The Impact of Grape Juice Extract on Liver Structure of Castrated Rats

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Abstract— **The effects of ingestion of freshly prepared ethanolic extracts of 6g/kg/day of black grape juice was investigated on some biochemical, parameters and histological structure of liver in castrated male rats for 42 days. Twenty-one male rats (Rattus norvegicus) were used in the present study. Rats are divided into three groups of animals (N= 7). Tap water was given to the intact and castrated rats in groups 1 and 2. Daily, 1 ml (6g/kg/day) of grape extract was administered intragastrically to the castrated rats in group three. The biochemical test for several parameters did not reveal any differences between the groups that were being compared. Whereas the values of HDL showed a significant difference in both castrated and castrated treated subjects as compared with control animals (P<0.01; P<0.05) respectively, as well as the results of VLDL showed a significant difference in castrated treated group (P< 0.02). However, the value of ALP also showed a significant difference in castrated treated group (P<0.03). Castration had a major impact on the histological structure of the liver, resulting in lobular inflammation, ballooning degeneration, and macrovesicular steatoses. The liver structure of castrated rats was found to be improved effectively by grape juice treatment, as indicated by histological sections showing a moderate normal architecture to some extent. Administration of grape extract was significantly affecting the histological regeneration of the liver in castrated rats.**

Keywords: **liver; castration; grape juice; central vein.**

INTRODUCTION

The liver is crucial to many physiological processes and is susceptible to damage from a variety of sources, including toxins, microorganisms, metabolic waste products, circulatory materials, and the metabolism of carbohydrates, lipids, and proteins [1, 2]. One of the main risks to global health is liver disease [3]. Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease globally, affecting 30% of all adults. Nonalcoholic fatty liver disease (NAFL) is a range of hepatic abnormalities observed on liver histological slides, which include steatosis, or simple intrahepatic fat accumulation, and necrotic inflammation, or NASH, or nonalcoholic steatohepatitis (NASH) [1, 4, 5]. Kupffer cells, known as hepatic resident macrophages, are the largest group of macrophages in the body and comprise 20–25% of the liver's non-parenchymal cells (intrasinusoidal cells) [1, 6]. Many endogenous and external stimuli can activate kupffer cells, which are an integral component of the innate immune system and are significant in the regulation of immunological responses [7]. Fat-storing, lipocyte-producing, perisinusoidal, and vitamin A-storing cells are all found in the space of Disse between hepatocytes and sinusoidal endothelial cells. Roughly 10% of all liver cells are these cells. Their cytoplasm is especially rich in lipid droplets, and they feature long, branched cytoplasmic processes that may narrow the lumen of the sinusoidal capillaries, changing the liver's sinusoidal blood flow [1, 8, 9, and 10]. When damage is done to the liver, a process called "activation" drives hepatic stellate cells to become myofibroblasts [11]. Hepatocyte steatosis which a disease where accumulation fat occurs in the liver it is also been associated with cirrhosis and hepatocellular carcinoma in addition to all other NAFLD stages [12]. The lack of androgen and estrogen, two important components in the regulation of the plasma lipid profile, is probably the cause of the poor lipid profile in castrated animals [13]. In the metabolism of fats and carbohydrates, testosterone is crucial [1]. According to Kelly et al [14], testosterone modulates the expression of genes involved in glucose and lipid metabolism, hence reducing the accumulation of body fat. Increased adiposity in the liver and peripheral tissues is the result of hypothalamic and peripheral tissue dysregulation of glucose and lipid metabolism caused by low testosterone levels [14, 15]. Metabolic syndrome, which in men and laboratory animals includes obesity, nonalcoholic fatty liver disease, and type-2 diabetes, is predicted to develop in those with low testosterone levels [3, 8, 2, and 6]. When animals undergo orchiectomy, even in non-reproductive organs, different degrees of hypertrophy have often been observed. The reduction of the sinusoidal bed and the hepatocyte volume indicated the consequences of castration. A low amount of plasma testosterone is associated with hyperinsulinemia and glucose intolerance [16]. After castration, insufficient testosterone caused excessive accumulation of liver triglycerides (steatosis), atherosclerosis, abdominal obesity, and poor fasting glucose tolerance. All of these disorders are characterized by decreased glucose utilization and increased fat production from excess glucose, [17]. Liver dysfunction has been treated using a variety of medicinal plants and fruits, including grapes (Vitis vinifera). Proanthocyanidins, procyanidins, anthocyanins, flavonoids, polyphenols, and various derivatives are among the many active ingredients found in grapes. Flavonoids, which comprise the largest group of grape polyphenols with biological properties, such as antioxidant, anti-inflammatory, anti-cancer, antibacterial, antiviral, cardioprotective, neuroprotective, and hepatoprotective, are the

