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Research Article

AMELIORATIVE EFFECT OF MELATONIN, VITAMIN - C AND THEIR COMBINATION ON ACRYLAMIDE TOXICITY IN TESTIS OF ADULT MALE RATS

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Abstract

The present study was designed to determine the ameliorative effect of melatonin (Mel.), vitamin C (Vit.C) alone and their combination on reproductive hormone concentration, epididymal sperm characteristic and testicular histological changes in acrylamide (ACR) intoxicated rats. Forty eight adult male rats were divided randomly into two main groups. Control group (no.16) subdivided into two groups: I group: eight animals of control administration distal water and group II - eight animals give 5 mg/kg BW Mel. for 21 days. second group: the ACR trested group subdivided into ACR + distal water orally, ACR + Mel (5 mg/kg BW/day), ACR + Vit. C (200 mg/kg BW/day), ACR + Mel. + Vit. C (5 + 200 mg/kg BW/day) for 21 days. The result revealed significant decrease in serum LH and testosterone hormones and non significant differences in serum FSH concentration in Mel group, significant elevation in serum LH, FSH and testosterone concentrations in all treatment groups compared with ACR – non treated group. No significant differences were observed in sperm concentration, viability and abnormality and significant decrease in sperm motility in Mel. treated normal male rats compared with control. A significant improvement in sperm concentration, sperm motility, viability and abnormality in all treatment groups compared with ACR-treated group. Hisopathological changes showed improvement in testicular tissues.

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1. Introduction

Acrylamide (ACR) is white, odorless, crystalline solid at room temperature widely used industrial chemical with a molecule of C_3H_5NO (Grivas *et al*., 2002). ACR is found in carbohydrate rich food such as potato chips and french fries prepared in high temperature that consumed by human (Rydberg *et al*., 2003). ACR is one of the most contaminated in the environment which was shown to be neurotoxicant, reproductive toxicant, hepatotoxicant and carcinogen in animals (El-Assouli, 2009).

It has been recently found that ACR-treatment affects the integrity of cell membranes of epididymal spermatozoa in mice (Kermani-Alghoraishi *et al.*, 2010). The adult male rats exposed to subchronic ACR-treatment resulted in decreased sperm viability and increased abnormal

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motility (Song *et al*., 2008). ACR also able to cross the placenta and the concentration reach to the significancy at concepts and in turn cause direct development and postnatal effect in rodent offspring. ACR also has an adverse effect on reproduction such as dominant lethal effects, testicular epithelial degeneration such as dominant lethal effects, testicular epithelial degeneration and abnormalities of sperm-head (Dearfield *et al*., 1988). Melatonin (Mel.) is a lipophilic hormone produced and secreted by the pineal gland. Its physiological function includes regulation of body weight, seasonal reproduction and energy balance (Hussein *et al*., 2006). Mel. is a potent free radical scavenges and antioxidant which is known to be highly effective in protecting against oxidative damage caused by a variety of toxins (Gonenc *et al*., 2005; Kus *et al*., 2007). Vit. C is hydrophilic and is an important free radical scavenger in extracellular fluid, trapping radical and protecting biomembranes from peroxide damage. It is effectively scavenges singlet oxygen, superoxide, hydroxyl, water soluble peroxyl radical and hypochlorous (Smirnoff and Wheeler, 2000). The aim of the present study was to determine the ameliorative effect of Mel, Vit. C and their combination on ACR induced testicular damage by biochemical and histological changes.

2. Materials and Methods

Animals and housing

Sixty adult male rats were used in this present study. All rats were weighed about (205.00 ± 19.00). They were kept in animal house under constant environmental condition for 2 weeks to acclimatization before beginning of the experiment. Food and drinking water were provided ad libitum throughout the experiment.

Experimental Design

Rats were divided randomly into two groups as follows:

1) **Control group (n = 20):** Adult male rats administered distilled water daily by gavage for 45 days.
2) **Treatment group (n = 40):** Adult male rats administrated ACR (5 mg/kg BW/day) for 45 days by gavage.

