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Research Article

Immunohistohemical and Molecular Studies of Mouse Liver in Castrated Subjects treated with Grape Juice -

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SUMMARY

The present study was designed to evaluate the alterations in the liver tissues especially Ito and Kupffer cells of both castrated and castrated mice treated with10 µl/g of grape juice. The present study was conducted on forty five healthy males of Swiss albino mice, which *were* divided into 3 groups (N= 15 mice per each group). The first group was intact (control); the second one was castrated and the third group was castrated treated with 10 µl/g of grape juice. The immunohistochemical and molecular sections of liver tissue from castrated group stained with vimentin antibody, showed a strong intensity of positive stain (+++), as compared with sections from intact group (control) which showed a weak intensity of positive stain (+). On the other hand sections from castrated subjects treated with 10µl/g grape juice revealed a moderate intensity of positive stain(++) as compared with control group. The present study concluded that surgical castration aggravated increased hepatic steatosis and increased inflammatory response by increased activation of Kupffer and Ito cells, these effects could be reserved by aggravated increased grape juice administration.

2. Keywords: Immunohistochemistry; molecular; Castration; Sections; Grape; Liver

INTRODUCTION

The mammalian liver is the largest internal digestive organ, which is indispensable in many essential physiologic processes and vulnerable to be impaired by a wide variety of factors, such as toxins, microorganisms, metabolic products, circulatory materials and metabolism of carbohydrates, lipids and proteins [1,2]. Kupffer cells, the hepatic resident macrophages, represent the largest group of macrophages in the body and account for about 20–25% of non-parenchymal cells in the liver (intrasinusoidal cells) [3,4,5]. As the critical component of the innate immune system, Kupffer cells can be activated by various endogenous and exogenous stimuli, and play a key role in regulating the phenotype and function of neighboring parenchymal and non-parenchymal cells [6]

Castration is a way of studying the consequences of extreme testosterone deficiency in animal models [7]. Castration promotes progression to steatohepatitis through activation of the ER (Endoplasmic Reticulum) stress pathway and enhancement of macrovesicular droplet. Some medicinal plants may exert promising pharmacological properties and improve the effectiveness of conventional medications as complementary agents [8]. Vitis vinifera (Grape) are one of the most consumed fruits globally. It possesses a wide range of pharmacological activities due to its rich polyphenol ingredients most of which have been demonstrated to have therapeutic or health promoting properties [9], among them, flavonoids are the most abundant and widely studied, recent studies have shown that the beneficial health effects promoted by consumption of grape and grape products are attributed to the unique mix of polyphenolic compounds. As the largest group of grape polyphenols, flavonoids are the main candidates considered to have biological properties, including but not limited to antioxidant, anti-inflammatory, anti-cancer antimicrobial, antiviral, cardioprotective, neuroprotective and hepatoprotective [10,11].Therefore, the aim of the present study was to determine the effects of castration, and treatment of castrated subjects by using grape juice on both Ito and Kupffer cells by using Scanning Electron Microscope (SEM).

MATERIALS AND METHODS

Swiss albino male mice weighting between (14-17) g., and aged (3weeks) were used in the present study, the mice were obtained from the Animal House, Faculty of Science/ University of Kufa. Animals were kept in ventilated cages, with a temperature of (25±2C°) at 12:12 h light, dark cycle was used balanced, rodent food pellet and water were provided ad libitum [12]. All experimental protocols using live animals were first reviewed, approved and accepted according to guidelines for the care and use of laboratory animals in biomedical research [13]. A total number of 45 Swiss albino mice were used in the present study. Animals were divided into 5 groups (N=15), and the treatment was started at the age of 21 days for 6 weeks as:

Group 1: Intact male mice received tap-water as control.

Group 11: Castrated male mice received tap-water as (positive group).

Group 111: Castrated male mice treated daily with (10µl/g) of grape juice,

The surgical castration method was done according to [14].

Black grape (*Vitis vinifera*) obtained from local market (Baghdad, Iraq) 100g of grape was blundered by using a commercial blender without separating the seeds, and then it was filtered to remove the residue. The resulting extract (10 mls was stored in the refrigerator at 4˚C, and used after one hour. The extract was prepared according to [15]. A previous study documented that 10µl/g/day of grape juice extract was effective dose [16,17]. For this protocol, we used in the present study 10µl /g/day and was given daily as orally administered for six weeks. Animals were sacrificed at the end of the experiments, with using ketamine and xylazine as anesthetic drugs to anesthetize the mice. The preparation procedure for immunohistochemistry has been described by [15]. Number of both Ito and Kupffer cells in cytoplasmic color in form of brown scale intensity was done according to [18].

Results were expressed as the mean ± Standard Deviation of the mean (S.D). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (SPSS version 16.0) the p values $p< 0.05$ was considered as significant for all statistical analysis in this study.