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main contenders [18, 19, and 20]. Even though grape skin and seeds are the primary source of these bioactive components, ordinary dietary consumption, wineries, and the grape juice business typically discard grape skin and seeds. According to following studies [20, 21] these wastes include bioactive substances with strong antioxidant and free radical scavenging properties. Studies on liver tissue indicate that pro-ions from grape chemicals help tissues avoid and develop diseases [22]. Because grape juice has been shown to have antioxidant properties, it may help increase the enzymatic activity of specific enzymes, which may help lower oxidative stress in the body [23]. More investigation is need to validate these claims about grape juice's health benefits for people, but.

To the best of our knowledge, little research has been conducted on the efficacy of grape juice extract in treating liver issues in animals that have been castrated. The current study evaluates the structure of liver tissue in castrated and castrated individuals treated with grape extract in order to identify any histological changes that may have resulted from such surgery and physiological conditions in both castrated and castrated treated subjects. This is done in order to assess the many hepatic criteria that serve as indicators of liver function. These physiological traits were also the investigation's main emphasis.

METHODOLOGY

Plant material extract: Grape juice (*Vitis vinifera)*, extract was prepared according to the procedure described by [24]. The fresh grape juice was prepared as follows: Black grape (*Vitis vinifera*) obtained from local market (Erbil city, Iraq), 600g of grape was blundered without separating the seeds, and then it was filtered to remove the residue. After an hour, the resultant extract was used and kept in a refrigerator at 4˚C. A prior study found that an effective amount of grape juice extract was 6g/kg/day [25, 26]. In the present study, 1 ml (6 g/day) was utilized for this therapy, as intragastric injection daily for six weeks. Rats were anesthetized with ketamine and xylazine before the animals were scarified at the end of the experiment. Experimental Animals and Ethical Statement: In this study, a three-week-old laboratory rat, Rattus norvegicus, belonging to the family Muridae and order Rodentia, were employed. The rats were acquired from the animal house, Cihan University, Erbil. They were housed in ventilated cages with a balanced light-dark cycle at a temperature of 25±2Cº at 12:12 h. Water and rodent food pellets were given to the animals as needed based on [27, 28] standards for the care and use of laboratory animals in biomedical research were the basis for the first evaluation, approval, and acceptance of all experimental protocols using live animals. After the trials were completed, the rats were rendered unconscious using ketamine and xylazine. The animals were then slaughtered.

EXPERIMENTAL DESIGN

A total of twenty-one three-weeks -old male rats were employed in this study. The animals were divided into three groups (N = 7) and the therapy was only given for six weeks. Intragastrically, castrated subjects were given 1 ml of grape juice daily. The protocol for this investigation was as follows: Group 1: Tap water was given as a control (intact); Group 3: Male castrated rats got intragastric injections of 1 m of grape juice (6g/kg/day) daily, while Group 2, castrated subjects received tap water as a positive control. The surgical castration technique was carried out in accordance with [29] .Prior to surgery, male rats (3 weeks old) were given an intramuscular injection of (50 mg ketamine + 20 mg 4/1 xylazine) for five minutes. In order to remove the testicles without tying the spermatic arteries, a transverse scrotal incision was created. Rats were returned to clean cages after their recuperation, and the wound was repaired with a simple stitch. Upon completion of the study, rats were given anesthesia, and 1 ml of blood was collected directly from the heart via a midline incision made above the belly, close to the diaphragm. Serum was extracted from the blood samples by centrifugation at 3000 rpm for 10 minutes, and it was stored frozen at -20°C (unless it was immediately tested and put to use). The blood samples were placed in a serum gel tube and left for 30 minutes. Fully automated equipment, the SMT-100, was used to conduct the biochemical experiments. The test was assessed using Saematy kits, which are designed specifically for use with animals. Numerous biochemical parameters were determined. Hematoxylin and Eosin stain [30]. Was used to stain the histological sections after they had been prepared in accordance with the method outlined by Chong *et al* [31]. The findings were presented as the mean ± standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to examine the data for comparisons involving several variables. According to the statistical package program (SPSS version 16.0), the p values p< 0.05 was deemed as significant for all statistical analysis in this approach. Duncan's test was employed as a post hoc test for the comparison of significance between groups.

