



RESEARCH ARTICLE

Assessing the Effects of *Mentha longifolia* on Liver Structure in Castrated Rats

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ABSTRACT

The liver is the largest internal organ in the human body. It has dual roles: An exocrine function, producing bile to aid digestion, and an endocrine-like function, helping regulate metabolism. It cleanses the blood, makes important proteins, and stores essential nutrients. This study aimed to assess the impact of *Mentha longifolia* extract on a variety of physiological, biochemical, and histological parameters. A total of 21 male rats (*Rattus norvegicus*), 3 weeks of age, from the order Rodentia and family Muridae, were used in this study. Rats were divided into three groups, each consisting of seven rats. Both Group 1 (control) and Group 2 (castrated) of male rats received tap water. However, male rats in Group 3 were castrated and administered a daily intragastric extract of *M. longifolia* (6 g/kg). In castrated-treated groups, the effects of consuming a freshly made extract of *M. longifolia* were examined for various biochemical parameters of liver enzymes and lipid profile, as well as the histological structures of liver tissue. The outcomes of the biochemical tests performed on the treated subjects did not reveal any notable variations. Histological sections of the castrated group's liver tissue showed cellular inflammation of lymphocytes, ballooning degeneration, and acidophil bodies (Councilman Bodies) in contrast to the control group. The histological structure of the injured liver did not fully recover in rats that had been castrated and fed 6 g/kg daily for 4 weeks. On the other hand, this treatment greatly reduced the adverse effects of castration, such as macrovesicular steatosis, Councilman Bodies, and mild ballooning degeneration. According to the current research, fresh *M. longifolia* extract may have some hepatoprotective benefits against castration.

Keywords: Extract, histology, inflammation, rat, steatosis

INTRODUCTION

The hepatic gland is a sophisticated anatomical and functional organ found in mammals. Circulatory materials, toxins, microbes, metabolic products, and the breakdown of proteins, lipids, and carbohydrates are just a few of the many substances that deplete it. Numerous vital physiological processes depend on it.^[1-3] In addition to hepatocytes, which comprise the majority of parenchymal cells, it also includes non-parenchymal cells such as Kupffer cells, sinusoidal endothelial cells, and Ito (perisinusoidal cells).^[4] Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. The metabolic syndrome, a complicated collection of disorders that includes elevated low-density lipoprotein (LDL) levels, central obesity, hypertension, hyperglycemia, hypertriglyceridemia, and hypercholesterolemia, is believed to be the hepatic manifestation of this illness. These illnesses are all predictive risk factors for NAFLD.^[5,6] To prevent reproduction and lessen aggressive behavior, farm animals that are castrated surgically have their testicles, or testis, removed through a bilateral orchiectomy.^[7,8] Following castration, hypogonadism results

in abnormal liver triglyceride accumulation, decreased fasting blood sugar, atrophy of the thigh muscles, and abdominal fat. Castrated mice also show an increase in visceral fat and a decrease in thigh muscle mass.^[9] Steatohepatitis develops more quickly after castration because it causes an increase in microvascular droplets and starts the endoplasmic reticulum (ER) stress pathway. By reducing ER stress, preventing the formation of macrovascular lipid droplets, encouraging lipid export, and stopping their growth, testosterone lessens

