in the cerebrospinal fluid of rats with inflammatory hyperalgesia: the effects of analgesic drugs. Anesth Analg 104 (4):949–54.

[9] **Bierhaus A, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, 2003.** A mechanism converting psychosocial stress into mononuclear cell activation. Proc Natl Acad Sci100(4):1920–5.

[10] **Bilir A, Erkasap N, Koken T, Gulec S, Kaygisiz Z, Tanriverdi B, Kurt I. 2007.** Effects of tramadol on myocardial ischemia-reperfusion injury . Scand Cardiovasc J, 41:242 -247.

[11] **Broadbear J.H., L.C. Hutton, I.J. Clarke and B.J. Canny (2004):** Sex differences in the pituitaryadrenal response following acute antidepressant treatm-ent in sheep. Psychopharmacology (Berl), 171 (4): 450-457.

[12] **Chi HJ, Kim JH, Ryu CS, Lee JY, Park JS, Chung DY, Choi SY, Kim MH, Chun EK, Roh SI .2008.** Protective effect of antioxidant supplementation in sperm -preparation medium against oxidative stress in human spermatozoa. Hum Rep rod, 23:1023-1028.

[13] **Chowdhury AR.** Effect of pharmacological agents on male reproduction. Adv Contracept Deliv 1987; 3(4):347-352.

[14] **Christensen P., A. Lolk, L.F. Gram and P. Kragh-Sorensen et al. (1989):** Cortisol and treatment of depression: predictive value of spontaneous and suppres cortisol levels and cause of spontaneous plasma cortisol. 97 (4): 471-475.

[15] **Dahiya P and Purkayastha S 2012.** Phytochemical analysis and antibacterial efficacy of dill seed oil against multi-drug resistant clinical isolates. Asian Journal of Pharmaceutical and Clinical Research. 5(2):62-64.

[16] **Daoud A.S., H. Bataineh, S. Otoom and E. Abdul-Zahra (2004):** The effect of Vigabatrin, Lamotrigine and Gabapentin on the fertility, weights, sex harmones and biochemical profiles of male rats. 25 (3):178-83.

[17] **Dayer P., J. Desmeules and L. Collart (1997):** Pharmacology of tramadol. Drugs, 53 Suppl 2:18-24.

[18] **Dehpour AZ, Ghafourifar P, Ahangari N 1996.** Inhibition by lithium and rubidium of gentamicin -inducedrelease of N -acetyl -P-D-glucosaminidase from perfused rat kidney. Toxicology 110: 9 -15.

[19] **El-Gaafarawi I.I., M. Hassan, Gh. Fouad and F. El-Komey (2005):** Toxic effects of paroxetine on sexual and reproductive fuctions of rats. The Egyptian Journal of Hospital Medicine. Vol. 21:16-32.

[20] **Gillman P.K.(2005):** Monoamine oxidase inhibitors, opioid analgesics and serotonin toxicity. British J.Anaesth.95 (4): 434-441.

[21] **Grond S.and A. Sablotzki (2004):** Clinical pharmacology of treatment. Clin. Pharmacokinet. 43(13): 879-923.

[22] **HABIG, W.H., PABST, M.J. and JAKOBY, W.B. 1974.** Glutathi-one-S-transferase, the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249,7130–7139.

[23] **Hajhashemi V and Abbasi N 2008.** Hypolipidemic activity of Anethum graveolens in rats. Phytotherapy Research. 22(3):372-375.

[24] **Halliwell, B. (1996).** Antioxidants in human health and disease. Ann. Rev. Nutr., 16: 33-50.

[25] **Heim KE, Tagliaferro AR, Bobliya DJ (2002)** Flavonoid antioxidants: chemistry, metabolism and structure activity relationships. J Nutr Biochem 13:572–584

[26] **Herzog A.G., F.W. Drislane, D.L. Schomer, P. B. Pennell (2004):** Differntial effects of antiepileptic drugs on sexual function and reproductive horm-ones in men with epilepsy: interim analysis of a comparison between lamot-rigine and enzyme inducing antiepileptic drugs. Epilepsia, 45(7): 764-768.

[27] **Kaur GJ and Arora DS 2010.** Bioactive potential of *Anethum graveolens*, Foeniculum vulgare and Trachyspermum ammi belonging to the family Umbelliferae- Current status. Journal of Medicinal Plants Research. 4(2):87-94.

[28] **Kojima H, Ohi H, Miyaji H, Seki M, Fujita T, Hatano M (1987).** Localization of C3d in renal tissues of patients with membranous nephropathy and IgA nephropathy. Nihon Jinzo Gakkai Shi;29 (9):1161- 1165.

[29] **Koleva, I.I; N iederlander, H.A.G. and Van Beek, T.A. (2001).** Application of ABTS radical cation for selective on line detection of radical scavengers in HPLC elutes.Anal. Chem., 73: 3373-3381.

[30] Kubera M, Maes M, Kenis G, Kim YK, Lason´ W 2005. Effects ofserotonin and serotonergic agonists and antagonists on the production of tumor necrosis factor alpha and interleukin-6. Psychiatry Res 134(3):251–8.

