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Research Article
Possible Protective Role of Whey Protein on the Rat's Liver Tissues Treated with Nandrolone decanoate

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Abstract

Background and Objective: Nandrolone and whey protein are used as supplementary food and athletic food. The aim of this study was to evaluate the possible histological and ultrastructural alterations in the liver of adult rats after treatment of the anabolic androgenic steroids (Nandrolone decanoate) and whey protein. **Materials and Methods:** Twenty eight Wistar Albino male rats were used in the present study divided into 4 groups: Control group received 0.5 mL of saline solution by oral, Nandrolone group injected intramuscular (10 mg kg^{-1} b.wt./week for 3 months), whey protein group treated by oral (5 mg kg^{-1} b.wt./week for 3 months) and Nandrolone and whey protein group. At the end of the experimentation, all the rats were sacrificed and liver samples were processed for histological and ultrastructural examination. Haematoxylin and eosin stains for general histological examination and Mallory trichrome stain for collagen fibers. **Results:** Light microscopy examination of the liver of the nandrolone group showed bleeding and widening of the blood sinusoids. Degeneration, vacuolation, coagulative necrosis and pyknotic nuclei were observed. In addition, increased collagen fibers were detected. Whey protein group showed more or less normal hepatocytes, blood sinusoids and collagen fibers. The nandrolone and whey protein group illustrated normal appearance of hepatocytes with vacuolation in some of the hepatocytes and normal blood sinusoids and collagen fibers were noticed. Electron microscopic examination of the nandrolone group showed depletion of the nuclear chromatin, damaged mitochondria, increased of lysosomes, some lipid droplets, damaged blood sinusoids and space of Disse and increased of Kupffer cells, whereas the whey protein group appeared normal. The nandrolone and whey protein group showed well developed hepatocytes, regular space of Disse and normal hepatic sinusoids. **Conclusions:** Whey protein may be ameliorate the hepatic architecture after treatment with nandrolone.

Key words: Nandrolone, whey protein, histopathology of hepatocytes, athletic food, alteration in liver and histochemistry, Ultrastructure

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nandrolone is one of the most used anabolic-androgenic steroids (AAS) and has been used for treating patients suffering from growth retardation[1]. It was reported that AAS is a synthetic substance related to the male sex hormones (androgens) that promote growth of skeletal muscle and the development of male sexual characteristics[2]. The AAS are used in medical clinics to improve physical performance of individuals subjected to physical training[3].

These drugs have displayed great effects on the protein-building properties of the body, but their clinical use has been quite limited[4,5]. Additionally, AAS may play a significant role in clinical situations, such as HIV-related muscle wasting, severe burn injury, trauma following surgery, neuromuscular disorders and malnutrition due to alcoholic cirrhosis[5].

Undesirable side effects have been demonstrated after short- and long-term AAS treatment. The most affected organ is the liver, which displayed alterations related to AAS[6,7].

Moreover, AAS modulation of hydroxylase activity can cause hypercalcaemia in men[8]. In addition, the hepatotoxicity is the most common side effect of AAS[9].

Likewise, AAS-induced jaundice, cholestasis, hepatic adenomas, hepatitis, hepatocellular necrosis, liver cysts,[7-10-12] peliosis hepatis and hepatocellular carcinoma[13,14] are common side effects of AAS. Moreover, toxicant-associated fatty liver disease (TAFLD), non-alcoholic steatohepatitis, cirrhosis, hepatocellular carcinoma and steatosis are caused by abuse of AAS[15].

Whey Protein (WP) is the milk serum protein defined as substances that remain soluble in milk serum[16]. During cheese production, milk serum proteins are naturally formed[17,18] and represent approximately 20% of all the protein in milk[19]. Whey protein containing supplements for the anabolic process in resistance training have been supported by several investigations[20].

Pacheco *et al.*[21] and Bowen *et al.*[22] mentioned that building and repairing muscles can be affected by WP, which has high concentrations of branched chain amino acids ($BCAA_S$) like valine, isoleucine and leucine. Milk serum proteins do not clot in acidic conditions through the stomach and quickly reach the jejunum. In a short time, they can be in the digestion process and increase the plasma amino acid concentration[18-23].

In addition, Sousa *et al.*[24] suggested that there is a relationship between WP and hepatoprotective effects, oxidative stress and increased resting energy expenditure. Moreover, the WP exhibited antioxidant and anti-inflammatory markers and reduced the blood pressure.

Recent studies indicated that the milk proteins have antioxidant properties released from peptides[25,26]. These peptides are factored to inhibit scavengers of free radical, lipid peroxidation and transition metal ions[27-29]. Moreover, WP has hepatoprotective effects against liver injuries[30].

The work was aimed to estimate the histological and ultrastructural alterations in the liver of rats induced by nandrolone decanoate (ND) as an AAS and evaluated the protective effect of WP against AAS-induced hepatotoxicity in rats.

MATERIALS AND METHODS

The chemicals: Nandrolone decanoate (Deca-Durabolin) is available as an oily solution in ampoule. Each ampoule contains 25 mg mL^{-1} of the active ingredient. The ND is manufactured by the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, A.R.E.® under license of N.V.Organon-OSS-Holland. The drug was intramuscular injected at a dose of 10 mg kg^{-1} b.wt./week for 3 months[31,32]. Whey protein 100% gold standard whey protein WP isolate primary source powder 0.8 g kg^{-1}/day/man according to Brody[33], National Academy[34] and Tarnopolsky[35]. Whey protein is manufactured by ON Company, USA®. The drug is dissolved in distilled water and administrated orally by gastric tube at a dose of 5 mg kg^{-1} b.wt./day for 3 months. The dose was calculated according to Paget and Barnes[36].

Experimental animals: Twenty eight male Wistar Albino rats *Rattus rattus* were included in this study and carried out in the Faculty of Science. Their weight ranged from 120-130 g.