At the end of experimental period the animals of each group were divided into the following sub groups:

The control group was divided into two equal groups: Control (-ve) (n = 10) G1 administrated distal water orally by gavage for 21 days. Control (+ve) (n = 10) G2 administrated (5 mg/kg BW/day) for 21 days orally by gavage.

The ACR group was divided into four subgroups: ACR + distal water (n = 10) G3: administrated distal water orally by gavage for 21 days. ACR + Mel. (n = 10) G4: administrated 5 mg/kg BW/day orally by gavage for 21 days. ACR + Vit. C (n = 10) G5: administrated distal 200 mg/kg BW/day Vit. C orally by gavage for 21 days. ACR + Mel. (n=10) G6: administrated both Mel. + Vit.C in the same dose cited above of each one by gavage for 21 days.

Collection of blood samples

At the end of treatmentt peroid the animals were anesthized using diethyl ether and sacrificed. The blood samples were collected directly by cardiac puncture into clean dry test tube and serum were separated and stored at -20 ˚C until used for hormonal analysis. The testes were excised directly and one of each animal was fixed neutral buffered formalin 10 % for histological study according to Luna (1968). The right testis was used for estimation of epididymal sperm characteristics.

Hormonal analysis

Testosterone ELISA Kit

The quantitative determination of total testosterone concentration in serum or plasma by a micoplate enzyme immunoassay (Lashansky *et al*., 1991).

Estimation of Follicle Stimulating Hormone Concentration (FSH) and Luteinizing Hormone Concentration (LH) (ng/ml)

Estimation of FSH concentration by using an enzyme test kit (Human GmbH.53020 Wiesbaden. Germany Gesellechalf for Biochemical and diagnostic mbH) (Odell *et al*., 1981).

Estimation of epididymal sperm characteristics

The epididymus of each testis was dissected and transferred into the petridish contain 5 ml normal saline. **Epididymal sperm concentration:** The sperms were counted according to the method of Robb *et al*. (1978) by using Neubauer hemocytometer chamber which uses for RBC and WBC count. **Sperm motility percentage**: The individual motility of epididymall sperms was measured depending upon the graduation basis that was suggested by Chemineau *et al*. (1991) as follows: **Sperm abnormality:** The percentage of abnormal spermatozoa were counted in the same slide that used for measurement of the viability of epididymal sperm using account 200 sperms under a light microscope using 100 X power (Evans and Maxwell, 1987).

Histopathological Study

Specimens from testes were collected and preserved in 10 % neutral buffered formalin and processed to obtain paraffin sections of 6 micron thickness, then stained with hematoxylin and eosin stain, then the slide was used for histolopathological examination under a light microscope (Luna, 1968).

Statistical Analyses Method

Statistical analyses were performed by the standard method. All the results were expressed as mean ± standard deviation (S.D.). The mean of the all groups compared using One - way ANOVA by SPSS (Special Program for Statistical system) 'P' value of less than 0.05 was considered to represent statistically significant change (Abo-Allam, 2003).

3. Results

Effect of Mel., Vit.C alone and their combination on reproductive hormones in ACR-treated adult male rats

The data illustrated in Table - 1 indicated that no significant differences in serum LH concentration between ACR-Mel. and ACR-Mel. plus Vit. C treated groups compared with control. However, a significant ($P < 0.05$) decrease in serum LH concentrations was recorded in normal Mel. treated, ACR-non treated and ACR - Vit.C treated groups compared with control and other treated groups. The low value of serum LH concentration was recorded in ACR-Non treated groups. Serum FSH concentration revealed no significant difference between control-Mel., ACR-Mel. and ACR- Mel. + Vit. C compared with the control group. A significant ($P<0.05$) decrease in serum FSH concentrations was recorded in ACR-non treated compared with control and other treated groups. The low significant ($P<0.05$) value of serum FSH concentration was recorded in ACR-non treated compared with control and other treated groups. Serum testosterone concentrations showed no significant differences between ACR-Mel. treated and ACR-Mel. Plus Vit. C treated groups compared with control, while a significant ($P<0.05$) decrease in testosterone concentrations was recorded in normal Mel. treated, ACR-non treated and ACR-Vit.C treated groups compared with control. The low significant value of serum testosterone concentration was recorded in ACR-non treated group compared with control and other treated groups.