RESULTS

Monoclonal mouse Anti Vimentin: The present findings of the scoring number of Ito cells in different experimental groups are shown in table 1. The results of table 1 and figure 1 showed a weak intensity to anti- vimentin in intact male (control) mice (+). Whereas sections from castrated subjects revealed strong intensity of positive stain (+++), figure 2: On the other hand, the Immunohistochemical sections from castrated subject treated with10µl/g grape juice (10) showed a moderate intensity of positive stain (++), figure 3.

Monoclonal mouse Anti- Human CD68: The present findings of the scoring number of Kupffer cells in different experimental groups are shown in table (2).

The present findings of table 2 and figure 4 from intact (control) group stained with CD68 antibody showed a weak intensity of positive stain (+). Whereas, sections of liver tissue from castrated subjects revealed a strong intensity of positive stain (+++) to CD68 antibody, figure 5. Moreover, sections from castrated subject treated with 10µl/g grape juice stained with CD68 antibody appeared a moderate intensity of positive stain (++) figure 6.

DISCUSSION

Vimentin antibody: In the present study the results of immunohistochemical sections of liver tissue from intact (control) group, stained with Vimentin antibody appeared a weak intensity of positive stain (+). With regard to sections of liver tissue from

Table 1: Showing the scoring number of Ito cells in different experimental groups.

A (percentage ofpositive cells)	B (intensity ofstaining)	score (multiplicationof A and B)	Power
0 = no positive cells	0 = no color reaction	0-1 = negative	**0 poor**
1 = <3 of positive cells	1 = mild reaction	2-3 = mild	**+ weak**
2 = 3-4 positive cells	2 = moderate reaction	4-8 = moderate	**++ moderate**
3 = 5-7 positive cells	3 = intense reaction	9-12 = strongly positive	**+++ strong**
4 = >8 positive cells	**Final IRS score (A × B): 0-12**		

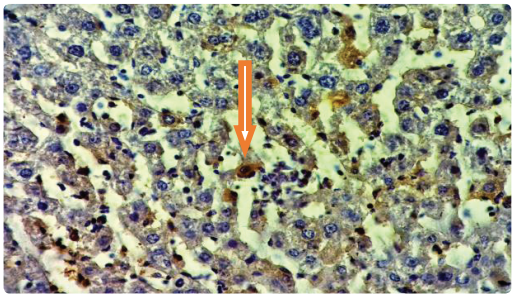

Figure 1: Immunohistochemical section of liver tissue from intact male mice stained with vimentin antibody showing weak intensity of positive stain (+) arrow indicated Ito cells (X400)

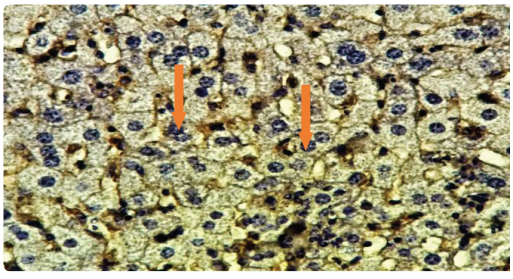

Figure 2: Immunohistochemical section of liver tissue from castrated group stained with vimentin antibody, showing strong intensity of positive stain (+++) arrow indicated Ito cells (X400).

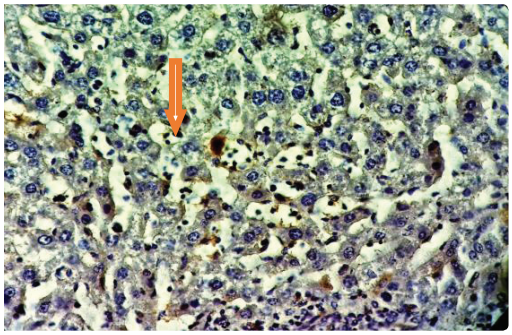

Figure 3: Immunohistochemical section of liver tissue from castrated group treated with10µl/g grape juice stained with vimentin antibody showing a moderate intensity of positive stain (++)arrow indicated Ito cells400x.

Table 2: showing the scoring number of Kupffer cells in different experimental groups.

A (percentage of positive cells)	B (intensity of staining)	score (multiplication of A and B)	Power
0 = no positive cells	0 = no color reaction	0-1 = negative	0 poor
1 = <7 of positive cells	1 = mild reaction	2-3 = mild	+ weak
2 = 7-8 positive cells	2 = moderate reaction	4-8 = moderate	++ moderate
3 = 9-11 positive cells	3 = intense reaction	9-12 = strongly positive	+++ strong
4 = >12 positive cells	Final IRS score (A × B): 0-12		

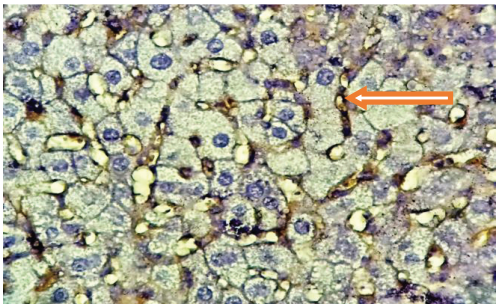

Figure 4: Immunohistochemical section of liver tissue from intact group stained with CD68 antibody showing weak intensity of positive stain (+) arrow indicated Kupffer cells (X400).