Experimental design: The experimental animals were divided into four groups of 7 rats. Every group were put in a separate clean cage in a clean solution of saline once daily orally for 3 months. According to the guide lines of the Animal Ethics Committee at Imam Abdulrahman Bin Faisal University and all work was conducted with the formal approval of (number: IRB-2016-10-086), the animals were kept in well- aired room. The animals were allowed to get water and maintained on a standard pellet:

- **Group 1 (G1):** The control group consisted of 7 rats. They received 0.5 mL saline solution once daily orally for 3 months
- **Group 2 (G2):** The animals received Nandrolone at (10 mg kg^{-1} b.wt./week) as intramuscular (i.m) injection for 3 months

- **Group 3 (G3):** The animals of this group received whey protein extract at (5 mg kg^{-1} b.wt.) daily orally by gavage needles for 3 months
- **Group 4 (G4):** The animals received whey protein (5 mg kg^{-1} b.wt.) daily orally by gavage needles for 6 weeks only. Continuously, the same treated animals received Nandrolone (10 mg kg^{-1} b.wt./week) (i.m) for the remaining 6 weeks

The animals were sacrificed after 12 weeks, liver specimens were taken for the light and the transmission electron microscopic investigations.

Light microscopic study: The specimens were immediately fixed in 10% neutral buffered formalin. The fixed tissue was then dehydrated, cleared and embedded in paraffin wax. Sections of 5 µm thickness were stained with haematoxylin and eosin[37] and Mallory trichrome stain for collagen fibers demonstration[38]. The sections were examined and photographed with an Olympus system microscope.

Electron microscopic study: Specimens were cut into very small pieces and fixed in 2.5 % glutaraldehyde and then fixed in 1% osmium tetroxide, dehydrated, cleared and embedded in Eponresin[39]. One micrometer thick sections were cut and stained with 0.5% toluidine blue and examined by light microscope. Ultrathin sections were cut and stained with uranyl acetate and lead citrate[40] and examined by JEOL 100 sec transmission electron microscope at Imam Abdulrahman Bin Faisal University.

The study experiment is done at 2016-2017 and taking 3 months to carried out.

RESULTS

Light microscopic results: Light microscopic examination of liver sections stained with haematoxylin and eosin of the control animal group showed the radial arrangement of hepatocytes surrounding the central vein (Fig. 1a), the hepatocytes characterised with large central highly chromatolized nuclei (Fig. 1b) and blood sinusoids, portal arteries and Kupffer cells were revealed (Fig.1a-c). The liver sections treated with ND (10 mg kg^{-1} b.wt.) for 3 months illustrated bleeding and widening of the blood sinusoids, hydropic degeneration and cloudy swelling of hepatocytes (Fig. 2a, b). Some sections revealed hyperactivity of the Kupffer cells (Fig. 2c). In addition, a high degree of the cellular degeneration of the hepatocytes, vacuolation and distinct coagulative necrosis of the hepatocytes were noticed (Fig. 2d).

The group that was treated with 5 mg kg^{-1} b.wt., of WP after 3 months showed more or less normal hepatocytes, blood sinusoids and Kupffer cells (Fig. 3a-c).

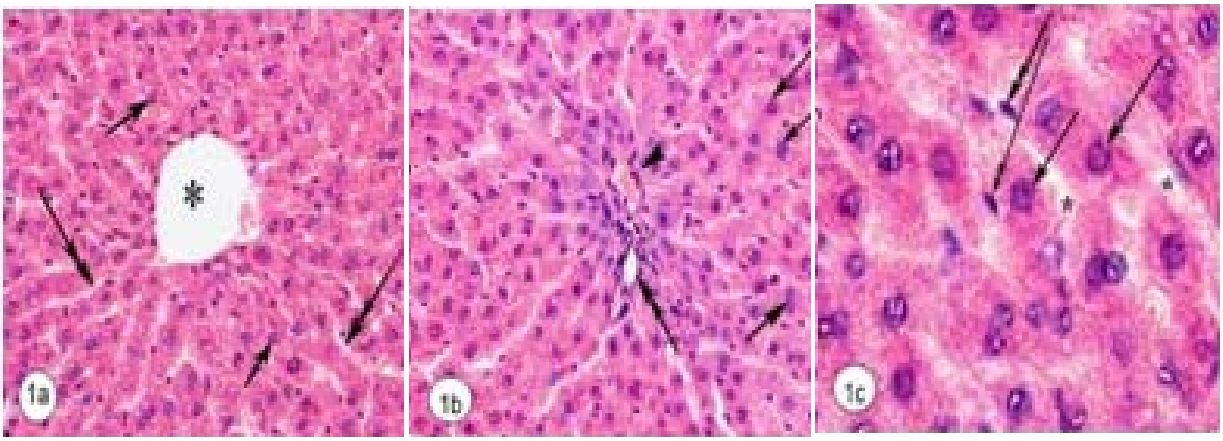

Fig. 1(a-c): Liver tissue sections of control group (G1), (a) Radial arrangement of the hepatocytes (arrow) surrounding the central vein (star). The blood sinusoids appear among the strands of the hepatocytes (double arrow) (H and E, X = 40), (b) Hepatocytes with central chromatolized nuclei (arrow). The portal space appears with the bile ductule (double arrow), Normal portal artery was revealed (arrow head). Active kupffer cells were demonstrated at the blood sinusoid (colored arrow) (H and E, X = 40) and (c) Hepatocytes with central nuclei and well-developed chromatin (arrow). Normal blood sinusoids (star) with active kupffer cells (double arrow) were observed (H and E, X = 100)