The results represented in Table - 2 revealed that after 21 days of treatment no significant differences was observed in sperm concentration between normal - melatonin treated, ACR - melatonin and ACR - treated with combination of Mel + Vit. C groups compared with control. While, the sperm motility concentration was recorded significantly ($P<0.05$) decreased in normal-Mel., ACR – non treated and ACR- Vit. C treated groups compared with control and other treated groups. The low significant ($P<0.05$) value of sperm concentration was recorded in ACR- non treated group. Sperm

motility as illustrated in the same table showed that no significant differences between ACR-Mel. And ACR-group treated with a combination of Mel. + Vit. C compared with control. The lowest significant ($P<0.05$) decrease in sperm motility percentage was recorded in ACR-non treated group. Sperm viability indicated that no significant differences in normal melatonin and ACR-Mel. treated groups compared with control after 21 days of treatment. On the other hand a significant ($P<0.05$) decrease in viability percentage were recorded in ACR-non treated, ACR-Vit. C and ACR-treated with melatonin plus Vit. C compared with control and other treatment groups. The low significant ($P<0.05$) value of sperm viability was recorded in ACR-non treated group compared with control and other treatment groups. After 21 days of treatment, no significant differences were recorded in sperm abnormalities between all treatment groups and control except in ACR-non treated group in which sperm abnormality percentage still significantly lower compared with those of control and other groups.

The results of the present study as illustrated in Table - 1 revealed a significant decrease in serum testosterone concentration in control (normal) group treated with Mel. compared with control. These results are consistent with Nasirae - Moghadam *et al*. (2014) who reported that normal adult male rats treated with Mel. (5 mg/kg BW) I/P for two weeks resulted in a light decrease in serum testosterone concentration compared with controls. Similarly, Hatamoto *et al*. (2006) who found that adult rats treated with Mel. caused a significant decrease in plasma testosterone concentration. In the same line of the present results, Ahmad *et al.* (2010) found that application of Mel. in dose of (2.5 mg/ml) inn inner testicular in rodents of *Fanmbulus pennati* during 10 and 28 days resulted in significant decrease in testosterone concentration, spermatogenesis and increase the expression of the receptors type (MT_1). The present results are also in agreement with that previously mentioned by Valente *et al.* (1999) who demonstrated that Mel. treatment was capable of reducing the testosterone level in Leydig cells *in vitro*. In contrast to the present results, Hardy *et al*. (2015) and Kalil and Abdu (2015) who all reported that normal adult male rats treated with Mel. resulted showed an insignificant elevation in serum concentration of testosterone, LH and FSH compared with control. These results agreed only in FSH level with the present results which revealed no significant differences in FSH level in Mel. treated group compared with control. Moreover, no significant difference was observed in serum testosterone level compared with control in adult male rats treated with Mel. (6 mg/kg Bw/day) for 28 days (Pati and Balaraman, 2009). Mel. binding to its receptor (MT_1) and the activation of Gi protein, decrease the level of cAMP and calcium. These effects promote the reduction of testosterone secretion which was an essential hormone for spermatogenesis (Chun *et al*., 2008; Reiter *et al*., 2009).