RESULTS

Biochemical tests: The results of different biochemical tests (TG, TC, LDL, GOT, GPT, Globulin, Bilirubin, Glucose, Protein, Albumin, Blood Urea, Creatin) are shown in (tables; 1; 2 and 3). Most of these results did not show any significant variations between the compared groups which might be contributed to many factors as: the used dosage; the duration of time or any other physiological factors. Whereas the values of HDL showed a significant differences in both castrated and castrated treated subjects as compared with control animals (P<0.01; P<0.05) respectively, as well as the results of VLDL showed a significant difference in castrated treated group (P< 0.02). Additionally, the value of ALP also showed a significant difference in castrated treated group (P<0.03) as compared with the control animals.

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TABLE 1: SHOWING THE LEVELS OF LIVER ENZYMES IN DIFFERENT EXPERIMENTAL GROUPS.

Parameters Treated	TG Mg / dl	TC Mg\dl	HDL Mg\dl	LDL Mg\dl	VLDL Mg\dl
Control	40.4 ±13.5	59.9 ±3.7	28.8±5.5	2.9±0.5	12.2±3.4
Castrated	93.3 ±40.8	79.7 ±8.6	43.4±7.4 43.4±7.4 P< 0.01 ***	10.3±4.9	6.5±1.1
Castrated treated	34.34 ±2.2	66.5 ±8.1	35.7±4.9 35.7±4.9 P< 0.05 *	6.8±3.2	7.1±1.1 7.1±1.1 P< 0.02 **

*HDL, P<0.05; ** VLDL, P< 0.02; *** HDL, P <0.O1, Mean ± SD

TABLE 2: SHOWING THE LEVELS OF SOME BIOCHEMICAL PARAMETERS OF LIVER ORGAN IN DIFFERENT EXPERIMENTAL GROUPS.

* ALP P< 0.03, Mean ± SD

Parameters Treated	GOT Mg\dl	GPT Mg\dl	ALP Mg\dl	Globulin Mg\dl	Albumin mg/dl
Control	82.7±18.6	140.8±19.1	177.7±18	3.7±0.6	4±1
Castrated	300.8±77.5	235.7±35.7	166.6±41	10.2±2.6	10.1±2.3
castrated treated	198.6±41.6	72.7±19.7	194.1±32.9 P< 0.03 *	2.4±0.2	3.3±0.3

TABLE 3: SHOWING THAT LEVELS OF SOME BIOCHEMICAL PARAMETERS OF LIVER ORGAN IN DIFFERENT EXPERIMENTAL GROUPS.

Parameters Treated	glucose mg\dl	T. protein Mg\dl	TSP Mg\dl	Blood urea Mg\dl	Creatin mg/dl
Control	121.6±14	5.9±0.5	3±0.6	21.8±3.5	0.3±0.1
Castrated	187.1±13.9	13.1±3.4	11.1±2.6	41.7±8.6	0.31±0.06
Castrated treated	117.8±16.3	6.3±0.2	12.7±26.3	43.8±14.1	0.26±0.01

Mean ± SD

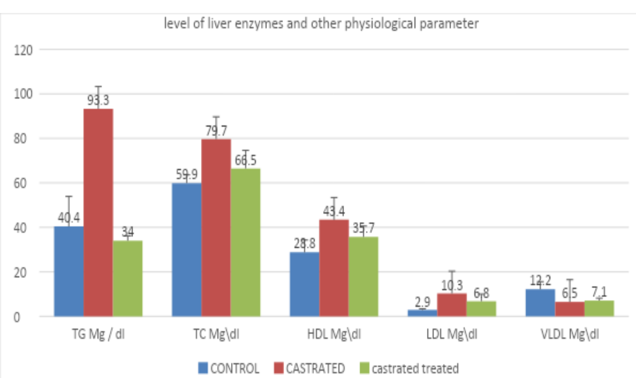

Figure 1: showing the levels of enzymes and other physiological parameter.