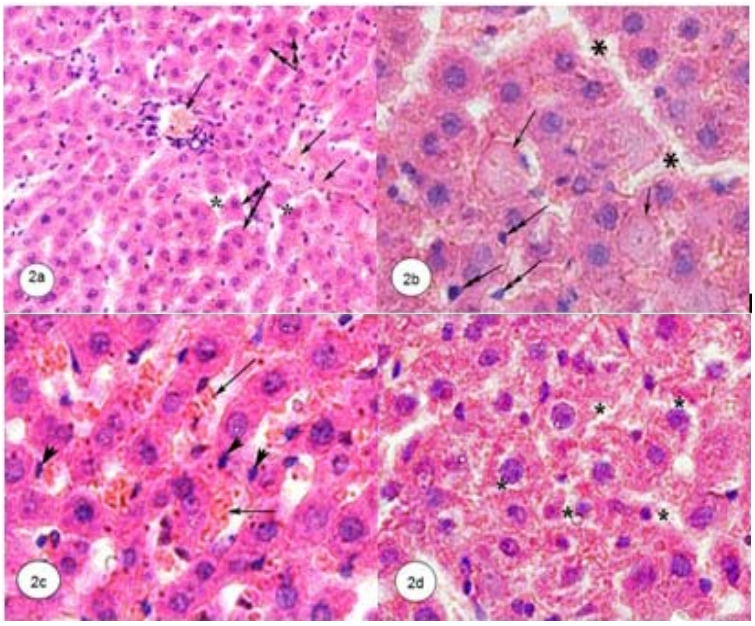

Fig. 2(a-d): Liver tissue sections of Nandrolone (10 mg kg^{-1} b.wt.) treated group (G2), (a) Bleeding (arrow) and widening of the blood sinusoids (star). Also, cloudy swelling of hepatocytes (double arrow) was revealed (H and E, X = 40), (b) Hydropic degeneration of the hepatocytes and pyknotic nuclei (arrow). Distinct widening of blood sinusoids (star) and active kupffer cells were noticed (double arrow) (H and E, X = 100), (c) Mild bleeding in the blood sinusoids (arrow) with kupffer cells hyperactivity (arrow head) (H and E, X = 100) and (d) High degree of cellular degeneration of the hepatocytes and vacuolation (star). Distinct coagulative necrosis of the hepatocytes is also shown (H and E, X = 100)

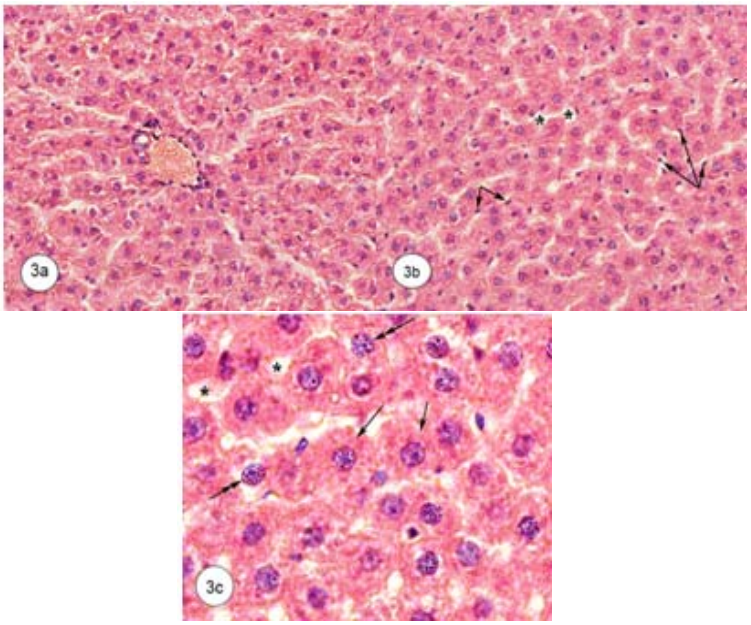

Fig. 3(a-c): Liver tissue sections of whey protein (5 mg kg^{-1} b.wt.) treated group (G3), (a) Normal architecture of hepatic tissue which illustrated the radially arranged hepatic strands with polygonal hepatocytes and centrally located nuclei (H and E, X = 40), (b) Normal blood sinusoids with some mild widening (star). Hyperactive kupffer cells (arrow) are also shown (H AND E, X = 40) and (c) Hepatocytes that show some cloudy swelling (arrow) and of some narrowing of the blood sinusoids (star). Well developed chromatic nuclei (double arrow) are also shown (H and E, X = 100)

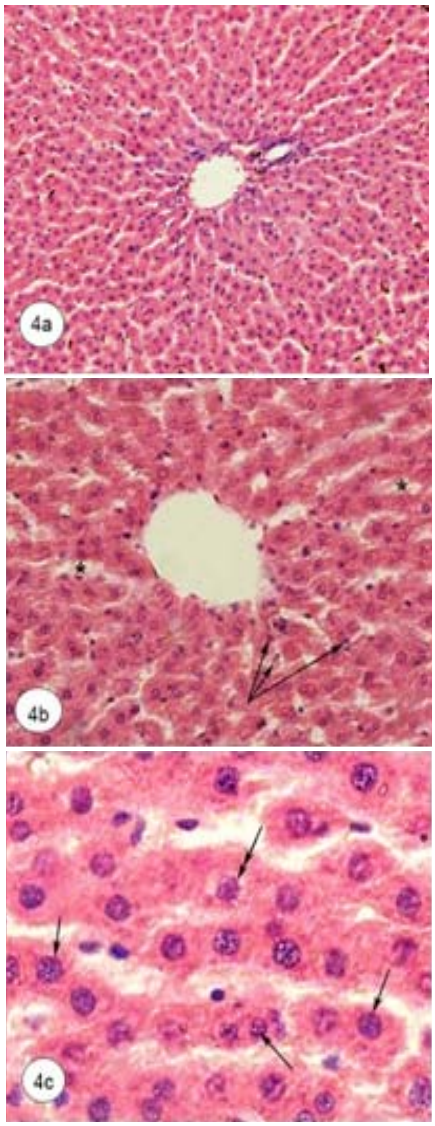

Fig. 4(a-c): Liver tissue sections of Nandrolone followed by whey protein treated group (G4), (a) Showing more or less normal architecture and improvement of the hepatic tissue. Normal central vein and hepatic strands indicated. (H and E, X = 40), (b) Hyperactive kupffer cells (arrow) scattered in the blood sinusoids. The blood sinusoids apparent normally undilated (star). (H and E, X = 40) and (c) Normal appearance of the hepatocytes with vesicular central nuclei and homogeneous cytoplasm (arrow). Some hepatocyte displayed slight cytoplasmic vacuolation (double arrow). (H and E, X = 100)

The light microscopic examination of the hepatic tissue of rats treated with ND and WP showed the normal appearance of hepatocytes. Some hepatocytes displayed slight cytoplasmic vacuolation. The blood sinusoids were normal and Kupffer cells were hyperactive (Fig. 4a-c).