The present results also demonstrated that ACR group which left for 21 days without treatment appeared significant decrease in serum LH, FSH and testosterone concentrations. The present results in ACR group treated with Mel. revealed a significant increment in serum LH and testosterone concentration compared with ACR-nontreated group. These results are in agreement with Pati and Balaraman (2009) who reported that adult male rats treated with doxorubicin causes significant decrease in serum testosterone level compared with control. While the coadmenisitration of Mel. restored testosterone value significantly toward the control value. Our results are also parallel to those mentioned by Khalil and Abdu (2015) who found that adult male rats treated with different doses of zonisamide (10, 20 and 50) mg/kg BW resulted in a significant decrease in serum LH, FSH and testosterone levels while Mel. treatment resulted in a significant elevation in these parameters toward the normal values compared with controls.

The present results of ACR group treated with Vit.C on serum levels of LH, FSH and testosterone as illustrated in Table - 1 are accordance with Al-Damegh (2014) who indicated that adult male rats exposed to acute and chronic immobilization stress showed a significant decrease in serum LH and testosterone levels

compared with control. While administration of Vit.C cause a significant increase in both serum LH and testosterone levels compared with stresses groups but still significantly lower than those of control. Similarly, Vijayprasad *et al.* (2014) found that swimming stress resulted in significant reduction of testosterone level compared with control. While administration of Vit.C in doses of (10, 20 and 30 mg/kg BW.) causes significant elevation in testosterone levels toward the normal values compared with stresses groups, but still significantly lower than those of control. Moreover, male rats exposed to nicotine for 35 days resulted in significant reduction of plasma FSH, LH and testosterone levels compared with control. While Vit.C administration leads to the elevation of the levels of the above cited parameters compared with nicotine group (Oyeyemi *et al*., 2014). In the same line of the present study the male rats exposed to the lead for six weeks resulted in the significant decrease in serum FSH and testosterone. However, no significant changes were obseved in LH hormone compared with control, while the treatment with Vit.C cause significant increase in serum FSH and LH levels to the normal value compared with the Lead group (Ayinde *et al*., 2012). The reduction in the serum LH level rest from ACR stress which may be responsible for the decrease in testosterone concentration. Previous studies indicated that many which occurred due to inhibition of LH-RH synthesis and release from the hypothalamus (Hardy *et al*., 2015). The inhibition of the HPG axis induced by stress may be mediated by CRH and endogenous opioid mainly β-endorphins which release in response to the stress (Vaccarino and Kastin, 2000). Also the stress causes excessive secretion of glucocorticoids which may act as another factor cause depression in T level by acting directly on Leydig cell function through glucocorticoide receptor-mediated pathway (Dong *et al*., 2014). The exposure to the stress cause increase in serum levels of glucocorticoid and corticosterne secretion from adrenal gland (Jana *et al*., 2010) glucocorticoide may suppress the senserity of the gonads to the gonadotropin-releasiing hormone (GnRH) and may prevent gonadotropinsecretion (Kamel and Kubajak, 1987).

4. Discussion

The results in Table - 2 summarized the effect of Mel., Vit.C, and their combination on ACR intoxicated groups. In normal, Mel. treated group for 21 days, the results are in agreement with Gwayi and Bernard (2002) who reported a significant decrease in sperm motility *in vitro* spermatozoa collected from adult male rats treated with Melatonin. The present results are also accorded with Nasiraei-Moghadam *et al.* (2014) who indicated that no significant differences were observed in epididymal sperm number, abnormality and viability while reduced sperm abnormality after 2 weeks of treatment with Mel. in adult rats compared with control. Similarly, no significant differences were observed in epididymal sperm count and sperm abnormality percentage in Mel. treated male rats but differ from the present study in sperm motility which also not significantly differed from that of control (Ilbey *et al*., 2009). However, male rats treated with Mel. (10 mg/kg BWl day) for 15 days lead to significant decrease in sperm concentration, progressive motility compared with those of control (Akman *et al*., 2015).