[31] **Levine R L, Garland D, Oliver C N, Amici A, Climent I, Lenz A G, Ahn B W, Shaltiel S and Stadtman E R (1990)** Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. (186), 464-478

[32] **Li, S.; Yang, T.; Uang, J.Q.; Oberley, T.D. and oberley, L.W. (2000).** The role of cellular glutathione peroxidase redox regulatation in the suppression of tumor cell growth by manganese superoxide dismutase. Cancer Res., 60: 3927-3939.

[33] **Meda L, Cassatella MA, Szendrei GI, Otvos L, Baron P, Villalba M,1995.** Activation of microglial cells by beta-amyloid protein and interferon-gamma. Nature 374(6523):647–50.

[34] **Naseri M, Mojab F, Khodadoost M, Kamalinejad M, Davati A, Choopani R 2012.** The study of anti-inflammatory activity of oil-based dill (Anethum graveolens L.) extract used topically in formalin-induced inflammation male rat paw. Iranian Journal of Pharmaceutical Research. 11(4):1169-1174.

[35] **Pathak V, Dwivedi R, Shukla P 2014**. Pharmacognostical study of Anethum sowa (dill) seed. International Journal of Recent Biotechnology. 2(3):6-14.

[36] **Radulescu, V., Lidiapopescu, M. and Lies, D. C. (2010)** Chemical composition of the volatile oil from different plant parts of Anethum graveolens l. (umbelliferae) cultivated in Romania. Farmacia. 58: 594-600.

[37] **Radulescu, V., Lidiapopescu, M. and Lies, D. C. (2010)** Chemical composition of the volatile oil from different plant parts of Anethum graveolens l. (umbelliferae) cultivated in Romania. Farmacia. 58: 594-600.

[38] **Raffa R.B., E. Friderichs, W. Reimann, R.P. Shank, E.E. Codd and J.I. Vaught (1992):** Opioid and non-opioid components independently contribute to the mechanism of action of tramadol, an "atypical" opioid analgesic. J. Pharmacol. Exp. Ther., 260 (1): 275-285.

[39] **Raffa R.B. (1996):** A novel approach to the pharmacology of analgesic. Am. J. Med., 101 (1A): 40S-46S.

[40] **Rekha MN, Yadav RA, Dharmesh S, Chauhan AS, Ramteke RS.** Evaluation of antioxidant properties of dry soup mix extracts containing dill (Anethum sowa L.) leaf. Food and Bioprocess Technology. 2010; 3: 441-449.

[41] **Singh S.; Maurya S.; De Lampasona M.P. and Catalan, C. (2005).** Chemical constituents, antimicrobial investigations, and antioxidative potentials of Anethum graveolens L. essential oil and acetone extract. Part 52. J. Food Sci., 70: 208-215.

[42] **Snedecor, G.W. and Cochran, W.G. 1980.** Statistical Methods, 7th Ed., Iowa State University Press, Ames, Iowa.

[43] **Stannard J 1982.** The multiple uses of dill (Anethum graveolens L.) in medieval medicine. Wurzburg Med Forsch. 24:411-424.

[44] **Stavri M and Gibbons S 2005.** The antimycobacterial constituents of dill (Anethum graveolens). Phytotherapy Research 19:938-941.

[45] **Tanwar, V.; Sachdeva, J.; K ishore, K.; Mittal, R.; Nag, T.C.; Ray,R.; Kumari, S. and Arya, D.S. (2010).** Dose dependent actions of curcumin in experimentally induced myocardial necrosis: a biochemical, histo-pathological, and electron microsocopic evidence. Cell Biochem. Funct., 28: 47-82.

[46] **Terao J (2009)** Dietary flavonoids as antioxidants. Forum Nutr 61:87–94

[47] **Trinder P (1969):** Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Ann. Clin. Biochem. 6: 24-25.

[48] **Uchiyama M and Mihara M (1978)** Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Anal. Biochem ., (86), 271 - 278.

[49] **Van Hoof F, and Hers HG (1968):** The abnormalities of lysosomal enzymes in mucopolysaccharides. Euro. J. Biochem., 7: 34-44.

[50] **WANG, C.C., HUANG, Y.J., CHEN, L.G., LEE, L.T. and WANG,L.L. 2002.** Inducible nitric oxide synthase inhibitors of Chineseherbs III. Rheum palmaturn. Planta Med. 68 (10), 869–874.

[51] **Wei SM, Yan ZZ, Zhou J. 2011.** Protective effect of rutin on testicular ischemia -reperfusion injury. J Pediatr Surg, 46:1419-1424

[52] **Wolfe KL, Liu RH (2008)** Structure–activity relationships of flavonoids in the cellular antioxidantactivity assay. J Agric Food Chem 56:8404–8411

[53] **World Health Organization (1999)** WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Intraction, 4th edn. Cambridge, University Press, New York.

[54] **Youdim KA, Spencer JP, Schroeter H, Rice-Evans C (2002)** Dietary flavonoids as potential neuroprotectants. Biol Chem 383:503–519
[55] **Zargari A 1991.** Medicinal Plants . Tehran University Publications, Iran.

Fawzy M lashin"The Possible Ameliorative Role Of Anethum Graveolens Against Tramadol-Induced Testicular Damage In Male Albino Rat." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.1 (2018): 82-90.