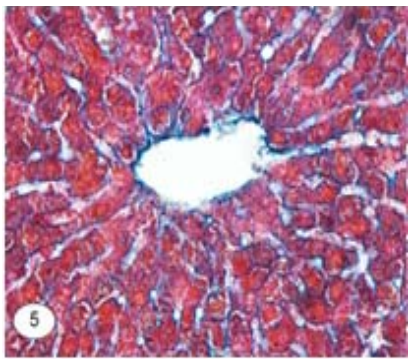

Fig. 5: Normal distribution of the collagen fibers of G1 were demonstrated with blue coloration (Masson trichrome X = 40)

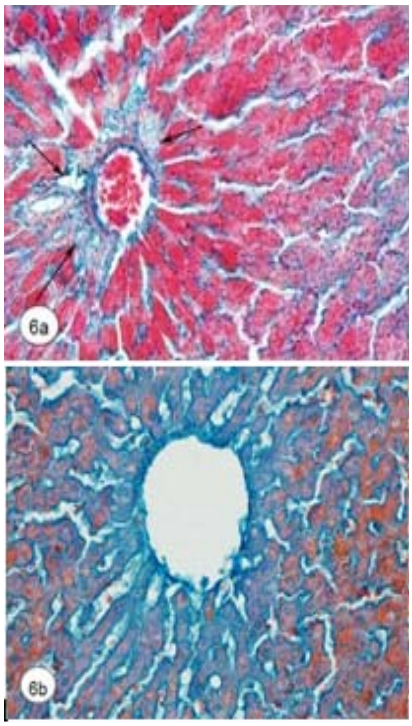

Fig. 6(a-b): Increasing collagen fibers at the portal and central spaces (arrow) and the inner lining of the blood vessels and blood sinusoids in G2

The examination of liver tissue sections stained with Masson's trichrome revealed homogeneous distribution of the collagen fiber within the hepatocytes (Fig. 5). On the other hand, the collagen fibers of the group that was treated with ND showed a significant increase, especially at the portal space, in the central region of the hepatic tissue and the inner lining of the blood vessels (Fig. 6a, b). While the liver sections

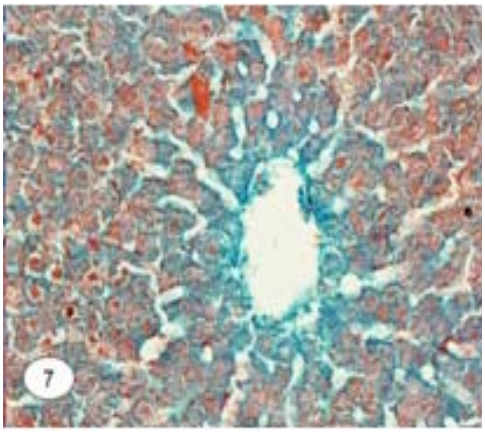

Fig. 7: Mild stained hepatic tissue in the central region in G3 (Masson trichrome, X = 40)

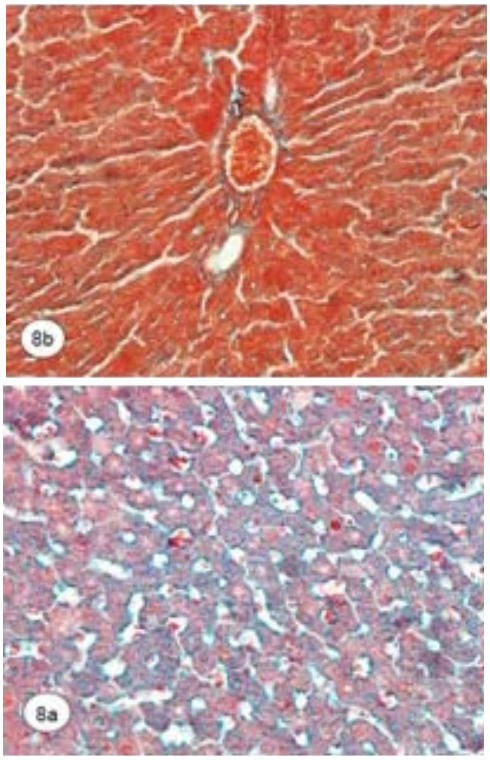

Fig. 8(a-b): Improvement in the distribution of fibers in G4 where the collagen fibers intensity and thickening were appeared normal (Masson trichrome, X = 40)

treated with WP indicated mild stained hepatic tissue, especially at the central region that surrounded the central vein, the collagen fibers revealed a great degree of improvement in the distribution of fibers (Fig. 7). However, the collagen fiber intensity and thickening appeared normal in the group that was treated with ND and WP (Fig. 8a, b).

Ultrastructural results: Ultrastructural examination of the liver tissue sections of the rats of Group 1 (control rats) showed a hepatic cell with a large central nucleus and prominent nucleolus, various mitochondria and cisternae of the rough endoplasmic reticulum (RER). In addition, a Kupffer cell exhibited a heterochromatic irregular nucleus. An endothelial cell with an elongated nucleus surrounded with small firm cytoplasm placed in the space of Disse was noticed (Fig. 9a, c).