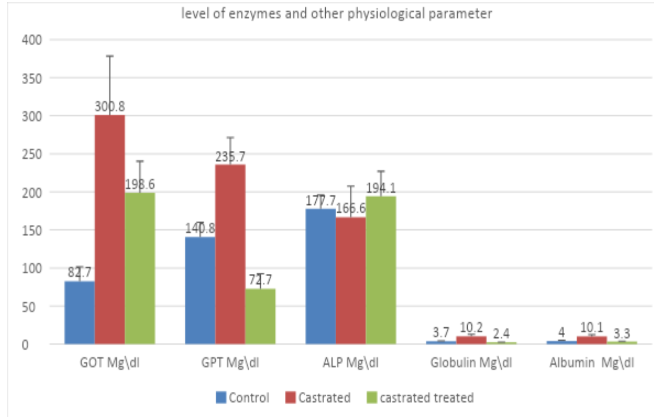

Figure 2: Showing the levels of enzymes and other physiological parameter.

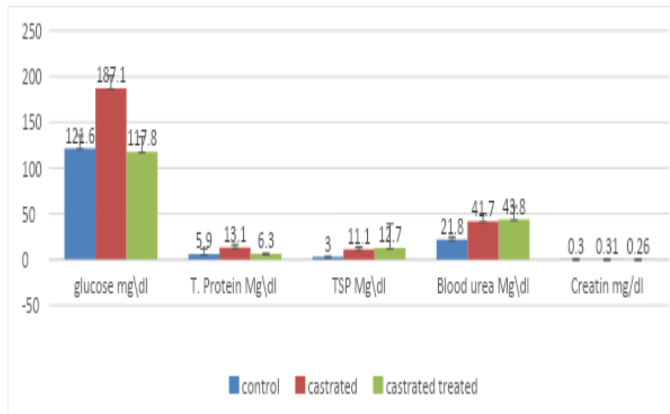

Figure 3: Showing levels of enzymes and other physiological parameter

HISTOLOGICAL RESULTS:

Figures (4, 5 and 6): showing the normal histological sections of liver tissue from intact male rat. It is made up from largely predominant parenchymal cells (hepatocytes), as well as non-parenchymal cells, including Ito (perisinusoidal cells), Kupffer cells and sinusoidal endothelial cells. The hepatocytes appear cuboidal in shape and are arranged in plates, one cell thick, usually, they separated by sinusoids.

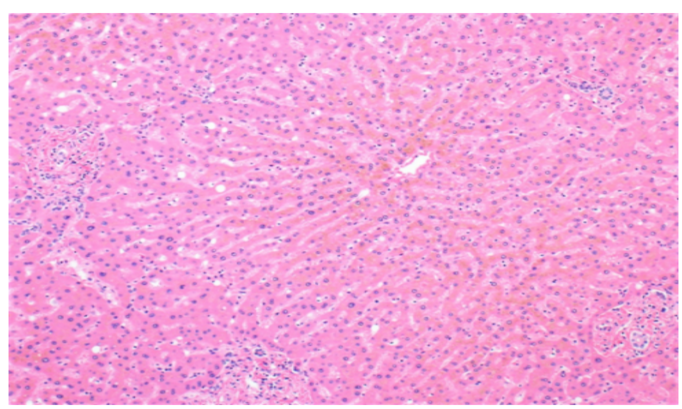

Figure 4: Cross histological section of liver tissue from intact rat showing the normal structure of hepatocyte, central vein, and sinusoids. (H&E, X100).

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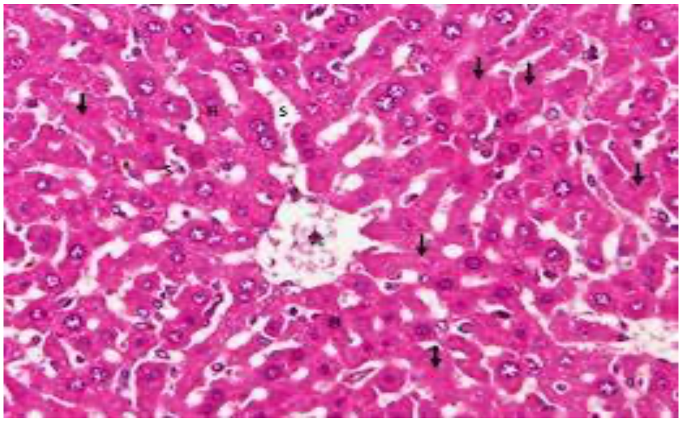

Figure 5: Cross histological section of liver tissue from intact rat showing normal histological structure central vein, sinusoid, Ito cells and Kupffer cells. (H&E, X200).

