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RESEARCH ARTICLE

Impacts of Grape Seed Oil Supplementation against the Acrylamide Induced Lesions in Male Genital Organs of Rats

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ABSTRACT

This work aimed to evaluate the ameliorating effect of grape seed oil (GSO) on the lesions of experimental Acrylamide (ACR) intoxication in male rat genital organs. Two experimental groups of concomitant administration of 2 dietary levels of GSO with the toxic dose of ACR were evaluated in comparison with a negative control group and other 3 positive control groups for each of the 2 dietary levels of GSO and the toxic dose of the ACR. The results, at the end of the experiment (3 weeks), revealed occurrence of the highest scale of the histopathologic changes in case of the group of ACR-intoxication. These changes were in the form of testicular degeneration, abnormal epididymal contents of immature spermatocytes and multinucleated giant cells. The prostate glands and seminal vesicles showed cystic dilatation with less secretory materials, epithelial hyperplasia, necrosis and desquamation. Nearly similar scales of lesions were seen in the group administrated by ACR with low levels of GSO, while the lesions in the other group of administration by ACR with the high levels of GSO were of less scales. The conclusion from this microscopic evaluation was the occurrence of a less and level-dependent impact ameliorating effect of GSO supplementation against ACR-induced lesions in male genital organs of the adult rats.

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INTRODUCTION

Grape seed extract (GSE) was reported to have many beneficial effects as antioxidant and free radical scavenger activity (Fauconneau *et al*., 1998), hepato-protective effect (Jamshidzadeh *et al*., 2008) or protection against ethanol-induced cell death (Chan and Chang, 2006). Grape seed oil (GSO) as an extract of the grape seed has many uses ranging from cooking (as a food additive), cosmetics and in controlling several diseases and wound-healing potential (Shivananda *et al*., 2011). Nowadays, many scientific researchers have revealed that the GSO has several health benefits and is considered as a good and potent antioxidant compound for its contents of polyphenols, flavonoids, unsaturated fatty acids (El-Ashmawy *et al*., 2007) and vitamin E (Dos Santos Freitas *et al*., 2008; Hassanein and Abedel-Razek 2009; Kikalishvili *et al*., 2011).

Acrylamide (ACR) is a chemical content elevated in fried, baked and heat-processed starchy foods. It is also used in various scientific and industrial processes. Although there are likely to be multiple sources of ACR exposure, the oral source of exposure, due to the presence of ACR in foods is considered as the most common source of exposure (WHO 2002). ACR consumption, in human and animals has been shown to be responsible for several toxic effects such as neuropathies and reproductive toxicity (Tyl *et al*., 2000). The reproductive toxicity in rats is considered as one of the methods for evaluation for the toxicity of the ACR (Yang *et al*., 2005). For evaluation of male fertility it has been reported that the histopathologic assay is more sensitive than breeding studies (Takayama *et al*., 1995). In addition, the histopathology is thought to be a useful tool for detection of low Dose effects and clarifying the mechanisms of toxicity (Takahashi and Matsui 1993). So the present work aimed to use the histopathologic tools to study and evaluate the impact and possible ameliorating effect of 3 weeks, daily supplementation with GSO, in 2 levels (5% and 10%) against experimentally induced lesions of ACR intoxication in the genital organs of adult male rats.

MATERIALS AND METHODS

Laboratory animals: A total of 180 adult male Sprague-Dawley rats (of about 150-200 g BW), were obtained from the Animal House of College of Veterinary Medicine & Animal Resources, King Faisal University. The rats were left for one week period to enable acclimatization under standard hygienic conditions. Rats were fed on commercial pellet (obtained from the Grain Silos and Flourmills Organization-Riyadh) with free access to food and water. The National Research Council guide for the care and use of laboratory animals was followed.

Chemicals: Acrylamide (ACR) was purchased from Sigma-Aldrich Chemie GmbH, Riedstr, 2, D-89555 Steinheim Germany, as a white powder with purity over 98%. Grape seed oil (GSO), commercial name (Pepin Raisin), purchased from Huilerie emile, NOEL SAS 30130 Pont-Saint-Esprit-France.