The transmission electron microscopic observations of Group 2 sections showed signs of degeneration, such as depletion of the nuclear chromatin, damaged mitochondria and the appearance of some lipid droplets and malformed RER were markedly observed (Fig.10a). Some pyknotic nuclei of hepatocytes were also noticed (Fig. 10b) and the blood sinusoids were damaged (Fig. 10b). The space of Disse appeared damaged with the fragmented microvilli and pyknotic nuclei of the endothelial cell (Fig. 10c). The Kupffer cells increased at the malformed hepatic blood sinusoids (Fig. 10d). Bile canaliculi revealed irregular villi and an increase of lysosomes and the intercellular junction displayed marked alteration (Fig. 10e).

Tissue sections of Group 3 appeared normal. The hepatocyte appeared with a large euchromatic nucleus, various forms of mitochondria and many clusters of the RER. In addition, the blood sinusoids appeared well developed and were associated with a Kupffer cell and endothelial cell (Fig.11a, b).

Examination of the tissue sections of the liver of group 4 showed a well-developed hepatocyte with normal nucleus, homogeneous cytoplasm, various forms of mitochondria and clusters of RER. In addition, the regular space of Disse with characteristic unaltered microvilli was clearly observed. In addition, normal hepatic sinusoids with improved endothelial cells appeared (Fig. 12a-c).

DISCUSSION

The present study reported that the hepatocyte lesions induced by ND revealed cellular degeneration, cytoplasmic vacuolation and necrosis. Previous studies of AAS agreed with the present finding[41,42]. They recorded that the alterations may be due to the capability of anabolic steroids in altering the liver capacity for metabolizing xenobiotics and indicated that high doses of anabolic steroids could exert a proliferative effect on liver cells[43]. Moreover, ND may cause lipid peroxidation via Reactive Oxygen Species (ROS), leading to

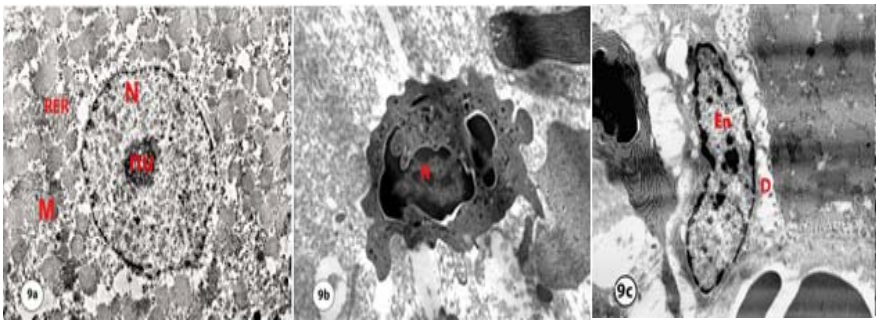

Fig. 9(a-c): Electron micrograph of control group (G1), (a) Hepatic cell with large central nucleus (N) and prominent nucleolus (nu). Various mitochondria (M) are distributed with the cytoplasm and cisternae of rough endoplasmic reticulum (RER) were noticed (X = 8900), (b) Kupffer cell with heterochromatic irregular nucleus (N) and boundaries (X = 8 900) and (c) Endothelial cell (En) with elongated nucleus surrounded with small firm of cytoplasm placed in the space of disse (D) (X = 7100)

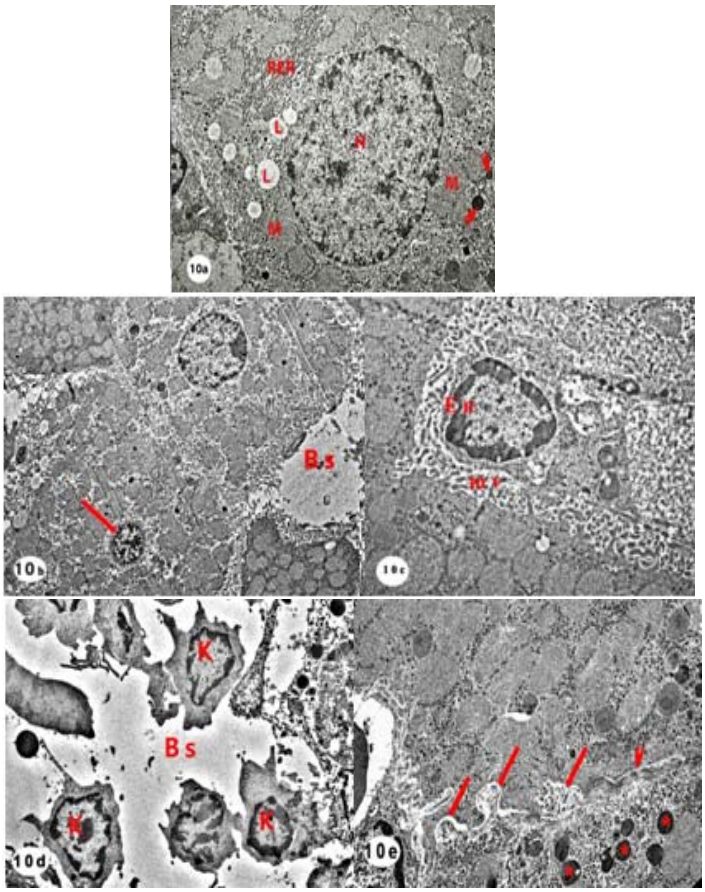

Fig. 10(a-e): Electron micrograph of Nandrolone treated group (G2), (a) Hepatocyte (H) displaying signs of degeneration such as depletion of the nuclear chromatin, damaged mitochondria (M). Appearance of some lipid droplets (L), lysosomes (arrow head) and malformed rough endoplasmic reticulum (RER) (X = 7100), (b) Hepatocytes with nuclear pyknosis (arrow) and damage of the blood sinusoids (Bs) (X = 3500) (c) Highly damaged space of Disse with fragmented microvilli (mv) and necrotic endothelial cell (En) (X = 8 900) (d) Increased kupffer cells (K) at the injured hepatic blood sinusoids (Bs) (X = 4400) and (e) Proliferation of the damaged bile canaliculi (arrow) characterized with the irregular villi. The intercellular junction display marked alteration (arrow head). Increase of the lysosomes (*) are also shown (X = 8 900)

