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The Intra Gastric Effects of Grape Juice on Histological Structure of Liver Tissue in Castrated Treated Mice

Homady MH*[1], ALquraishi LO[2], Tanya S Salih[1] and Juma ASM[1]

[1]College of Science, Department of Biomedical Sciences, Cihan University-Erbil, Kurdistan Region, Iraq

[2]College of Dentistry, Babylon University, Iraq

***Corresponding author:** Homady MH, College of Science, Department of Biomedical Sciences, Cihan University-Erbil, Kurdistan Region, Iraq, Tel: 009647517118678; Email: merzahh@yahoo.com

Research Article

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Abstract

The effect of ingestion of freshly prepared ethanoic extract of grape juice was investigated on the histology of liver tissue on both castrated and castrated treated with 10 µl/g of grape juice. The histological sections of liver tissue from castrated group showed ballooning degeneration, macro and micro vesicular steatosis, lobular inflammation, hydropic degeneration, cellular inflammatory of lymphocytes and acidophil bodies (Councilman Body) as compared with the control group. The orally administration of 10µl/g grape juice for 6 weeks to castrated subjects was able to restore the histological structure of liver tissue to normal structure as compared with the control group. These results suggest that the fresh extract of grape juice can exert hepatoprotective effects of castration.

Keywords: Liver; Histology; Castration; Steatosis; Grape Juice

Introduction

The liver tissue is made up of the largely predominant parenchymatous cells (hepatocytes), as well as Ito (perisinusoidal cells), Kupffer cells and sinusoidal endothelial cells [1-3]. Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. It is present in 30% of the general adult population. In reality, NAFLD comprise as a spectrum of hepatic abnormalities that are observable in liver histological slides, from a simple intrahepatic accumulation of fat (steatosis or nonalcoholic fatty liver, NAFL) to various degrees of necrotic inflammation (NASH nonalcoholic steatohepatitis) [4-7]. Simple steatosis rarely progresses to advanced disease, whereas, in approximately 20% of patients with NASH, it progresses to fibrosis and cirrhosis and potentially to hepatocellular carcinoma. NAFLD is also deemed to be hepatic manifestation of metabolic syndrome, which is a cluster of complex conditions, including central obesity, hypertension, hyperglycaemia, hypertriglyceridemia, hypercholerteremia and high LDL (low density lipoprotein) that are predictive risk factors of cardiovascular disease, stroke, and diabetes [8].

Testosterone deficiency has been associated with increased accumulation of Visceral Adipose Tissues (VAT), insulin resistance and accumulation intrahepatic lipids, thus the lower testosterone levels are associated with nonalcoholic fatty liver disease [9,10]. Farm animals have been castrated to eliminate breeding and reduce aggressive behavior [11,12] Hypogonadism after castration caused abdominal obesity, impaired fasting glucose, excess accumulation of liver triglyceride, and thigh muscle loss. Castration also induced increase of visceral fat mass and decreased thigh muscle mass in mice [13]. Castration promotes progression to steatohepatitis through activation of the ER (endoplasmic reticulum) stress pathway and enhancement of macro

vesicular droplet. Testosterone suppresses ER stress, inhibits the formation of macro vesicular lipid droplets, promotes lipid export, and ameliorates steatohepatitis induced by castration [14] Low serum testosterone levels have a higher risk of developing hepatic steatosis [15].

Vitis vinifera (Grape) is 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 [16]. 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, and hepatoprotective [17]. Moreover, Flavonoids also have shown to influence the apoptotic effects of cytokines, chemotherapeutic agents, and gamma radiation [18]. The present study was aimed to study the effects of both castration and manipulation of fresh grape juice on structure of liver tissue.

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±2Cº) at 12:12 h light, dark cycle was used balanced, rodent food pellet and water were provided ad libitum [19]. 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 [20].

A total number of 45 Swiss albino mice were used in the present study. Animals were divided into 3 groups (N=15), castration and 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. grape juice, the surgical castration method was done according to Al-Fatlawi AA [21].