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the effects of castration-induced steatohepatitis.^[10] Blood triglycerides then cause an increase in visceral fat deposition and an excess of fat to build up in ectopic storage in the liver and arteries. Serum testosterone levels and insulin resistance are inversely correlated.^[11] Hepatic steatosis is more likely to occur when serum testosterone levels are low.^[12] The disruption of lipid and glucose metabolism brought on by low or absent testosterone results in increased adiposity in the liver and other peripheral organs. Higher visceral fat is linked to lower testosterone levels. Reduced testosterone levels predict the development of metabolic illness.^[13,14] Kupffer cells, another name for hepatic resident macrophages, comprise 20–25% of the liver's non-parenchymal cells and are the largest subset of intrasinusoidal cells in the body.^[15,16] Throughout history, medicinal plants have been widely used to treat human illnesses.^[17,18] About 80% of the world's population gets their medical care from plant-based products.^[19] As secondary metabolites, plants produce a wide range of chemical compounds, many of which have pharmacological characteristics.^[20] Iraqi flora contains six species of *Mentha*, a genus in the Lamiaceae family.^[21] Many species used in traditional medicine can be found in local markets and traditional herb shops.^[22] *Mentha longifolia* (Lamiaceae family) is a common plant in North Africa, Europe, Australia, and the Mediterranean region.^[23] The perennial has a peppermint scent and is variegated. The leaves are oblong-elliptic to lanceolate, sparsely to thickly tomato-colored, white underneath, and green to grayish-green above. Tall, branching, tapering spikes bear several clusters of lilac, violet, or white blooms, each 3–5 mm long. *M. longifolia* has specific uses in the pharmaceutical, cosmetic, culinary, and tobacco industries. In traditional folk medicine, usually, parts of a plant have been used extensively as stimulants, carminatives, antibacterials, and antispasmodics to treat a range of ailments, including stomach issues and headaches.^[23] The perennial has a peppermint scent and is variegated. This plant has been used historically to treat gastrointestinal infections, respiratory problems, headaches, diarrhea, dysentery, cardiac disorders, high blood pressure, and muscular and joint discomfort. Its leaves are green to grayish-green above and white below, oblong-elliptic to lanceolate, and sparsely to thickly tomentose.^[24,25] Since ancient times, plants have been utilized as successful treatments for a variety of illnesses due to their pharmacological properties. The leaves are oblong-elliptic to lanceolate, sparsely to thick, and green to grayish-green on top and white underneath. This plant has long been used to treat gastrointestinal infections, respiratory problems, headaches, diarrhea, dysentery, cardiac disorders, high blood pressure, and pain in the muscles and joints. Plants have long been employed as an effective remedy for numerous illnesses owing to their therapeutic properties.^[26,27] The bioflavonoids luteolin-7-O-glycoside, luteolin-7, 3'-O-diglycoside, quercetin-3-O-glycoside, apigenin, and kaempferol-3-O-glycoside are the primary constituents of *M. longifolia* essential oils, according to David *et al.*^[28] Environmental factors (weather, soil, nutrition, etc.) that can change the regulation of essential oil biosynthesis have been linked to seasonal variations in the chemical composition and content of *M. longifolia* essential oil across nations.^[23] Traditional medicine uses *M. longifolia* to treat liver problems in castrated animals. The effects of this plant's ethanolic extracts on a variety of histological, physiological, and biochemical parameters were

assessed in this study. It was anticipated that this kind of study would at least partially support or contradict the purported advantages of the herbal remedies and possibly offer guidance for further therapeutic research.

METHODOLOGY

Plant Material Extracts

The method outlined by Homady *et al.* 2000^[29] was used to make the *M. longifolia* extract. This is how the ethanolic extract was made: A market in Erbil City, Kurdistan Region, Iraq, provided the dried *M. longifolia* material. A suspension containing 600 g of powdered material was heated in 2 L of 97% ethanol for 5 minutes and then left undisturbed for 2 to 3 hours. Finally, it was filtered and dried over MgSO₄. The solvent was eliminated by evaporation at 14 mmHg and 30°C. The resulting 60 g of extract were stored at 4°C and used within 24 h. Before being given to the animals throughout the experiment, 6 g of the substance was freshly dissolved in 100 mL of distilled water. Intra-gastric application of animal feeding needles (Popper and Sons, New York) was done every day at 10:00 a.m. for 4 weeks before scarification. Castrated subjects were given 1 mL of the extract every day (6 g/kg) for 4 weeks.

Animals

In this study, 21 male rats (*Rattus norvegicus*), 3 weeks of age, belonging to the order Rodentia and family Muridae, were used. To maintain a controlled temperature of 21 ± 1°C, they were kept on a 12-h light:12-h dark schedule, with white lights on from 06.00 to 18.00 h local time. In the animal house at Cihan University-Erbil City, the subjects were kept in type M1 plastic cages (North Kent Plastics, Erith, Kent, U.K.) that were 30 × 12 × 11 cm in size and had a wire grid top. Sawdust bedding was used, and food and water were freely distributed.^[30]