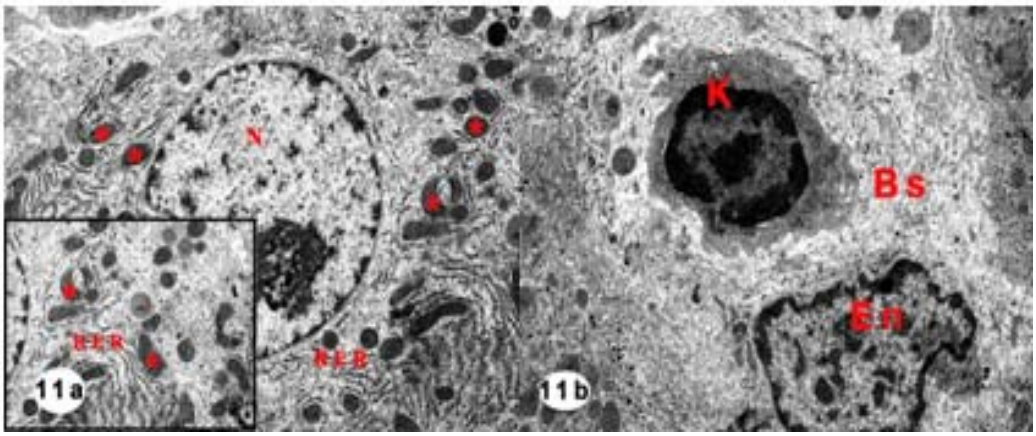

Fig. 11(a-b): Electron micrograph of Whey protein treated group (G3), (a) Showing more or less control hepatocyte with large euchromatic nucleus (N), various forms of mitochondria (*) and many clusters of the rough endoplasmic reticulum (RER) (X = 7100-11000) and (b) Well developed blood sinusoids (Bs) associated with a kupffer cell (K) and endothelial cell (En) (X = 7100)

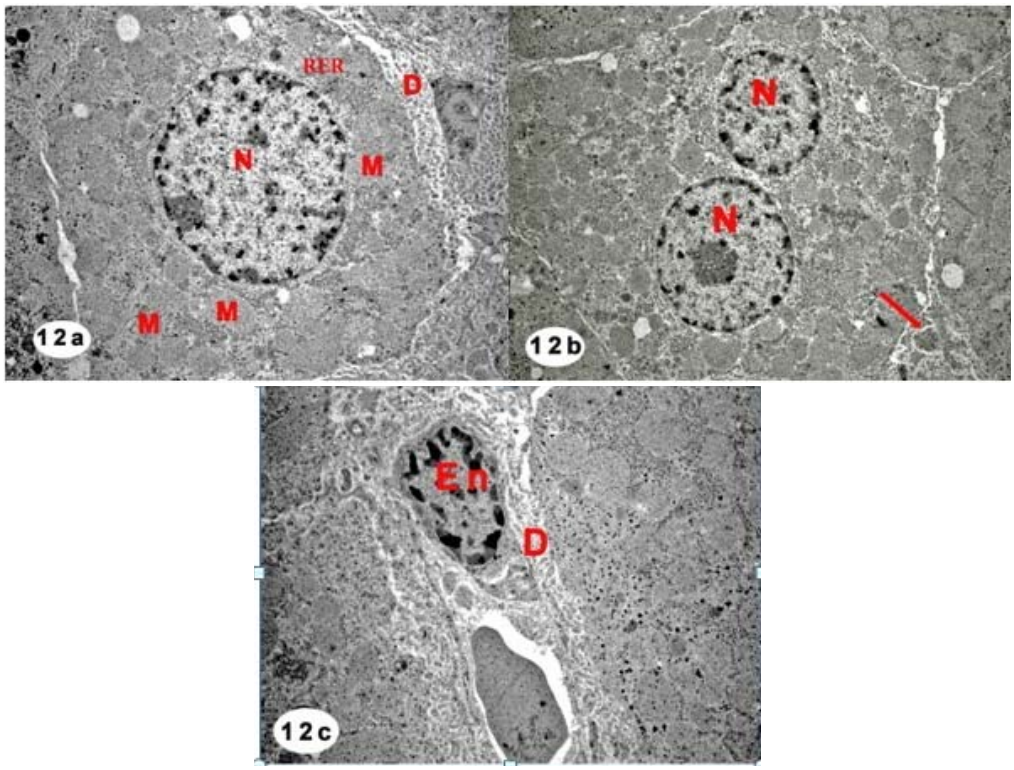

Fig. 12(a-c): Electron micrograph of Nandrolone followed by whey protein treated group (G4), (a) Well developed hepatocyte with more or less normal nucleus (N), homogeneous cytoplasm with various forms of mitochondria (M) and clusters of (RER). Space of Disse (D) appear with characteristic unaltered microvilli (X = 5600), (b) Binucleated hepatic cell with normal hepatic sinusoids (arrow) (X = 4400) and (c) One of the blood sinusoid with an endothelial cell and regular space of Disse (D) (X = 7100)

fatty changes in addition to hypoxia[42]. Histopathological lesions observed in the present study revealed how ND has ability to induce progressive liver cell injury because it is considered the first organ responsible for metabolism of ND and these effects may be induced by accumulation of toxic metabolite[44].

In addition, various studies agreed with the present findings and illustrated that acidophilic degeneration could be considered a previous phase of controlled cell death (apoptosis)[45]. Similar observations have been reported by Al-Kennany and Al-Hamdany[42] who revealed cell swelling, sinusoidal dilation and blood-vessel congestion in addition to centrilobular necrosis. Moreover, Hild *et al.*[46] mentioned that anabolic steroids have the ability to cause programmed cell death using immunohistochemistry by increasing a protein P_{53}, which is responsible for programmed cell death through the ability to damage cell DNA and mitochondria. These lesions occur due to the AAS and can cause injury to hepatocytes through the effect on mitochondria, particularly the mitochondrial membrane and can inhibit mitochondrial respiration[47].