Experimental design: The used rats were randomly divided into 6 groups and housed in separate cages (n=30) and treated for 3 weeks as followings: Group 1 (Gp1), received drinking water (free from any chemicals) *ad libitum*, fed on commercial pellet of rat diet allover the experiment, and maintained as a negative control. Groups 2 and 3 (Gp2 and Gp3) received the commercial diet mixed with 5% and 10% GSO (Kikalishvili *et al*., 2011), and maintained as positive controls for GSO. Group 4 (Gp4) daily intoxicated with ACR (45 mg/Kg BW in drinking water) according to Rahangadale (2012), and maintained as a positive control for ACR-intoxication. Group 5 (Gp5) daily intoxicated with ACR and fed on diet mixed with 5% GSO, and remained as the 1st experimental group for the effect of the low level of GSO. Group 6 (Gp6) daily intoxicated with ACR and fed on diet mixed with 10% GSO, and assigned as the 2nd experimental group for the effect of the high level of GSO. Rats of all groups were clinically observed till the end of the experiment, at which all rats were euthanized and necropsied. Tissue specimens were collected from the male genital organs (testes, epididymis, prostate glands and seminal vesicles) and prepared for histopathologic examination.

Histopathologic techniques: The collected tissue specimens were fixed in Bouin's solution, washed and processed for embedding in paraffin blocks. Paraffin sections of 5 microns-thick were cut and stained with Hematoxylin and eosin (Bancroft and Stevens, 1996) and examined microscopically. The microscopic changes were recorded and comparatively evaluated using a common scales for changes (Misawa *et al*., 2000; Yíldíz *et al*., 2011) in the examined organs (+ 1 = normal to very less changes, + 2 = mild changes, + 3 = moderate changes and + 4 = severe changes).

RESULTS

Regarding the clinical observations in the present work, some neurologic signs of paralysis, splaying and dragging, especially of the hind limbs were observed (starting from the end of the 1st week and later on) in the ACR intoxicated group (Gp4). Similar observations, but in a mild form were also noticed in rats administrated with the ACR and the low level of GSO (Gp5). The other group of rats administrated with the ACR and the high level of GSO (Gp6) exhibited a similar mild form of the neurologic signs from the end of the 1st week but gradually disappeared all over the following days. The rats in the other groups (Gp1, Gp2 and Gp3), did not exhibit any abnormal signs all over the experiment.

Rats in Gp1, Gp2 and Gp3 showed nearly similar scale (+1) of testicular changes. The testes in these groups showed nearly normal and complete spermatogenesis. Most of all the seminiferous tubules appeared filled with normal contents of Sertoli cells, spermatogonial cells, spermatocytes, spermatids, and mature spermatozoa (Figs. 1 and 2). The testes of the rats in Gp4 as well as in Gp5 were nearly similar and showed scales of changes ranged from +3 to +4. The testes showed impairment in spermatogenesis with presence of degenerated spermatogonial cells, immature round spermatids and excess of the multinucleated spermatocytic giant cells in most of all the seminiferous tubules in Gp4 (Figs. 3 and 4), or in a few of the tubules (Fig. 5) in Gp5. The rats in Gp6 showed scale (+2) of testicular changes. The changes were also in form of impairment of spermatogenesis but with presence of round spermatids and few multinucleated spermatocytic giant cells in most of all the seminiferous tubules (Fig. 6).

The epididymal changes in rats of Gp1, Gp2 and Gp3 were nearly of similar scale (+1), where the cauda showed intact epithelium (Fig. 7), while the caput appeared plugged with mature spermatozoa (Fig. 8). The rats in Gp4 and Gp5 showed nearly similar scale (from +3 to +4) of epididymal changes at the two regions. In Gp4, variable degrees of epithelial degeneration, few of the mature spermatozoa and excess of the immature spermatocytes, round spermatids and multinucleated spermatogonial giant cells (Figs. 9 and 10) were seen. The rats in Gp6 showed less and mild scale (+2) of changes. The caput epididymis appeared plugged with excessive number of the mature spermatozoa with a few of the immature round spermatids (Fig. 11), while the cauda showed dilated tubules, some of which were also plugged with the mature spermatozoa and a few round spermatids (Fig. 12).

The rats in Gp1, Gp2 and Gp3, showed similar scale (+1) of the prostatic changes. The prostatic acini were lined with normal epithelium and filled with normal secretory materials (Fig. 13). The prostate glands of the rats in Gp4 and Gp5, showed nearly similar scale of changes (+4). Numerous changes of epithelial hyperplasia, necrosis and desquamation (Fig. 14) were seen, while the secretory materials were very less. The scale of prostatic changes in Gp6 was milder (+3), where the acini showed cystic dilatation with presence of variable amounts of the secretory materials and in some areas appeared separated by edema (Fig. 15).