The first methods using live animals were evaluated, approved, and accepted based on the Institute of Laboratory Animal Resources' 1986 guidelines for the care and use of laboratory animals in biomedical research.^[31] At the end of the experiment, rats were killed after being put to sleep with anesthetics such as xylazine and ketamine. Rats (N = 7) were divided into three groups: Group 1 received tap water as a control, Group 2 (castrated) received tap water as a positive control, and Group 3 received 1 mL in solution (6 g in 100 mL D. W.) of *M. longifolia* extract intragastrically daily.^[23] Surgical castration was carried out in compliance with Al-Fatlawi, 2015.^[32] A transversal scrotal incision was made, exposing and removing the testicles without tying the spermatic vessels. The rats were transferred to clean cages once they had recovered, and a simple stitch was used to seal the wound. After the experiment (3 weeks), the rats were put to sleep, and a midline incision was made above the abdomen near the diaphragm to directly extract 1 mL of blood from the heart. After that, the blood was placed in a serum gel tube and left for half an hour (to obtain clear serum for tests such as biochemical assay). The serum was extracted by centrifugation at 3000 rpm for 10 min, and it was then frozen at -20°C until it was examined and put to use. Animal-specific Saematy Kits were used to conduct and evaluate the biochemical analyses using the fully automated SMT-100. Triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), LDL,

very LDL (VLDL), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), globulin, bilirubin, glucose, protein, albumin, blood urea, and creatinine were among the 15 parameters that were estimated. The histological sections (liver tissue) were made using the method outlined by Chang *et al.* [33] Standard deviation (S.D.) ± mean was used to express the results. The data were analyzed for comparisons between several variables using one-way analysis of variance. Duncan's test was employed as a *post hoc* test to compare the significance between groups, in accordance with the Statistical Package Software (SPSS version 16.0). In this method, $P < 0.05$ was considered significant for all statistical analyses.

RESULTS

Figures 1 and 2 present the results of biochemical testing for a number of markers, including TG, TC, HDL, LDL, VLDL, GOT, GPT, ALP, globulin, bilirubin, glucose, protein, albumin, blood urea, and creatinine. These findings showed non-significant differences between the groups compared could be attributed to differences in treatment, dosage, or duration.

Histological Results

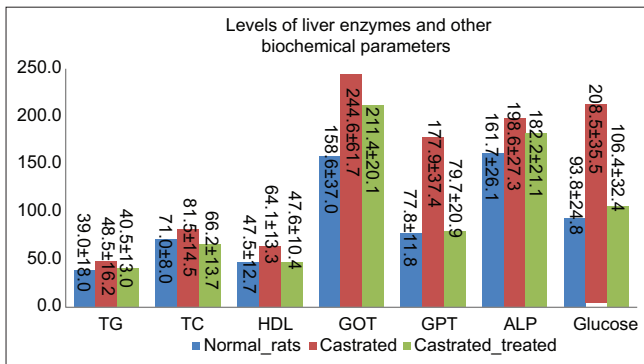


Figure 1: The levels of different parameters of the liver organ in different experimental groups for 4 weeks of treatment (mean ± standard deviation)

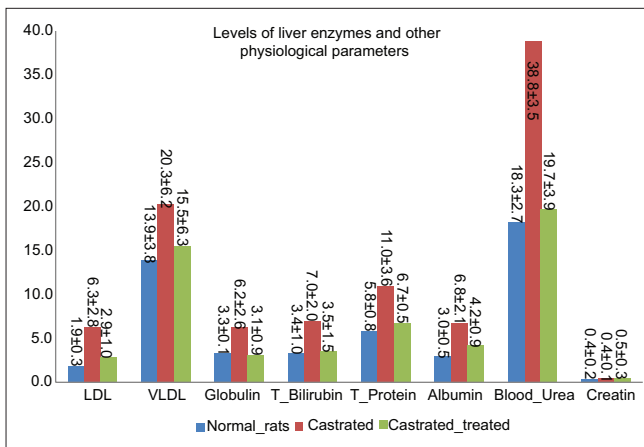


Figure 2: Levels of different parameters of the liver organ in different experimental groups for 4 weeks of treatment (mean ± standard deviation)

The liver lobule's boundaries can be defined by locating the portal triads at its periphery and the central vein at its center, as demonstrated by the histological sections of liver tissue from an intact male rat [Figures 3 and 4]. The hepatocytes are arranged into one-cell-thick, cuboidal-shaped plates that are frequently separated by sinusoids. A few blood cells and phagocytic von Kupffer cells had large, spherical nuclei; occasionally, mid-endothelial cells were visible in a few hepatic sinusoids. Fat-storing cells extend from the sinusoids and occupy the vacant space.