In the present work, some lipid droplets were observed in ND-treated liver. Previous studies showed that TAFLD is connected to the abuse of AAS[15]. In addition, when AAS is used externally, most prevent the normal steroid biosynthesis process[48].

On the other hand, the results recorded increased collagen fibers in hepatocyte. This agrees with the findings by Neri *et al.*[9] and Vieira *et al.*[49] who demonstrated that the administration of ND leads to an increase in collagen deposition in the liver, which is probably related to the increment in Kupffer cell numbers while activated and Kupffer cells produce many harmful products that are compounds that act directly by stimulating the liver fibrosis process.

The current work also demonstrated damaged mitochondria and a marked increase of lysosomes. These findings agreed with those of Gragera *et al.*[50] and Turillazzi *et al.*[41] who reported that the mitochondria of rat liver induced by anabolic steroids were heterogeneous in size and shape. The most striking was the swelling and the cristae embedded in a matrix of low electron density. In addition, there was an increase in the number of lysosomes through the cytoplasm of the hepatocyte. Various studies recorded that the hepatic stellate cells (HSC) are perisinusoidal cells residing in the space of Disse, which, during injury, in response to inflammatory and other stimuli, like AAS, adopt a myofibroblast-like phenotype and represent the cornerstone of the fibrotic response in the liver[51].

Abnormal changes of the mitochondria were reported as increasing in oxidative capacity[50]. Furthermore, they reported that a disordered metabolism in the liver because of the changes in mitochondria leads to increased lysosomes. Increasing the dose of the anabolic steroid means damage to the hepatocytes that takes place by damaging the mitochondria, which leads to leakage of the liver enzymes outside of hepatocytes[42-52]. An experimental study demonstrated that prolonged AAS administration provokes dysfunction of the mitochondrial respiratory chain complexes and mono-oxygenase systems leading to an increased ROS generation[53]. Afterwards, when ROS production exceeds the high levels of enzymatic and non-enzymatic antioxidant defences and liver repair capacity, the oxidative stress-induced liver damage appears[54].

Histopathological lesions reported widening and bleeding of the malformed hepatic blood sinusoids. Consequently and rogen steroids may be cause peliosis, which is characterized by the existence of many distributed blood-filled cystic spacers over the liver parenchema[55].

In addition, it was recorded that oxidative stress could represent another mechanism of liver toxicity[54], resulting in the impairment of the canalicular bile salt export[56]. The present study revealed these results and showed the fragmentation of the microvilli of the space of Disse and the alteration of intercellular junctions. Histological findings in rats treated long-term show chronic adaptive changes in liver tissue (cytoplasmic vacuolation with lipidic degeneration in many cases). In addition, the cytoplasmic P450 and b5 enzyme contents decreased in long-term treatment[45-57]. Various studies recorded that AAS are capable of altering the liver metabolizing power and inducing cell proliferation in rat livers[45].

On the other hand, according to the current study, the hepatic tissue has clearly manifested improvement in the histological architecture of the liver tissue. These findings agree with those of Oryan *et al.*[58]. Moreover, Sousa *et al.*[24] and Peng *et al.*[59] suggested that there is a relationship between WP, oxidative stress, hepatoprotective effects and increased resting energy expenditure. In addition, it was found that the histopathological feature was improved, whereas the hepatocytes restored the normal structure with no necrotic cells. Decreased collagen fibers were observed after the treatment with WP.

Mansour *et al.*[60] reported that WP has antioxidant activity due to its ability to elevate cellular glutathione (GSH) synthesis hormone levels[61,62]. Moreover, it was found that most WPs are cysteine rich, including α-lactalbumin, β-lactoglobulin and bovine serum albumin[63]. Cysteine is known as an amino acid that regulates *in vivo* concentrations of GSH. Thus, the supplementation of the diet with WP high in cysteine may promote GSH biosynthesis. The latter has been reported to be an antioxidant and anticarcinogenic tripeptide, thus improving protection against oxidant-induced cell damage[64]. Moreover, the anti-inflammatory properties of WP due to its minor component lactoferrin that possesses anti-inflammatory

activities through inhibition of cytokine production, such as IL-Iβ, TNF-α and IL-6 from lipopolysaccharide sensitised Kupffer cells[65]. Interestingly, recent studies reported hepatic effects of lactoferrin in rats through inhibition of inflammation and reduction of inflammation and reduction of inflammatory markers, such as TNF-α, IL-18 and IL-4[66,67].

Mobley *et al.*[68] recorded that the WP supplementation acutely increases lipolysis markers in rodents[69] and reduces fat mass in younger males following two weeks of resistance training[70]. Petrella *et al.*[69] interpreted that the lipolysis character of WP may be due to unidentified peptides (produced during the hydrolysis manufacturing process) being absorbed from the digestive system and acting as ligands for cell-membrane receptors.

This explanation may provide further confirmation of the potential mechanism through which WP improved the histopathological architecture of ND-treated rats liver. Thus, the present findings demonstrated that WP offers ameliorative effects against ND-induced hepatic damage in rats.

In summary, using anabolic steroids randomly for a long time can induce hepatotoxicity and morphological alterations in the hepatic tissue and many improvement features appeared after treatment with WP. Therefore, the point of this study needs more investigation.

CONCLUSION

This study focused on the important role of WP in protection against the side effects of nandrolone that enable most people to reach a muscular size that simply does not come naturally. Abuse of AAS may cause side effects in the liver tissue. Oxidative stress is closely related to the histological alterations due to the pathophysiology. Several studies recorded the physiological changes caused by abuse of nandrolone and related drugs. On the other hand, histological confirmatory studies are rare. In addition, WP may have benefits for people with liver damage. The human body uses the cysteine found in WP to make GSH, a powerful natural antioxidant that mainly works in the liver to protect the body from free radicals and toxins. Thus, our study used the histological laboratory role to clarify the effect of WP on regaining the normal histological architecture of hepatocytes after alterations observed due to nandrolone treatment.