The rats in Gp1, Gp2 and Gp3 showed similar scales (+1) of changes in the seminal vesicles. The acini showed normal epithelium and contained variable amounts of the normal secretory material (Fig. 16). In case of Gp4 and Gp5, nearly similar scales (+2) of changes were seen. The acini showed epithelial changes of hyperplasia, necrosis

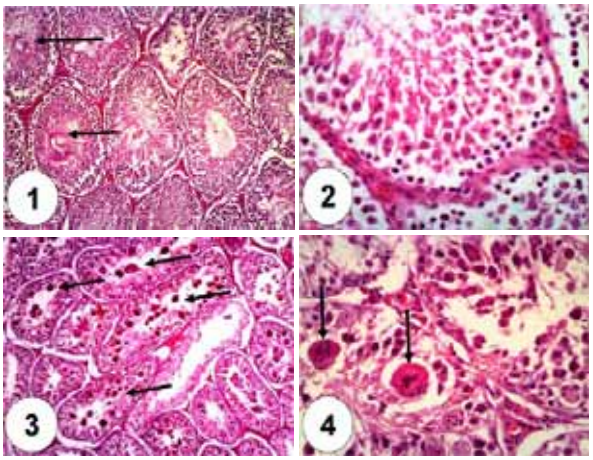

Fig. 1: Testis, Gp1 (Scale +1): Complete spermatogenesis in most of all the seminiferous tubules (arrows). H and E. X 250; **Fig. 2:** Testis, Gp1 (Scale +1): Complete spermatogenesis in the seminiferous tubule. H and E. X 400; **Fig. 3:** Testis, Gp4 (Scale +3): Large number of the multinucleated spermatocytic giant cells (arrows) with no spermatids or spermatocytes inside the lumina of most tubules. H and E. X 250; **Fig. 4:** Testis, Gp4 (Scale +4): Testicular degeneration with few mononuclear round cells and excess of multinucleated giant cells (arrows) in most of the seminiferous tubules. H and E. X 400.

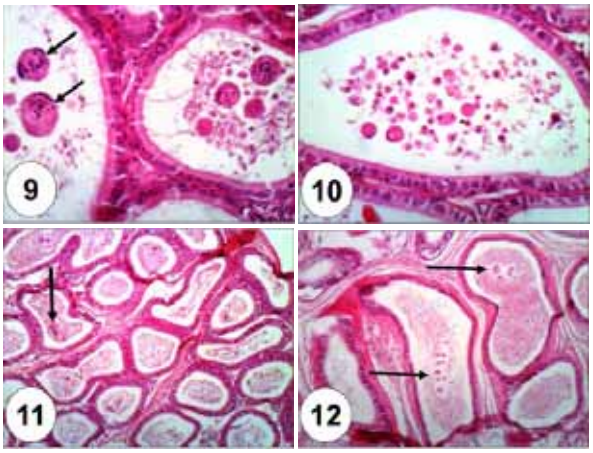

Fig. 9: Caput epididymis, Gp4 (Scale +3): Degenerated and necrotic round spermatids with excessive multinucleated spermatocytic giant cells (arrows). H and E. X 400; **Fig. 10:** Cauda epididymis, Gp4 (Scale +4): Large numbers of the immature round spermatids and multinucleated spermatocytic giant cells (arrows). H and E. X 250; **Fig. 11:** Caput epididymis, Gp6 (Scale +2): Tubules plugged with mature spermatozoa mixed with few immature round spermatids (arrows). H and E. X 160; **Fig. 12:** Cauda epididymis, Gp6 (Scale +2): Tubules plugged with mature spermatozoa mixed with numerous immature round spermatids (arrows). H and E. X 160.