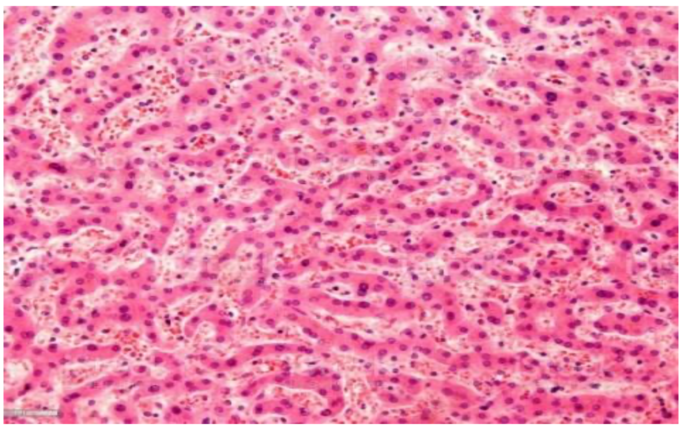

Figure 6: Cross histological section of liver tissue from intact rat showing the hepatocytes are arranged in cords, separated by clear areas where hepatic sinusoids showing red blood cells are located. (H&E, X400).

Effects of Castration on The Histological Structure of Liver:

It is generally known that castration in rats causes the sexual organs and, to a lesser extent, other organs, such as the liver, to atrophy. Castration has a considerable impact on liver histology, according to present findings, as seen in the figures (7, 8, 9 and 10). Display lobular inflammation, ballooning degeneration, and macrovesicular steatoses, which is a buildup of fat within hepatocytes. The pyknotic nuclei are located on the perimeter, additionally, the majority of the castrated hepatocytes produced Councilman Bodies (acidophil bodies, or apoptosis).

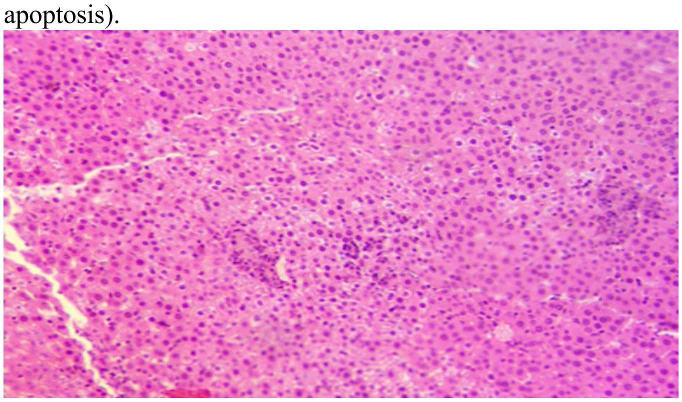

Figure 7**:** Cross histological section of liver tissue from castrated rat showing inflammation and ballooning degeneration. (H&E, X100).

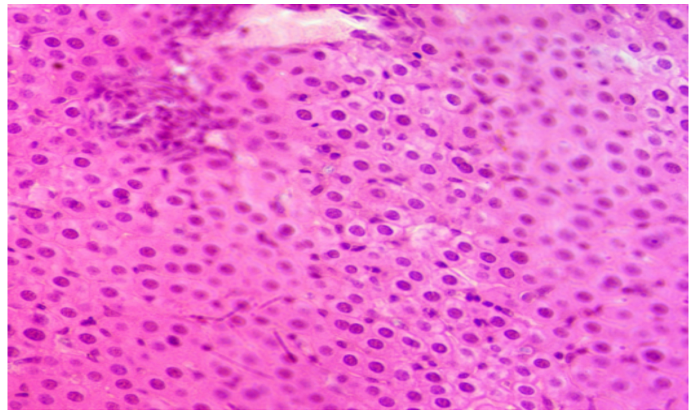

Figure 8: Cross histological section of liver tissue from castrated rat showing Macrovesicular steatosis. (H&E, X200).