Plant material extract: 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 Al-Ahmadi AA [22].

Dose Selection: A previous study documented that 10µl/g/ day of grape juice extract was effective dose [23,24]. For this protocol, 10µl /g/day are used in the present study 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.

Histological sections were prepared according to the procedure described by Chong WC [25] as following:

a) Fixation: samples were fixed in formalin 10% for 24-48 hours.
b) Washing and dehydration: samples were washed by water to prevent over fixing. And transferred to graded series of increasing ethanol concentrations 50%, 70%, 80%, 90%, 95%, for 1-2 hours than 100% for overnight.
c) Clearing: using xylene for twice at half – one hour.
d) Embedding: the samples were embedded by using soluble paraffin wax in 60C˚ in the thermal console for 1-2 hour.
e) After Embedding tissues, samples transferred to blocks, let go in room temperature to acquire solidly by cryo console.
f) Sectioning: histological sections were prepared by using auto-microtome at thickness of (5µm), cut by tapes transferred to water bath in temperature (55-50C˚).
g) Then, dry by slide dryer.

Sections are stained by using Acid Eosin Hematoxylin stain [25] as following:

A. Paraffin wax was removed from glass slides by placing in the oven at 58-65 C˚ temperature for a half hour and placed in xylene (twice) for a half hour to remove all wax.
B. Section are dried from xylene then rehydration by passing through a series of alcohol from descending concentrations for (2-3 minutes), then placed in tap water for (5 minutes).
C. Sections are placed in Hematoxylin for (10-15) minutes.
D. Sections are washed by tap water (5) minutes.
E. Sections are ducked in acid – alcohol for little seconds to prevent over the stain.
F. Sections are washed by tap water for 5 minutes until return the blue color.
G. Sections are placed in Eosin stain for (10-30) seconds.
H. Sections are washed by tap water.
I. Dehydration from glass slides by passing through series of ascending concentrations of ethanol alcohol for 2-3 minutes for each concentrate.
J. Sections are placed in xylene for half hour.
K. Then, cover specimen by cover slide with Histofluid (DPX).

Results

The histological sections of liver tissue from intact male mice (Figure 1) showed a normal histological structure that can identify the boundaries of the liver lobule by locating

the central vein at its center, and portal triads (portal vein, hepatic artery and bile duct) at its periphery. The hepatocytes are cuboidal in shape and are arranged in plates, one cell thick, usually, separated by sinusoids contain some blood cells (Figure 2).

Sections of liver tissue from castrated male mice (Figures 3 & 4) appeared ballooning degeneration of hepatocyte and lipid accumulation (Macro vesicular steatosis), with peripherally located nuclei. Moreover, castration also resulted in cellular inflammation (Figure 5) as well as hydropic degeneration and Micro vesicular steatosis with cellular inflammatory of lymphocytes (Figure 6). Mean time the sections also showed acidophilic bodies *(Councilman* Body) as seen in (Figure 7). Treatment of castrated subjects with 10µl/g grape juice (Figure 8) was able to restore the liver tissue to the normal structure as compared with the control group.

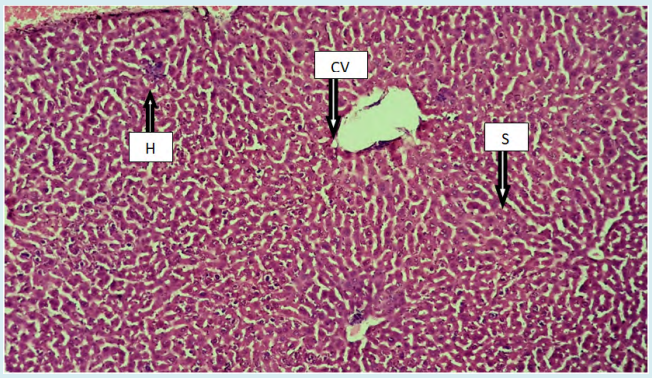

Figure 1: Cross histological section of liver tissue from intact male mice showing the normal structure of hepatocytes (H), central vein (CV) and sinusoid(S), (X100).