Effects of Castration on the Histological Structures of the Liver

Sections of male castrated rats' liver tissue displaying the characteristics listed below: Lobular inflammation, ballooning degeneration, lymphocyte cellular inflammation,

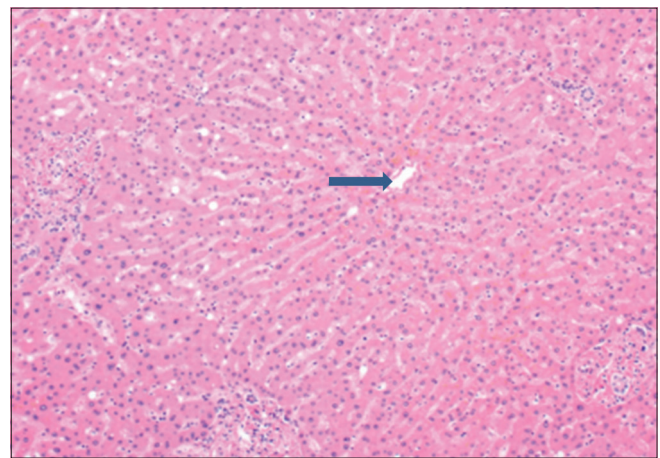


Figure 3: An intact male rat's liver tissue cross-section showing lobule borders, hepatic cords, and central veins (arrow). (×200, H&E).

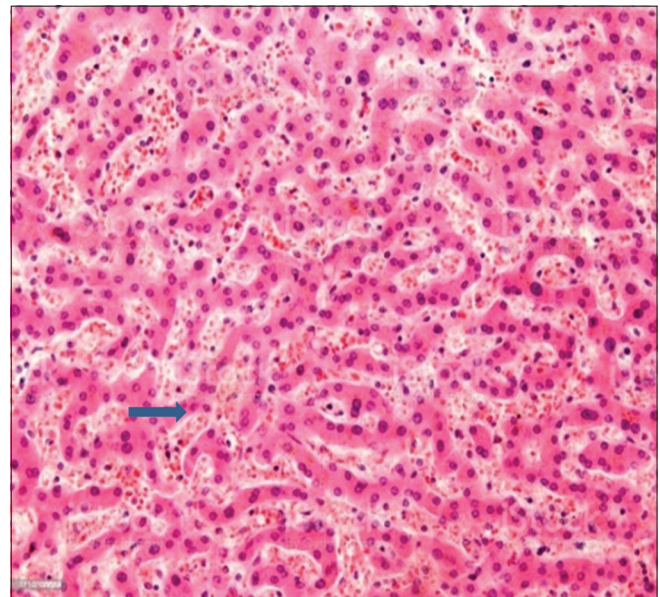


Figure 4: The intact male rat liver tissue's transverse histological section reveals hepatocytes arranged in cords (arrow) distinct from the liver's sinusoids, which display red blood cells. (×400, H&E).

and hepatocyte fat accumulation (macrovesicular steatosis). However, castration had a greater detrimental effect on the structure of the liver, resulting in the majority of hepatocytes going through apoptosis or becoming Councilman bodies, also known as acidophil bodies [Figures 5-8].

The histological structure of the livers of castrated rats receiving a daily dose of 6 g/kg of *M. longifolia* did not return to normal. However, this form of care decreased the deleterious effects of castration to some extent. These outcomes included cholestasis, Councilman bodies, bleeding with a rise in Kupffer

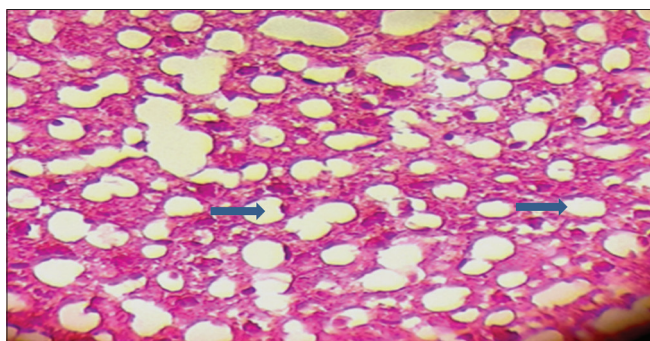


Figure 5: Liver tissue from a castrated rat indicating lipid buildup (Macro vesicular steatosis)(arrow). (H&E, $\times 400$).