Moreover, exogenous Mel. admiinistration in adult male rats (9 and 12) month old showed no significant differences in sperm motility. However, insignificant reduction in sperm count was recorded in 3 month, 9 month and 12 month old rats treated with Mel. (Idowu and Olatunji-Bello, 2015). This result indicated that exogenous Mel. treatment preserve the function of reproductive system during ageing in male rats. It was reported that Mel. has a direct effect on epididymal and testicular aromatase leading to alteration in the androgen/estrogen ratio and decrease sperm motility (Luboshitzky, 2002). In adult male mice treated with Mel. (10 mg/kg BW) for 66 days showed no significant differences in sperm count, sperm morphology and sperm stability compared with control group (Bustos-Obregon *et al*., 2013). The results of ACR-group treated with Mel. showed a significant amelioration in epididymal sperm characteristics. These results correlated well with Bustos - Obregon *et al*. (2013) who found that arsenic sodium treated adult male rats for different periods

resulted in significant reduction of sperm count, stability of DNA significant increase in sperm morphology percentage. But, Mel. treatment partially protects sperm toxicity caused by arsenic due to the ability of Mel. of scavenging the free radical species. Mel. treatment also ameliorate the epididymal sperm motility and sperm concentration in homocystein treated Wister rats (Sonmez *et al*., 2007).

Adult male rats treated with doxorubicin alone showed significant decrease in sperm count which coadministration of Mel. with doxorubicin cause a significant increase in sperm count compared with control (Patil and Balaraman, 2009). Mel. may exert its action on the reproductive system through its receptors in the hypothalamus, pituitary, ovary granulosa cells, prostate and spermatozoa (Fineberg *et al*., 2005; Tam *et al.*, 2008). The results of the ACR-group treated with Vit. C causes a significant amelioration of sperm concentration, sperm motility, viability and abnormality compared with ACR-non treated group but still significantly lower than those of control. These results are in agreement with Ashamu *et al.* (2013) who found that Vit.C treatment of Lead acetate intoxicated male mice resulted in significant improvement in sperm count and sperm abnormality compared with the Lead acetate group but still siginificantly lower than those of control. Similarly, swimming stress causes significant reduction in sperm count, sperm motility and sperm viability while administration of Vit.C in doses of 10, 20 and 30 mg/kg BW. cause significant elevation of above parameters in a dose dependent manner compared with stress groups but still siginificantly lower compared with control (Vijayprasad *et al*., 2014).

The reduction of sperm count and elevation in sperm abnormalities in animals treated with pesticides, chemical mutagenus xenobiotic and metals could be resulted from oxidative damage to the polyunsaturated fatty acids of germ cell membranes which affect membrane fluidity and permeability leading to damage of germ cells, spermatozoa and matures sperm. So, the reduction sperm count may be due to the interaction of ROS with sperm cell membrane. The improvement of these parameters in Vit.C treated group may be occurred due to the antioxidant effect of Vit.C which can neutize the free radicals that generated from the oxidative stress and also causes regeneration of the reduced form of glutathion (GSH) (Well and Xu, 1994).

Histopathological study: The microscopical examination of the testes in the control group showed normal testicular tissue architecture including seminiferous tubules with normal series of spermatogenesis and normal Leydig cells in the interstitial tissue as shown in Figure - 1. The histopathological study of the group treated for 21 days with 5 mg/kg BW. day Mel. Appared normal seminiferous tubules with reduction of spermatogenesis in some seminiferous tubules and reduction in interstitial tissue (Fig - 2). These results are nearly accordinated with the results that obtained by Patil and Balaram (2009); Rao and Bhatt (2012) and Akman *et al*. (2015) who all found that Mel. treatment to adult male rats revealed that no histopathological changes found in the testicular section compared with those of control.

However, in the male rats of ACR group treated with distal water for 21 days (Fig. 3) the histological changes include degeneration of germinal epithelium and sertoli cells suppression of spermatogenesis and vacuolation of spermatogonia. These results may indicate that ACR causes permanent degenerative changes in the seminiferous tubules. These results are inagreement with the previous studies. Wang *et al*. (2010); Lal *et al*. (2012); Rajesh *et al.* (2014) and Abd-Elghaffar *et al*. (2015) that histopathological changes in the testicular tissue of rats treated with ACR including degenerative changes of the majorty of seminiferous tubules, incomplete spermatogenesis, vacuolar degeneration and sloughing of seminiferous epithelium.