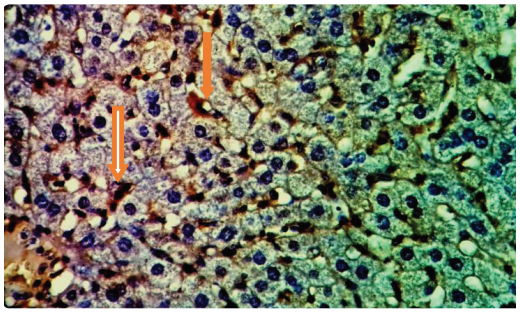

Figure 5: Immunohistochemical section of liver tissue from castrated group stained with CD68 antibody showing strong intensity of positive stain (+++) arrow indicated Kupffer cells (X400).

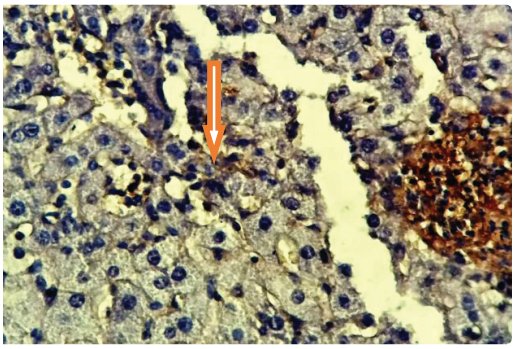

Figure 6: Immunohistochemical section of liver tissue from castrated mice treated with 10µl/g grape juice stained with CD68 antibody showing moderate intensity of positive stain (++) arrow indicated Kupffer cells (X400).

castrated group, they showed a strong positive immune expression of HSC markers Vimentin. These findings could be explained that the vimentin expression is linked to liver fibrosis and inflammatory infiltration, as activated Hepatic Stellate Cells (HSC) secreted cytokines that attracted inflammatory cells. Also the activation of HSC may be closely related to the role of ROS and oxidative stress in stimulating the expression of pro inflammatory and profibrotic molecules. In response to liver injury, [19,20] demonstrated that an excess lipids, lipid metabolites, and inflammatory cytokines released by hepatocytes and Kupffer cells in the setting of NAFLD, as well as activation of stellate cells. Another study by [21], reported that these cells were able to lose their abilities for producing and storing vitamin A acquire a proliferative myofibroblast-like phenotype and synthesize large amounts of extracellular matrix.

The present results of immunohistochemical sections of liver tissue from castrated subject treated with grape juice showed a moderate positive immune expression of HSC markers Vimentin. These findings may be attributed to the protective effect of grape juice and its components (polyphenol ,diferuloylmethane, flavonoides and others) that have manifested a diverse range of pharmacological activities including anti- inflammatory, antioxidant, antibacterial, and antitumor properties. [22] Stated that excess ECMs, including collagen, are mainly produced by activated HSCs. These findings are in agreement with other studies who stated that, there was an increase in positive number of Ito cells which lead to increase the expression of Vimentin antibody [22,23].

CD68 antibody: Results of immunohistochemical sections of liver tissue from intact (control) group stained with CD68 antibody showed a weak intensity of positive stain. Whereas such sections from castrated group showed a strong positive immune expression of Kupffer marker CD68. These results could be explained that CD68 linked to liver damage and inflammation, since NAFLD is associated with inflammation, an important relationship between lipotoxcitiy and macrophage activation, toll like receptors, TLR4 represent a major role to activate Kuppfer cells. Steatosis may interfere with sinusoid microcirculation and hepatocellular clearance microbial and host drive danger singles, which lead to activation Kupffer cells. These findings are in agreement with the findings of [24] who concluded that a decreased in androgen receptor has a main role in progression steatohepatities and inflammation which considered the main reason to activation Kuppfer cells.

Immunohistochemical sections of liver tissue from castrated group treated with grape juice showed a moderate positive immune expression of Kupffer marker CD68. These results may be due to the protective effect of polyphenolic compounds against the inflammation and lipotoxicity. Grapes are rich within polyphenol compounds and flavonoides which reduces the metabolic effect, lipid accumulation, insulin resistant regulate glucose levels and inflammation by antioxidant activity, anti-diabetic and hepatoprotective [25,26]. Flavonoids act as anti-oxidants by neutralizing oxidizing free radicals, including the superoxide and hydroxyl radicals. These findings are in agreement with the results of [27,28].

CONCLUSION

The present study demonstrated that impaired metabolic process related with surgical castration enhanced immune and inflammatory response, represented by activation of Kupffer and Ito cells which lead to hepatic apoptosis and contribute to increase nonalcoholic fatty liver disease. On the other hand the intra gastric application of Grape juice has dramatic effects to restore liver structure in castrated animals.

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