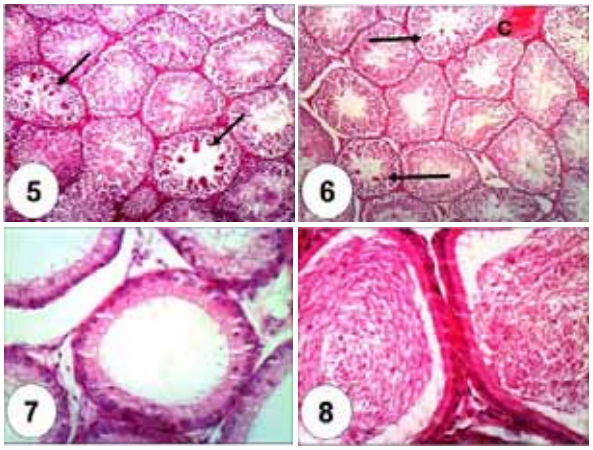

Fig. 5: Testis, Gp5 (Scale +3): Large number of the multinucleated spermatocytic giant cells (arrows) in few of the seminiferous tubules. H and E. X 250; **Fig. 6:** Testis, Gp6 (Scale +2): Few multinucleated spermatocytic giant cells (arrows) and congested interstitial blood vessel (C). H and E. X 250; **Fig. 7:** Caput epididymis, Gp1 (Scale +1): Normal intact epithelium and free lumina (Scale +1). H and E. X 400; **Fig. 8:** Cauda epididymis, Gp1 (Scale +1): Normal tubules plugged with mature spermatozoa. H and E. X 400

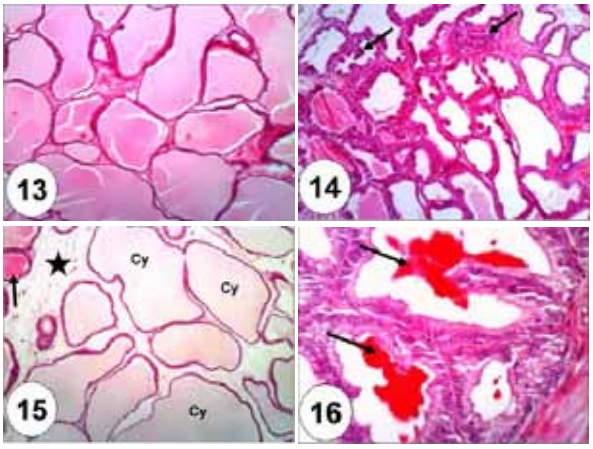

Fig. 13: Prostate gland, Gp1 (Scale +1): Normal dilated acini filled with normal secretory materials. H and E. X 160; **Fig. 14:** Prostate gland, Gp4 (Scale +4): Mild epithelial hyperplasia with absence of the secretions in some of the acini (arrows). H and E. X 160; **Fig. 15:** Prostate gland in Gp6 (Scale +3): Cystic acini (Cy) widely separated with edema (asterisk). The acini filled with normal secretions (arrow). H and E. X 160; **Fig. 16:** Seminal vesicle, Gp1 (Scale +1): Normal epithelium and secretory materials (arrows). H and E. X 400

and desquamation (Fig. 17). The rats in Gp6 showed scale (+1) of changes, where the acini showed nearly normal epithelium but accompanied with less amounts of secretory materials (Fig. 18).

DISCUSSION

The current work aimed to study and evaluate the possible ameliorating effect of GSO supplementation on the experimentally induced lesions of ACR intoxication in the male genital organs of the rats. The intoxicated rats in Gp4 exhibited gradual neurologic signs of paralysis, splaying and dragging of the hind limbs starting from the

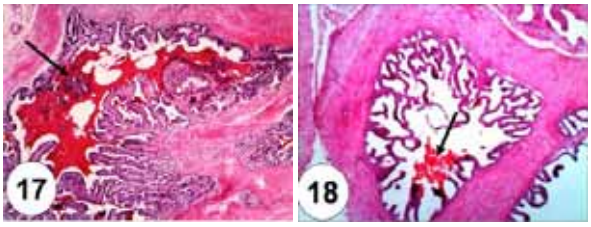

Fig. 17: Seminal vesicle, Gp4 (Scale +2): Hyperplastic epithelium with some necrotic and desquamated cells mixed with the secretory materials (arrow). H and E. X 250; **Fig. 18:** Seminal vesicle, Gp6 (Scale +1): Less or non-hyperplastic epithelium and few secretions (arrow). H and E. X 160.

end of the 1st week of administration. These neurologic signs were similar to the reported signs for ACR intoxication (Tyl *et al*., 2000; LoPachin 2004; Rahangadale *et al*., 2012), that were mainly attributed to the induced lesions in the spinal cord. However, a mild form of these signs were observed in rats administrated by ACR with low level of GSO (Gp5), while the group of rats administrated with the ACR and the high level of GSO (Gp6) exhibited a great diminish of these neurologic signs starting from the end of the 1st week and disappeared gradually in the following days of the experiment. These observations indicate clearly the level-dependent positive impact of GSO on ameliorating the neurotoxic effect of ACR.