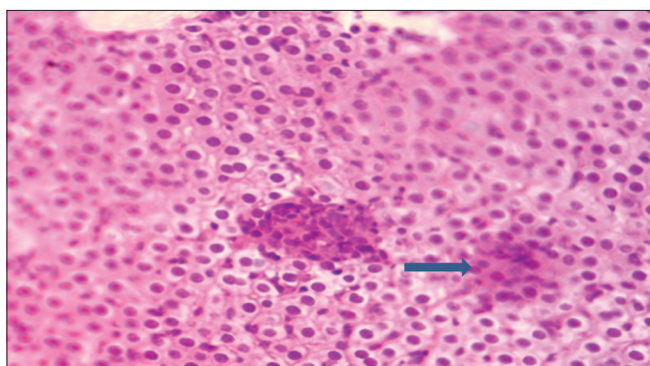


Figure 6: The liver tissue of the castrated rat shows lipid accumulation in the hepatocytes, ballooning degeneration, and lobular inflammation (arrow). with peripherally located nuclei in a cross histological section (arrow). (H&E, $\times 200$)

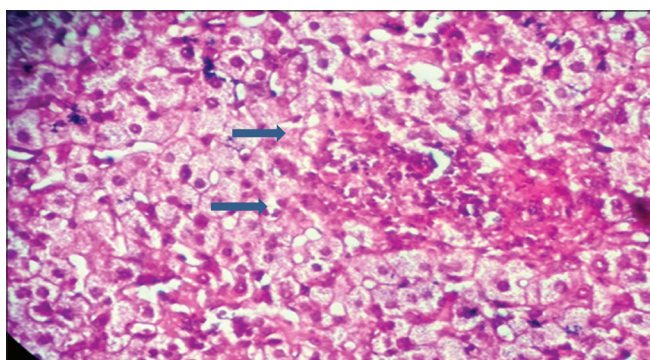


Figure 7: A section of the castrated rat's liver tissue demonstrating hydropic degeneration, microvesicular steatosis (arrow) and inflammatory lymphocyte cells. (H&E, $\times 400$)

cells, macrovesicular steatosis, and moderate ballooning degeneration [Figures 9-12].

DISCUSSION

The liver is an essential organ for regulating nutrient fluxes and serving as the key organ for nutritional metabolism in order to preserve body homeostasis. The food requirements of the extrahepatic tissues, especially the LM (likely "locomotor muscles" or skeletal muscle), change depending on the animal's physiological condition, and the liver adjusts its metabolism to meet these differences. *M. longifolia* is a fragrant herb that is frequently used in herbal therapy to treat a variety of conditions, including gastrointestinal problems, inflammatory disorders, irregular menstruation, coughs, the flu, nausea, and gallbladder infections. According to Mokaberinejad *et al.*,^[34] this plant is also applied externally to wounds and swollen glands. There were no discernible differences between the groups under comparison, according to the results of biochemical tests for various biochemical parameters. The extract's composition, which contains flavonoids and phenols that have protective qualities against oxidative damage by preventing the synthesis and deposition of liver fat and inhibiting the production of excessive amounts of free radicals, may be the cause of these results, as may the dosage or duration of treatment.^[30] Following castration, there was a decrease in testosterone levels. This decline was attributed to an increase

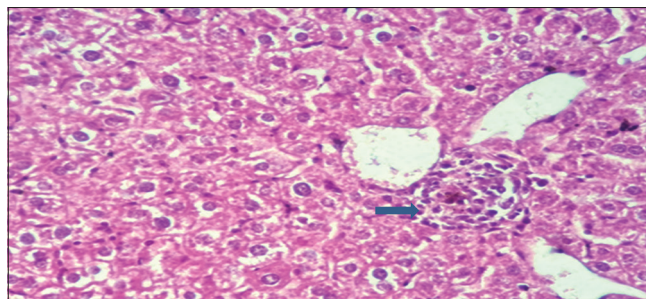


Figure 8: Councilman Bodies (arrow) also known as acidophil bodies or apoptosis in the majority of hepatocytes, are visible in a cross-histological section of the liver tissue from a castrated rat. ($\times 400$, H&E)