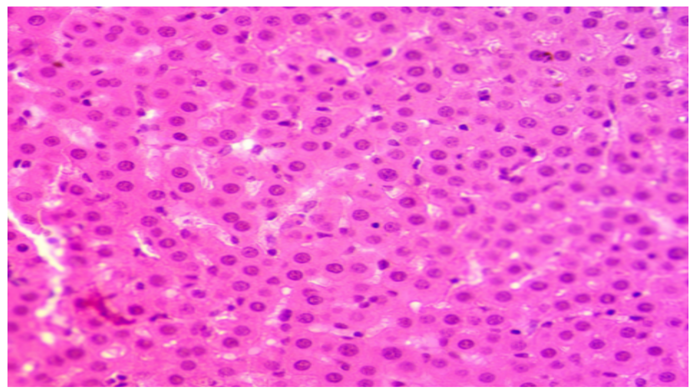

Figure 9: Cross histological section of liver tissue from castrated rat showing Ballooning degeneration. (H&E, X200).

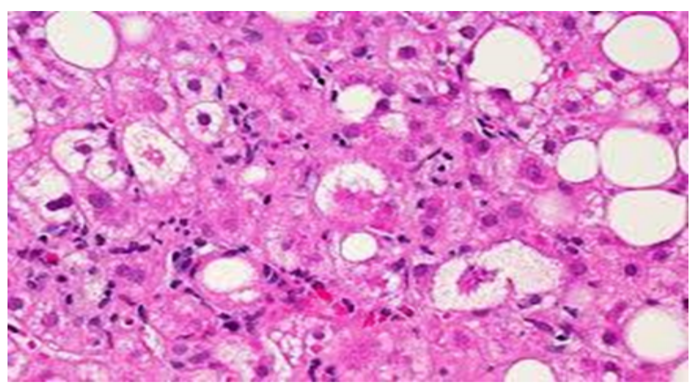

Figure 10 **:** Cross histological section of liver tissue from castrated rat showing acidophil bodies, (apoptosis) in most hepatocytes. (H&E, X400).

Effects of grape juice (*Vitis vinifera*):

Treatment of castrated rats with grape juice was more effective and helpful to somewhat improve liver structure due to their antioxidant properties. A dose of 6 g/kg of this extract may reduce the hepatic lesion and inflammation in hepatocytes caused by castration, (figures: 11, 12, 13, and 14). Microscopic examinations of liver sections from rats that had been castrated and given extract of grape juice showed that the architecture was nearly or partially normal. Hepatocytes and blood sinusoids alternate around the major veins, as shown in histological slices

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of the lobules. Hepatic cells have a limiting membrane and a large central nucleus. Additionally, the hepatic sinusoids of the lobules showed only slight degeneration and no evidence of Councilman Body's development

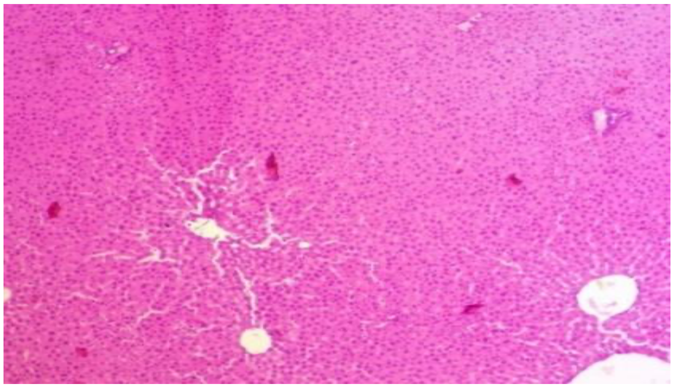

Figure 11: Cross histological section of liver tissue from castrated rat treated with grape juice showing the structure of hepatocyte, central vein, and sinusoids. (H&E, X100).