The testicular lesions in rats of the intoxicated rats in Gp4 were indicative for the severe toxic effect (scale +3 to +4) of the ACR. The seminiferous tubules showed degrees of spermatogonial cell degeneration, absence of the mature spermatocytes and spermatids with presence of excessive multinucleated giant cells. These findings were consistent to what reported by Al-Damegh (2012) with regard to the oxidative stress of the toxic agents on the testes. Moreover, the present data for the testicular changes in Gp5, of the administrated rats with ACR and the low level of GSO revealed the testicular affection with a nearly similar scales ranged from +3 to +4. These findings indicate the less or no effect for the low level of GSO. The testes of the administrated rats with ACR and the high level of GSO (Gp6), showed scales of testicular changes ranges from +1 to +2. Very few numbers of the multinucleated giant cells were seen inside the seminiferous tubules. These findings assure the positive ameliorating effect of high level of GSO over the low level (level-dependent effect). In fact, the ameliorating effect of GSO against ACR-induced lesions is mainly attributed to its known anti-lipid peroxidation and antioxidant mechanisms (Li *et al*., 2001). These results agree with Al-Damegh (2012) who mentioned that it is beneficial to use exogenous antioxidants that have been assessed before for their ability to counteract the induced oxidative stress in the testes. Our findings for the ameliorating effect of GSO against ACR intoxication are supported by the report of Lee *et al*. (2005) about the chemoprevention of acrylamide toxicity by using of antioxidants. These findings are also partially agree with what reported by El-Ashmawy *et al*. (2007) for the impact of GSE in case of ethanol toxicity in the testes.

Concerning the present histopathologic findings in the epididymis, the intoxicated rats in Gp4 as well as the rats administrated by ACR with the low level of GSO (Gp5) showed nearly similar scale (ranged from +3 to +4) of epididymal changes. These epididymal changes were consisted of variable degrees of epithelial degeneration with less numbers of the mature spermatozoa and excess of the immature spermatogonial cells and multinucleated giant cells. On the other hand, the rats administrated by the ACR with the high level of GSO (Gp6) showed milder scale (+2) of the epididymal changes. The epididymal tubules were plugged with excess of the mature spermatozoa and few immature round spermatids. These occurred lesions in the epididymis not previously mentioned in other works. We observed that changes seem to be come consistent with the previously mentioned lesions in the testes. These findings reflect the positive impact for the high level of GSO over than the low level. Therefore, it was clearly that GSO has also a level-dependent ameliorating effect on the ACR-induced lesions in the epididymis.

The results for the prostatic microscopic changes (scale +4), in the intoxicated rats of Gp4 were nearly similar to those seen in the rats administrated by the ACR and the low level of GSO (Gp5). The epithelium of the acini was affected by several degrees of hyperplasia, necrosis and desquamation. These changes, especially of epithelial hyperplasia suggesting predisposing lesions for the proposed carcinogenic effect of ACR intoxication (Klaunig, 2008). The rats in other group administrated by ACR with high level of GSO (Gp6), showed milder changes (scale +3) than those in Gp4 and Gp5. The acini showed edema and cystic dilatation with mild degrees of the epithelial degeneration and hyperplasia. The present microscopic changes in the seminal vesicles were somewhat less severe than in the prostate gland. These changes were nearly similar (scale +2) in the rats of Gp4 and Gp5. The seminal vesicles in these rats showed epithelial changes nearly similar to those seen in the prostate glands (degeneration, hyperplasia, and desquamation) but less severe. These findings were somewhat milder or absent (scale +1) in the rats of Gp6. The acini were lined with apparently normal epithelium but contained less secretory materials. The mentioned findings in the prostate glands and the seminal vesicles were also indicative of the more ameliorating effect of high level of the GSO, on ACR intoxication than the low one.

Conclusion: The present work concluded that the ACR-induced lesions in male rats were obviously seen in the testes and epididymis, while less change was seen in the prostate glands and the seminal vesicles. The testicular changes for the impairment of the spermatogenesis sure lead to infertility of the exposed males. The comparative microscopic evaluation, for the concurrent administration of the GSO led to main conclusion for the positive impact and ameliorating effect of the GSO (especially in high levels) against the induced lesions of the ACR in the male reproductive organs of the rats, so it could be used in human diet as a food supplement to improve the fertility, nutritive and protective value.

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