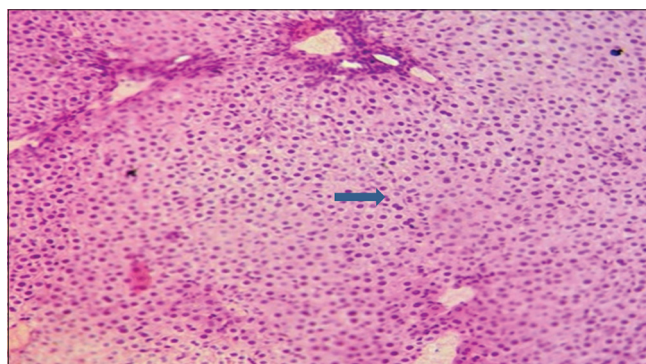


Figure 9: Cross-sectional liver from a castrated rat given 6g/kg revealed pyknosis (arrow), fatty infiltration in the cytoplasm, and degeneration of some nuclei. (H&E, $\times 200$)

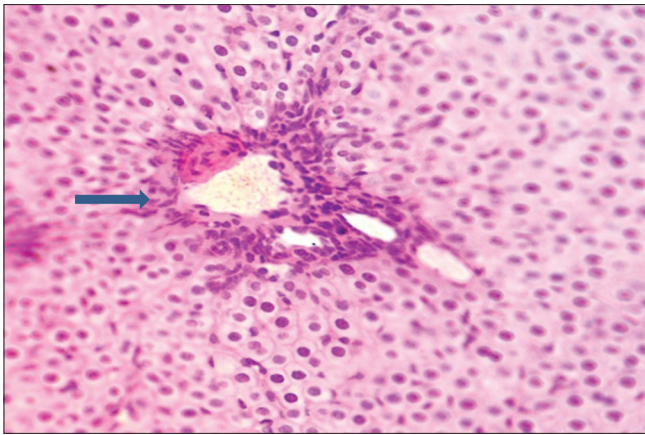


Figure 10: Demonstrating portal tract dilatation (arrow). With a corresponding rise in connective tissue quantities. ($\times 400$, H&E)

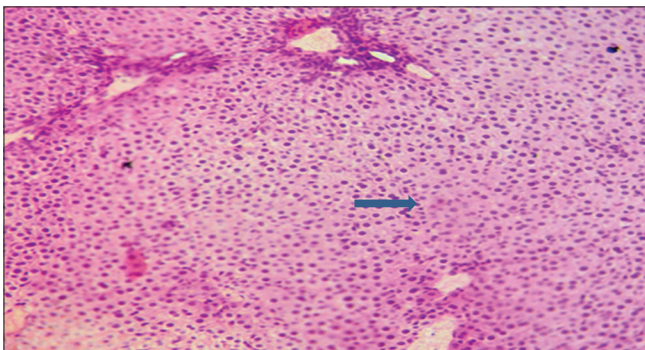


Figure 11: Shows mild Ballooning degeneration of hepatocyte with necrosis and accumulation of lipids within the hepatocytes (arrow). (H&E, $\times 200$)

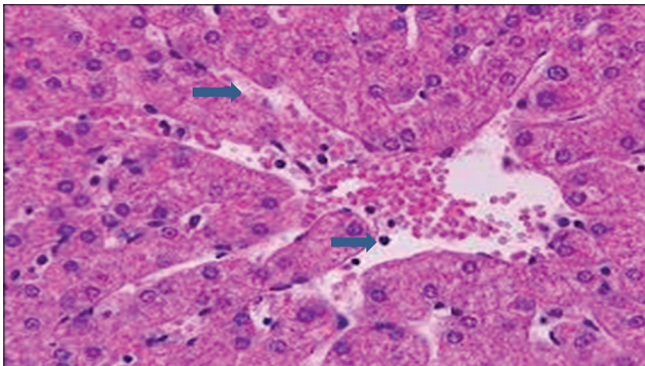


Figure 12: exhibiting both cholestasis and an increase in Kupffer cells in the liver sinusoids. (arrow). ($\times 400$, H&E)

in lipogenesis in the liver due to an increased build-up of triglycerides inside hepatocytes, which was primarily brought on by a breakdown of the cell membrane and elevated liver enzyme levels.^[31,32] These results may also be explained by ischemia induction or by increased reactive oxygen production in triglyceride-loaded hepatocytes due to lipid infiltration. According to Mokaberinejad *et al.*,^[34] testosterone therapy can reverse and control a variety of changes in the liver's normal condition.^[34] These changes involve reduced liver fat synthesis, lower fat breakdown in adipose tissue, and diminished lipid