The histopathological examination of the ACR group treated with Mel. for 21 days showed the nearly normal architecture of testicular tissue as shown in fig. (4). These results are inconsistent with Patil and Balaram (2009) who reported that administration of Mel. adult male rats intoxicated with doxorubicin cause restoration of the

Table - 1: Effect of Mel., Vit.C alone and their combination on reproductive hormones in ACR - treated adult male rats (M±SD) (n=10)

Groups	LH (ng/ml)	FSH (ng/ml)	Testosterone (ng/ml)
G1 (Control)	7.31±0.59 [a]	4.48±0.47 [a]	9.33±0.58 [a]
G2 (Control + Mel.)	6.18±0.61 [b]	4.22±0.64 [a]	7.61±0.40 [b]
G3 (ACR + D.W.)	4.75±0.74 [c]	2.56±0.61 [b]	5.25±0.69 [c]
G4 (ACR + Mel.)	7.46±0.88 [a]	4.40±0.58 [a]	9.60±0.62 [a]
G5 (ACR + Vit.C)	5.95±1.07 [b]	3.98±0.47 [a]	7.16±0.70 [b]
G6 (ACR + Mel. + Vit.C)	7.70±0.76[a]	4.50±0.59 [a]	9.16±0.92 [a]
LSD	1.12	1.42	1.55

Values expressed in small letters mean significant differences at (P<0.05) levels

Table - 2: Effect of Mel., Vit.C alone and their combination on epididymal sperm characteristic (M ± SD) (n = 10)

Groups	Sperm Concentration ($\times 10^6$/ ml)	Sperm motility (%)	Viability (%)	Abnormality (%)
G1Control + D.W	68.25±9.61 [a]	81.87±4.58 [a]	84.43±3.81[a]	12.75±5.14 [b]
G2 Control + Mel.	64.87±10.90 [a]	67.50 ±12.24 [b]	81.37±2.50 [a]	14.62±5.20 [b]
G3 ACR + D.W.	47.50±7.32 [c]	45.12±9.7 [c]	48.25±6.67 [c]	33.00±5.83 [a]
G4 ACR + Mel	66.75±9.92 [a]	80.50±7.21 [a]	81.00±5.04 [a]	12.75±3.84 [b]
G5 ACR + Vit.C	57.00±11.48 [b]	62.37±11.43 [b]	73.62±4.13 [b]	13.87±2.10[b]
G6 ACR + Mel. + Vit. C	60.37±6.06 [a]	79.75±6.01 [a]	74.12±7.41 [b]	14.82±4.31 [b]
LSD	11.25	12.25	6.62	16.37

Values expressed in small letters mean significant differences at (P<0.05) levels.

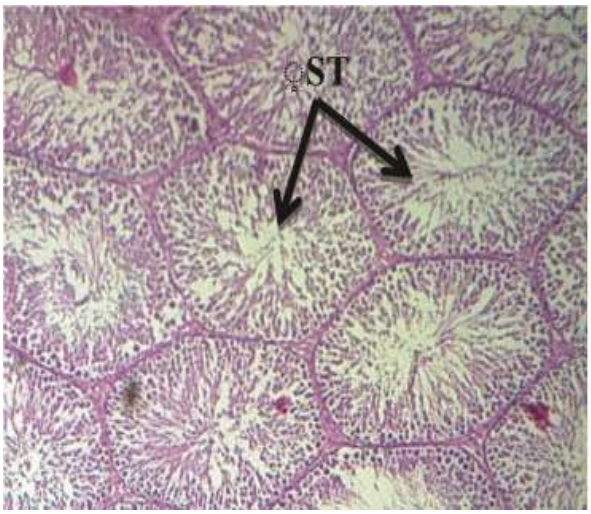

Fig. (1): Testes of control group showing normal sertoli cells (S), normal architecture of seminiferous tubules (ST) with different stage of spermatogenesis (H&E stain 400X).