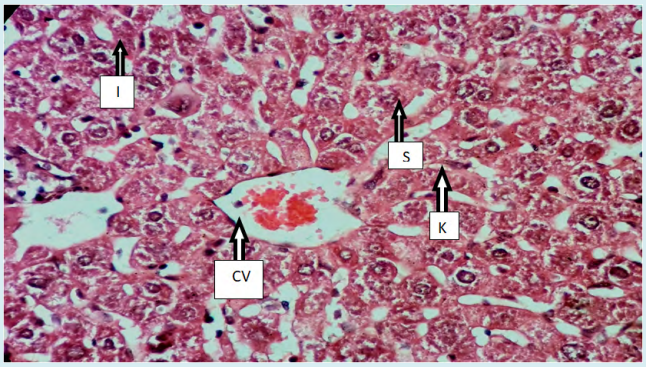

Figure 2: Cross histological section of liver tissue from intact male mice showing Normal histological structure central vein (CV), sinusoid (S), Ito cells (I) and Kupffer cells (K) (X400)

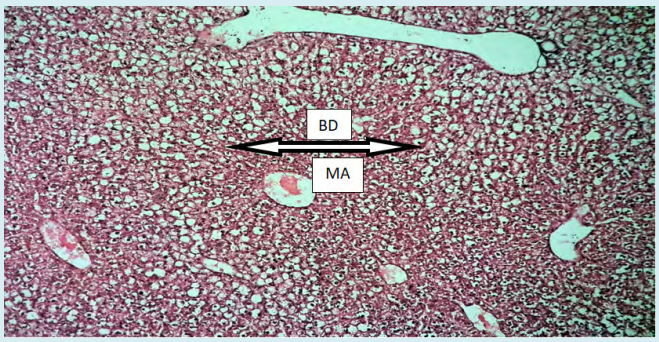

Figure 3: Cross histological section of liver tissue from castrated male mic showing Ballooning Degeneration (BD) and lipid accumulation in hepatocytes. Macro vesicular steatosis (MA) (X100).

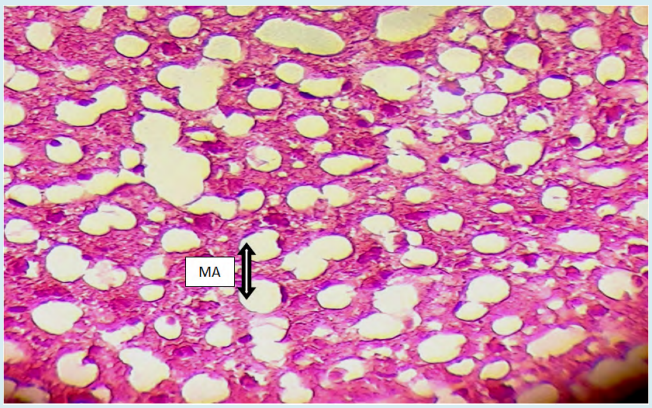

Figure 4: Cross histological section of liver tissue from castrated male mice showing lipid accumulation Macro vesicular steatosis (MA) (X400).

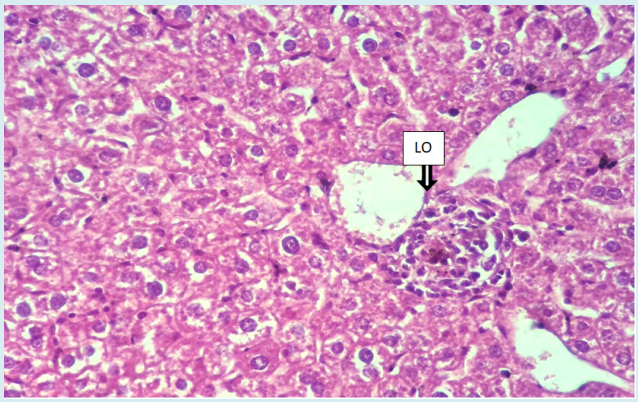

Figure 5: Cross histological section of the liver tissue from castrated male mice showing lobular inflammation (LO), (X400).