transport from the liver into the bloodstream. Testosterone treatment has been linked to improvements in metabolic disorders, including obesity, dyslipidemia, hyperglycemia, and non-alcoholic fatty liver disease. Nevertheless, increases in biomedical parameters appeared to result from oxidative stress caused by free radicals and liver cytochrome P-450 induction, producing highly reactive trichloromethyl radicals. This, in turn, causes lipid peroxidation of the membrane in the presence of oxygen created by metabolic leakage from mitochondria, which damages the liver cells and results in a loss of cell membrane integrity that releases the enzymes into the bloodstream.^[34,35] A study by Bakrania *et al.*, 2022, and Villa *et al.*, 2012, showed that lipid oxidation, enzymatic activity, and liver tissue architecture are all negatively impacted by testosterone hormone concentration levels.^[36,37] Furthermore, the results of this investigation do not align with those of Gao *et al.*, 2017, who reported that the activities of aspartate aminotransferase and alanine aminotransferase in castrated mice were significantly higher than in the controls. This discrepancy can be attributed to the dose-dependent risk effects of testosterone on the liver,^[38] which can enhance liver fibrosis and hepatocellular necrosis rather than intrahepatic cholestasis. The current study's findings concur with those of Homady *et al.*, 2021, who observed that hepatic steatosis, the buildup of triglycerides in hepatocytes, is caused by surgical castration.^[39,40] Steatosis damages cells, which impairs cell membrane integrity and allows liver enzymes to leak into the cytoplasm. The same conclusions were found in numerous additional studies, including those by Ogunwobi *et al.*, 2019; Domonkos *et al.*, 2017.^[41,42] Similar outcomes were seen in other studies.^[10,40] The histological sections from the castrated male rats' livers displayed hydropic degeneration (microvesicular steatosis with inflammatory cellular infiltration of lymphocytes and apoptosis in hepatocytes; Councilman body), ballooning degeneration in hepatocytes, and lipid accumulation (macrovesicular vesicular steatosis) (lobular inflammation). The molecular pathways via which low testosterone contributes to the pathophysiology of NAFLD may be the cause of these alterations. Numerous routes have been suggested: Increased hepatic lipogenesis, increased hepatic fatty acid β -oxidation, and decreased hepatic lipid export were all brought on by increased adipose tissue. Hepatic steatosis and lipogenesis are frequently elevated with obesity, hyperglycemia, and insulin resistance.^[43] According to Zhang *et al.*, 2013, there was an additional factor that led to an increase in the reactive oxygen species pathway, which was triggered by castration-induced liver cell damage.^[44] According to the "two-hit" theory of NAFLD progression, which is supported by the current study's increased inflammatory cells in liver tissue, oxidative stress, apoptosis, and inflammation all play important roles in the disease's pathological progression.^[45] Histological sections of liver tissue from the castrated group that received *M. longifolia* treatment demonstrated the extract's beneficial role in somewhat mitigating the effects of castration. This may be because of the extract's protective properties and its composition, which can either directly or indirectly reduce oxidative damage by inhibiting the synthesis and deposition of liver fat and preventing the overproduction of free radicals. Therefore, controlling the expression of immune response-related genes. The results of numerous studies support these findings, which show that a variety

of antioxidant classes, including polyphenols, resveratrol, quercetin, coumestrol, anthocyanins, epigallocatechin gallate, and curcumin, carotenoids, and carotenoids, have positive effects on the reversion of fatty liver, sometimes through an indirect interaction with mitochondrial metabolism.^[46]

CONCLUSION AND RECOMMENDATIONS

The present study demonstrated that hydropic hepatocyte degeneration, lymphocyte cellular inflammation, and microvesicular steatosis were all brought on by testicular excision surgery. Rats that were castrated exhibited minimal response to *M. longifolia* administration, whereas histological examination suggested that this extract might have some hepatoprotective benefits after castration. We can suggest using this extract at different concentrations for different periods of time to show how it affects both physiological and histological characteristics. It is necessary to do additional research to ascertain whether this extract, in combination with testosterone, is beneficial in treating particular liver diseases. In addition, electron microscopy will be used to examine the Ito cell lineage and its activation.

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