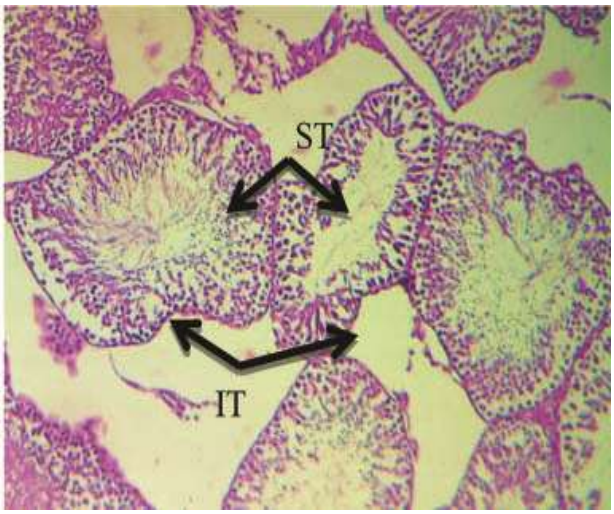

Fig. (2): Testes of normal control+Mel. treated group showing normal seminiferous tubules (ST), with normal spermatogenesis, destruction of some seminiferous tubules and reduction of interstitial tissues(IT) , stain (H&E) 100X.

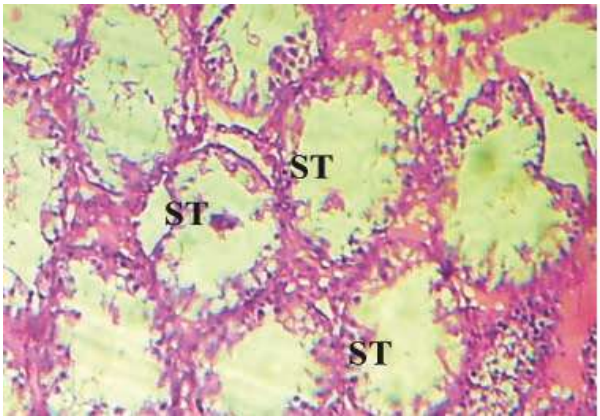

Fig. (3): Testes ACR+D.W. treated group showing distruction in semeniferous tubules (ST) with absence of spermatogonia, stain (H&E) 100X.

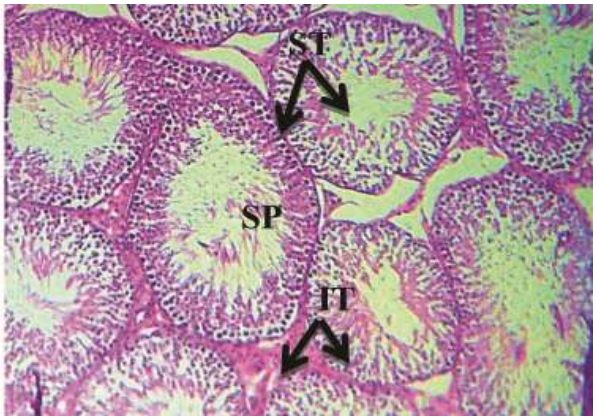

Fig. (4): Testes ACR+Mel. treated group showing normal semeniferous tubules (ST) spermatogonia (SP) and interstitial tissues (IT), stain (H&E) 100X.