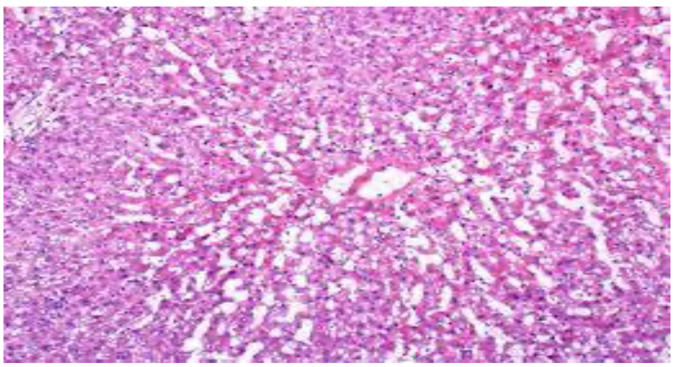

Figure 12: Cross histological section of liver tissue from castrated rat treated with grape juice showing, central vein, Sinusoids and hepatocytes with little inflammation. (H&E, X 200).

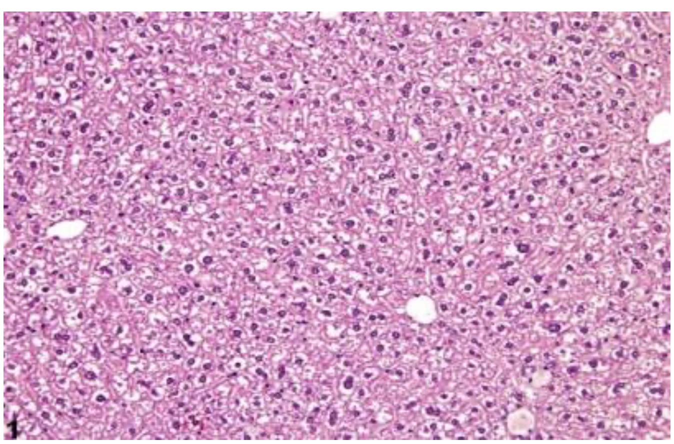

Figure 13: Cross histological section of liver tissue from castrated rat treated with grape juice showing, mild degeneration. (H&E, X200).

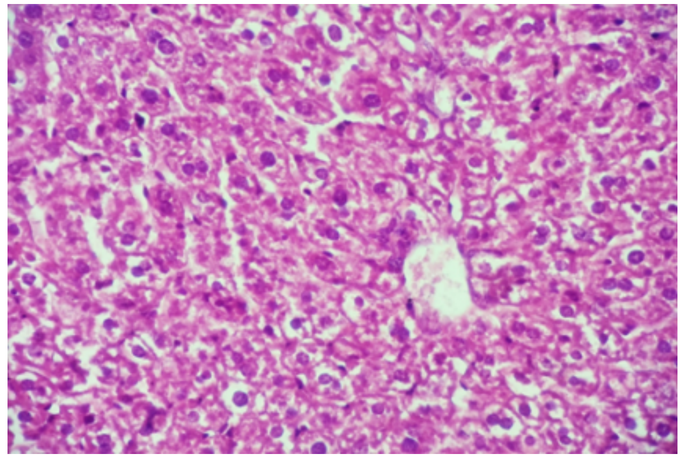

Figure 14: Cross histological section of liver tissue from castrated rat treated with grape juice showing nearly anormal structure with no evidence of Councilman Bodies. (H&E, X400).

DISCUSSION:

In the present study the outcomes of several biochemical indicators did not significantly differ between the castrated and the castrated individuals treated with grape extract as compared to the control group. The effects of castration on liver enzymes and lipid profiles may not be immediately apparent. One of the main risk factors for numerous diseases, including steatosis, atherosclerosis, and hyperglycemia, is obesity. The other cause is the male sex hormone, testosterone. The hepatocytes' increased lipid accumulation, as proven by histological investigation, can be intensified by an imbalance between the fatty acid intake, hepatic lipid synthesis, and β-oxidation. According to Kargulewicz *et al.,* [32] increased hepatic lipid production causes fat degeneration in hepatocytes, as the present results showed. Kim *et al.,* [33] observed that TG is exported to the bloodstream from the liver in the form of very low-density lipoproteins (VLDL). Consequently, low testosterone may impact blood TG levels by changing the expression of genes related to lipoprotein secretion and assembly. These findings contradict those of Langer *et al*., [34] who hypothesized that testosterone may speed up the reverse cholesterol transport process. It has also been proposed that declines in HDL-C brought on by a testosterone shortage may actually slow down this process. The levels of HDL and testosterone are positively correlated. These results are consistent with those of Zhang *et al*., [35] who showed that HDL surface phospholipid hydrolysis and testosterone purified by scavenger receptor (SR. B1) play important roles in atherosclerosis prevention. Similar results were seen in castrated subjects by other researchers [36, 37, and 16]. The reason behind elevation of ALP level in castrated treated subjects, it might be that the grape therapy could reverse the changes in the liver and brought it back to normal state by lowering the amount of lipid exported from the liver into the bloodstream, and adipose tissue. The elevated HDL values in the various experimental groups, It might be that grape extract will fasten the process of reverse cholesterol transfer; additionally, it's been proposed that low testosterone levels may actually slow down the process by lowering HDL levels. The levels of HDL and testosterone are positively correlated.