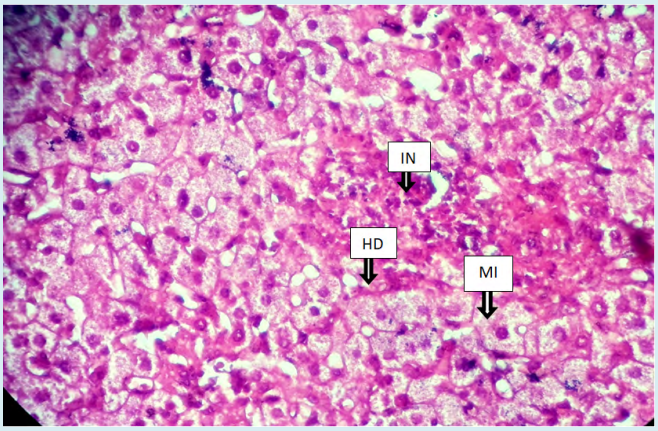

Figure 6: Cross histological section of the liver tissue from castrated male mice showing Hydropic degeneration (HD), Micro vesicular steatosis (MI) with inflammatory cellular of lymphocyte (IN), (X400)

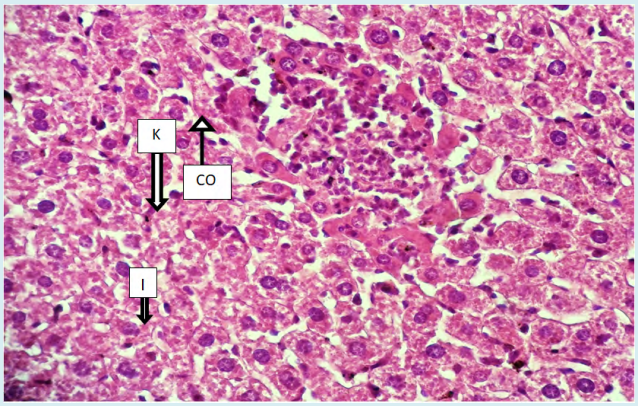

Figure 7: Cross histological section of liver tissue from castrated male mice showing *Councilman B*ody (CO), Kupffer cell (K), Ito cell (I), (H&E, X 400)

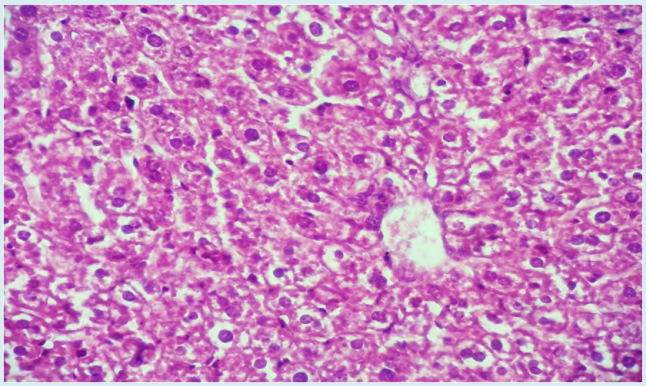

Figure 8: Cross histological section of liver tissue from castrated group treated with 10µl/g grape juice showing normal histological structure (X400).