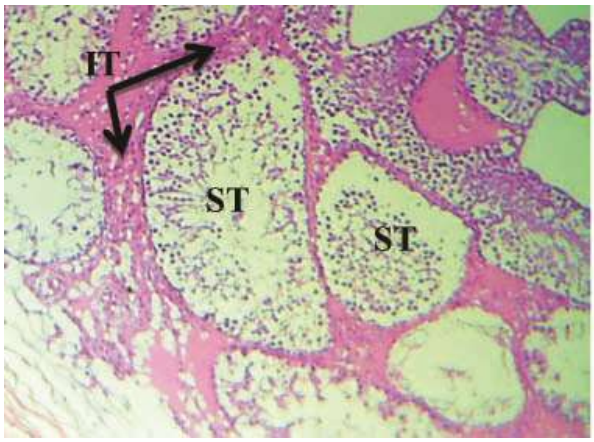

Fig. (5): Testes ACR+Vit.C treated group showing abnormally miss shaped semeniferous tubules (ST) with arrest spermatogonesis of some of them and thick interstitial tissues of leydig cells (IT), stain (H&E) 100X.

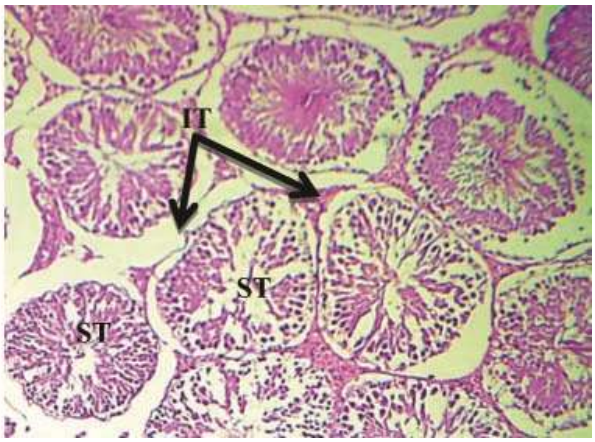

Fig. (6): Testes ACR+Mel.+Vit.C treated group showing normal of seminiferous tubules (ST), with nearly normal interstitial tissues (IT), disarrangement of some epithelial lining cells of seminferous tubules observed, stain (H&E) 100X.

with doxorubicin cause restoration of the histological changes of testis toward the normal architecture. Moreover, Ilbey *et al*. (2009) showed that adult male rats treated with cyclophosphoamide and cisplatin cause testicular damage with sever degeneration, necrosis and reduction in seminiferous tubules and germinal cells thickness. While, the co-treatment with Mel. resulted in amelioration of these histopathological changes due to its ability to scaveng the free radicals generated due to lipid peroxidation or due to the stimulatory effect of Mel. on enzymatic antioxidants such as glutathione peroxidase (GP_X). Similarly found by Akman *et al*. (2015) who indicated that Mel. treatment of diabetic male rats resulted in amelioration in the changes male rats resulted in amelioration in the changes of the testicular tissue compared with those of a *Diabetic mellitus* group.

The present results are in line with those of Ashamu *et al.* (2013) who showed that administration of Mel. protects the testicular tissue of Lead acetate intoxicated mice. The histopathological changes of ACR group treated with Vit. C showed abnormal miss shaped seminiferous tubules, arrested spermatogenesis in some seminiferous tubules and thick interstitial tissue Fig. - 5. These results were matched with results obtained by Zhou *et al.* (2014) who found

that adult male mice exposed to arecoline showed vacuolation, no spermatogenesis in the lumen of seminiferous tubules. The administration of Vit.C and E has ability to ameliorate oxidative stress releated testiicular impairment due to their antioxidant activities (Marchlewicz *et al*., 2007). In contrast, Uzum *et al*. (2009) indicated that administration of vitamin C and E have no beneficial effect on the testicular toxicity induced by malathion. The Vit.C is a nutritive antioxidant act by neutralize the free radical generated by heavy metals and by regenerating the reduced form of glutathion (Wells and Xu, 1994; Xu and Wells, 1996). In ACR group treated with both Mel. + Vit. C the histopathological examination revealed seminormal seminiferous tubules and interstitial tissue with disarrangement of some epithelial lining seminiferous tubules Fig. (6). These results may be resulted mainly due to the scavenging activity of Mel. to the free radicals or regenerating of the antioxidant defense system.

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