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These results could be attributed to the antioxidant properties of grapes and their flavonoid and phenolic content, which can lessen oxidative damage either directly or indirectly by inhibiting the production of too many free radicals and the synthesis and deposition of liver fat. According to Buchner *et al.*, [38], flavonoids function as antioxidants by scavenging oxidizing free radicals, such as hydroxyl and superoxide radicals. Due to their ability to suppress lipid peroxidation, flavonoids and phenolic compounds have been shown to be effective scavengers of superoxide, hydroxyl ions, and peroxyl radicals. Flavonoids can also operate as reducing agents.

The results of this study are consistent with those of Ebdel Moneim [39], and Rodrigues *et al.,* [40], who showed that resveratrol in grape juice plays a role in preventing endothelial dysfunction by acting as an antioxidant and up regulating the expression of nitric oxide synthesis in the endothelium. The current study findings are consistent with those of numerous other researchers [41, 42, 43], who showed that testosterone replacement or grape juice reduced the development of steatosis and steatohepatitis in rats as well as hepatic lipogenesis. Based on the current data, it is possible to draw the conclusion that flavone supplementation decreased liver steatosis by controlling genes related to fatty acid β-oxidation and lipogenesis in adipose tissue, which is consistent with the findings of Rodriguez *et., al* [44]. The histological findings of the liver tissue from the castrated rat showed hydropic degeneration, lipid buildup, and ballooning hepatocyte degeneration; these changes in the liver's structure can be linked to a low testosterone level. On the other hand, when castrated subjects were given grape juice, the negative effects of castration were somewhat mitigated. For example, there was less microvesicular steatosis and less macrovesicular fatty accumulation, and both hepatocyte and sinusoidal regeneration processes were observed. According to other researchers [41, 45, 46, 47] these results are consistent. Because grape juice has more antioxidants, it was more beneficial and helped to strengthen liver structure somewhat. A dose of 6 g/kg of this extract may reduce the hepatic lesion and inflammation in hepatocytes caused by castration. This impact may be due to the bioactive compounds present or the hypothesized lipid-lowering action [17]. Grape juice contains phenolic components, including flavonoids, anthocyanins, tannins, and phenolic acids, which have significant antioxidant activity and protect the liver from oxidative damage. Red grape juice has been shown by [48] to have a hepatoprotective effect, meaning that it can act as a therapeutic agent or protective measure to lessen organ damage and dysfunction caused by chemical pollutants. The rate at which a response manifests itself varies greatly throughout tissues and is frequently contingent upon the amount of extract administered to the tissue [49]**.** The management and prevention of nonalcoholic fatty liver disease (NAFLD) have been shown to depend heavily on nutrition and dietary supplements [50]**.** Recent research has examined the beneficial effects of several nutraceuticals on increasing liver enzyme levels. In this regard, compounds high in antioxidants have attracted special attention [50].

CONCLUSION AND RECOMMENDATION

The present study demonstrated that castration induced aggravated increased hepatic steatosis; impaired metabolic process related with surgical castration enhanced immune and inflammatory response, represented by hepatic apoptosis and contribute to increase nonalcoholic fatty liver disease. The intra gastric application of Grape juice has dramatic effects to restore histological structure of liver in castrated animals to some extent but did not show any changes in biochemical parameters as compared with the control subjects this might be due to many different physiological factors or as due to the used dosage or the duration of time. The present study recommends to using different concentrations of grapes with different duration to show their effects on both biochemical and histological changes. Also, to study the combination of testosterone and *grape juice* to treat disease like hypogonadism and prostate cancer.

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