Discussion

The histological sections of liver tissue from castrated male mice showed ballooning degeneration in hepatocytes and lipid accumulation (macro vesicular steatosis) lobular inflammation, Hydropic degeneration, micro vesicular steatosis with inflammatory cellular of lymphocyte and apoptosis in hepatocytes (*Councilman* Body). These changes might be due to the molecular mechanisms by which testosterone deficiency is involved in the pathogenesis of NAFLD. Several pathways have been proposed: increased adipose tissue increased hepatic lipogenesis, increased hepatic fatty acid B-oxidation, and decreased export of lipids from the liver caused Obesity, hyperglycemia, and insulin resistance often increased lipogenesis and hepatic steatosis. Another reason may be attributed to increase the reactive oxygen species (ROS) pathway, in which was stimulated by castration to induced cells injuries in the liver. Minehira and Gual [26] noticed that disturbances in fatty acid oxidation lead to excess lipid storage in the liver. Moreover, this observation demonstrated that there are many genes involved in fatty acid oxidation were regulated in the liver of castrated animals, and these effects were prevented by testosterone replacement like peroxisome proliferator-activated receptor (PPARD) regulates lipid oxidation processes. Saez Lopez, et al. [27] found that the levels of PPARD, mRNA and protein were significantly reduced in the livers of castrated animals and treatment with testosterone was able to restore the expression of PPARD. This provides a novel view of PPARD function in the modulation of hepatic lipid metabolism.

In the present study, the increased inflammatory cells in liver tissue considered "two-hit" theory of NAFLD progression proposes that inflammation, oxidative stress, and apoptosis play critical roles in the pathological progression of NAFLD [28]. Harada, et al. [29] who demonstrated that low testosterone levels increase the risk for cardiovascular disease in men and lead to shorter life spans. Androgen deprivation via castration caused obesity, impaired fasting glucose, excess hepatic triglyceride accumulation, thigh muscle weight loss, gene expression profiling indicated that several immune and inflammatory response genes were activated in the livers of castrated mice. Zhang N, et al. [30] concluded that castration promote progression to steatohepatitis through activation of endoplasmic reticulum stress pathway and enhancement micro vesicular droplet steatosis. Cia Z, et al. [31] noticed the pro-inflammatory of chemokines contributes to the mechanism of inflammatory recruitment in hepatic steatosis induced by testosterone deficiency.

On the other hand, tumor necrosis factor (TNF) may have a pathogenic role during NAFLD development,

specifically by modulating chronic lobular inflammation with hepatocellular injury. Testosterone has been suggested to act directly on immune cells by repressing transcription factors (such as FOS, JUN oncogenic transcription factors). Furman D, et al. [32] concluded that increased oxidative stress may induce hepatocyte apoptosis, resulting in more severe liver injury hepatic apoptosis was important to note that several genes associated with apoptosis, including B-cell leukemia/lymphoma-2 (*BCL2*), caspase-2 (*CASP2*), and bcl-2 associated-x (*BAX*), were up regulated in the livers of castrated pigs in response to testosterone deficiency. The present findings are in aggrement with the findings [33-35].

Histological sections of liver tissue from castrated group treated with grape juice showed the protective effect of this extract on the liver tissue by returning it to its normal structure. These results may be due to the role of poly phenolic and flavonoids compounds were commonly have been reported to multiple biological effects, including antioxidant activity, anti-diabetic and hepatoprotective. Flavonoids act as anti-oxidants by neutralizing oxidizing free radicals, including the superoxide and hydroxyl radicals. The properties of flavonoids also allow them to act as reducing agents and presence of flavonoids and phenolic compounds have been recognized as excellent scavengers of superoxide, hydroxyl ion and peroxyl radicals there by inhibiting lipid peroxidation.

Polyphenols are reported to exert hepatoprotective effects through increasing fatty acid oxidation in the liver and improving insulin sensitivity. The preventive effect of grape juice against hepatic steatosis may be predominantly due to proanthocyanidins. These findings are within the agreement of Ferramosca A [36] who stated positive effects of several classes of antioxidants, such as polyphenols (resveratrol, quercetin, coumestrol, anthocyanins, epigallocatechin gallate and curcumin), carotenoids on the reversion of fatty liver, and in some cases as an indirect interaction with mitochondrial metabolism.

Conclusion

Therefore, in the present study we can conclude that Grape juice appears to act as a protective agent against the effects of whole castration.

References

1. Wang H, Thorling CA, Liang X, Bridle KR, Grice JE, et al. (2015) Diagnostic imaging and therapeutic application of nanoparticles targeting the liver. Journal of Materials Chemistry B 3(6): 939-958.

2. Kaplan JB, Kalra A, Biggins SW (2017) Liver Anatomy and Function. In Radiation Therapy for Liver Tumors Springer, Cham pp: 3-11.

3. Kubes P, Jenne C (2018) Immune Responses in the Liver. Annual review of immunology 36: 247-277.

4. Demir M, Lang S, Steffen HM (2015) Nonalcoholic fatty liver disease–current status and future directions. Journal of digestive diseases 16(10): 541-557.

5. Yu J, Marsh S, Hu J, Feng W, Wu C (2016) The pathogenesis of nonalcoholic fatty liver disease: interplay between diet, gut microbiota, and genetic background. Gastroenterology research and practice.

6. Marengo A, Rosso C, Bugianesi E (2016) Liver cancer: connections with obesity, fatty liver, and cirrhosis. Annual review of medicine 67: 103-117.

7. Ibrahim SH, Hirsova P, Gores GJ (2018) Non-alcoholic steatohepatitis pathogenesis: sublethal hepatocyte injury as a driver of liver inflammation. Gut 67(5): 963-972.

8. Trauner M (2018) New Insights into the Pathogenesis of Non-Alcoholic Fatty Liver Disease. Annual Review of Pathology: Mechanisms of Disease 13(1).

9. Kelly DM, Jones TH (2015) Testosterone and obesity. Obesity Reviews 16(7): 581-606.

10. Jaruvongvanich V, Sanguankeo A, Riangwiwat T, Upala S (2017) Testosterone, sex hormone-binding globulin and nonalcoholic fatty liver disease: a systematic review and meta-analysis. Annals of hepatology 16(3): 382-394.

11. Moya D, González LA, Janzen E, Caulkett NA, Fireheller E, et al. (2014) Effects of castration method and frequency of intramuscular injections of ketoprofen on behavioral and physiological indicators of pain in beef cattle. Journal of animal science 92(4): 1686-1697.

12. Sawhney P (2016) Burdizzo Versus Pinhole Castration in Bucks and Cattle Calves (Doctoral dissertation, Nanaji Deshmukh Veterinary Science University Jabalpur).

13. Harada N, Hanaoka R, Hanada K, Izawa T, Inui H, et al. (2016) Hypogonadism alters cecal and fecal microbiota in male mice. Gut Microbes 7(6): 533-539.

14. Jia Y, Yee JK, Wang C, Nikolaenko L, Diaz-Arjonilla M, et al. (2017) Testosterone Protects High Fat/Low Carbohydrate Diet Induced Non-Alcoholic Fatty Liver Disease in Castrated Male Rats Mainly via Modulating ER Stress. American Journal of Physiology-Endocrinology and Metabolism 314(4): 366-376.

15. Hua X, Sun Y, Zhong Y, Feng W, Huang H, et al. (2014) Low serum sex hormone-binding globulin is associated with nonalcoholic fatty liver disease in type 2 diabetic patients. Clinical Endocrinology 80(6): 877-883.

16. Ananga A, Obuya J, Ochieng J, Tsolova V (2017) Grape Seed Nutraceuticals for Disease Prevention: Current Status and Future Prospects. In Phenolic Compounds-Biological Activity.

17. Hasona NA, Alrashidi AA, Aldugieman TZ, Alshdokhi AM, Ahmed MQ (2017) Vitis vinifera Extract Ameliorate Hepatic and Renal Dysfunction Induced by Dexamethasone in Albino Rats. Toxics 5(2): 11.

18. Georgiev V, Ananga A, Tsolova V (2014) Recent advances and uses of grape flavonoids as nutraceuticals. Nutrients 6(1): 391-415.

19. Martin DP (2010) Guidelines for Animal Care and Use in Biomedical Research. Current Protocols Pharmacology 49(1): 1-4.

20. National Research Council (2010) Guide for the care and use of laboratory animals. National Academies Press.

21. Al-Fatlawi AA (2015) Effects of Some Heavy Metals Especially Cr and Ni on some Histological and Physiological Parameters in male Mice Ph.D. Thesis, College of Science, Kufa University, Iraq.

22. Al-Ahmadi AA, Ali SS, Ayuob NN, Al Ansary AK (2014) Amelioration of hypercholesterolemia-induced hepatic changes with red grape juice: A histopathological study. Histol Histopathol 29(9): 1169-1183.

23. Park YK, Park E, Kim JS, Kang MH (2003) Daily grape juice consumption reduces oxidative DNA damage and plasma free radical levels in healthy Koreans. Mutation Res 529(1): 77-86.

24. Rodrigues AD, Scheffel TB, Scola G, Dos Santos MT, Fank B, et al. (2012) Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazole. Neurochemistry International 60(8): 799-805.

25. Chong WC, Wu R, Tu AY (2012) A Study on Tissue Processing. International Journal of Innovative Interdisciplinary Research 1: 37-43.

26. Minehira K, Gual P (2018) Role of Lipid Droplet Proteins in the Development of NAFLD and Hepatic Insulin Resistance. In Non-Alcoholic Fatty Liver Disease-Molecular Bases, Prevention and Treatment.

27. Saez-Lopez C, Barbosa-Desongles A, Hernandez C, Dyer RA, Innis SM, et al. (2017) Sex hormone-binding globulin reduction in metabolic disorders may play a role in NAFLD development. Endocrinology 158(3): 545-559.

28. Borrelli A, Bonelli P, Tuccillo FM, Goldfine ID, Evans JL, et al. (2018) Role of gut microbiota and oxidative stress in the progression of non-alcoholic fatty liver disease to hepatocarcinoma: Current and innovative therapeutic approaches. Redox biology 15: 467-479.

29. Harada N, Hanaoka R, Hanada K, Izawa T, Inui H, et al. (2016) Hypogonadism alters cecal and fecal microbiota in male mice. Gut microbes 7(6): 533-539.

30. Zhang N, Zhang H, Zhang X, Zhang B, Wang F, et al. (2014) The relationship between endogenous testosterone and lipid profile in middle-aged and elderly Chinese men. European Journal Endocrinology 170(4): 487-494.

31. Cai Z, Jiang X, Pan Y, Chen L, Zhang L, Zhu K, et al. (2015) Transcriptomic analysis of hepatic responses to testosterone deficiency in miniature pigs fed a high-cholesterol diet. BMC Genomics 16(1): 59.

32. Furman D, Hejblum BP, Simon N, Jojic V, Dekker CL, et al. (2014) Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proceedings of the National Academy of Sciences 111(2): 869-874.

33. Fan W, Evans R (2015) PPARs and ERRs: molecular mediators of mitochondrial metabolism. Current opinion in cell biology 33: 49-54.

34. Zawacka M, Murawska D, Gesek M (2017) The effect of age and castration on the growth rate, blood lipid profile, liver histology and feed conversion in Green-legged Partridge cockerels and capons. Animal 11(6): 1017-1026.

35. Rodriguez-Ramiro I, Vauzour D, Minihane AM (2016) Polyphenols and non-alcoholic fatty liver disease: impact and mechanisms. Proc Nutr Soc 75(1): 47-60.

36. Ferramosca A, Di Giacomo M, Zara V (2017) Antioxidant dietary approach in treatment of fatty liver: New insights and updates. Worid J Gastroenterol 23(23): 4146-4157.