The present study is important to interpret and link the histological, histochemical and ultrastructural examination with the physiological results recorded in several previous studies.

SIGNIFICANCE STATEMENT

This study demonstrated the protective effect of WP to improve the histopathological architecture of ND-treated liver. This study will help the researcher to uncover the critical areas of histological and ultrastructural alterations in the liver of adult rats after treatment with AAS (ND) and WP that many researchers were not able to explore.

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REFERENCES

1. Fontana, K., M. Aldrovani, F. de Paoli, H.C. Oliveira, B. de Campos Vidal and M.A. da Cruz-Hofling, 2008. Hepatocyte nuclear phenotype: The cross-talk between anabolic androgenic steroids and exercise in transgenic mice. Histol. Histopathol., 23: 1367-1377.
2. Van Amsterdam, J., A. Opperhuizen and F. Hartgens, 2010. Adverse health effects of anabolic-androgenic steroids. Regulat. Toxicol. Pharmacol., 57: 117-123.
3. Samieinasab, M.R., M.R. Shahraki, F. Samieinasab and S. Najafi, 2015. Influence of nandrolone decanoate administration on serum lipids and liver enzymes in rats. ARYA Atherosclerosis, 11: 256-260.
4. Kicman, A.T., 2008. Pharmacology of anabolic steroids. Br. J. Pharm., 154: 502-521.
5. Basaria, S., 2010. Androgen abuse in athletes: Detection and consequences. J. Clin. Endocrinol. Metab., 95: 1533-1543.
6. Gerez, J.R., F. Frei and I.C.C. Camargo, 2005. Histological assessment of ovaries and uterus of rats subjected to nandrolone decanoate treatment. Contraception, 72: 77-80.
7. Kafrouni, M.I., R.A. Anders and S. Verma, 2007. Hepatotoxicity associated with dietary supplements containing anabolic steroids. Clin. Gastroenterol. Hepatol., 5: 809-812.
8. Bhasin, S., L. Woodhouse, R. Casaburi, A.B. Singh and D. Bhasin *et al.*, 2001. Testosterone dose-response relationships in healthy young men. Am. J. Physiol. Endocrinol. Metab., 281: E1172-E1182.
9. Neri, M., S. Bello, A. Bonsignore, S. Cantatore, I. Riezzo, E. Turillazzi and V. Fineschi, 2011. Anabolic androgenic steroids abuse and liver toxicity. Mini Rev. Med. Chem., 11: 430-437.
10. Nakao, A., K. Sakagami, Y. Nakata, K. Komazawa and T. Amimoto *et al.*, 2000. Multiple hepatic adenomas caused by long-term administration of androgenic steroids for aplastic anemia in association with familial adenomatous polyposis. J. Gastroenterol., 35: 557-562.

11. Stimac, D., S. Milic, R.D. Dintinjana, D. Kovac and S. Ristic, 2002. Androgenic/Anabolic steroid-induced toxic hepatitis. J. Clin. Gastrointerol., 35: 350-352.
12. Masumori, N., H. Ikeda and T. Endo, 2009. Acute hepatitis induced by replacement oral testosterone product in a female to male patient with gender identity disorder. Int. J. Urol., 16: 530-531.
13. Tsirigotis, P., T. Sella, M.Y. Shapira, M. Bitan and A. Bloom *et al*., 2007. Peliosis hepatis following treatment with androgen-steroids in patients with bone marrow failure syndromes. Haematologica, 92: e106-e110.
14. Gorayski, P.M., A.C. Thomas, C.H. Thompson and H.S. Subhash, 2008. Hepatocellular carcinoma associated with recreational anabolic steroid use. Br. J. Sports Med., 42: 74-75.
15. Schwingel, P.A., H.P. Cotrim, B.R. Salles, C.E. Almeida and C.R. dos Santos, Jr. *et al*., 2011. Anabolic-androgenic steroids: A possible new risk factor of toxicant associated fatty liver disease. Liver Int., 31: 348-353.
16. Luhovyy, B.L., T. Akhavan and G.H. Anderson, 2007. Whey proteins in the regulation of food intake and satiety. J. Am. Coll. Nutr., 26: 704S-712S.
17. Haraguchi, F.K., W.C. de Abreu and H. de Paula, 2006. Proteinas do soro do leite: Composicao, propriedadesnutricionais, aplicacoes no esporte e beneficiospara a saudehumana. Rev. Nutr., 19: 479-488.
18. Haraguchi, F.K., M.L. Pedrosa, H.de Paula, R.C. dos Santos and M.E. Silva, 2009. Influencia das proteinas do soro sobre enzimas hepaticas, perfil lipidico e formacao ossea de ratos hipercolesterolemicos. Rev. Nutr., 22: 515-525.
19. Pal, S., V. Ellis and S. Ho, 2010. Acute effects of whey protein isolate on cardiovascular risk factors in overweight, post-menopausal women. Atherosclerosis, 212: 339-344.
20. Naclerio, F. and E. Larumbe-Zabala, 2016. Effects of whey protein alone or as part of a multi-ingredient formulation on strength, fat-free mass, or lean body mass in resistance-trained individuals: A meta-analysis. Sports Med., 46: 125-137.
21. Pacheco, M.T.B., N.F. Dias, V.L.S. Baldini, C. Tanikawa and V.C. Sgarbieri, 2005. Propriedades funcionais de hidrolisados obtidos a partir de concentrados proteicos de soro de leite. Cienc. Tecnol. Aliment., 25: 333-338.
22. Bowen, J., M. Noakes and P.M. Clifton, 2006. Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. J. Clin. Endocrinol. Metab., 91: 2913-2919.
23. Pal, S. and V. Ellis, 2010. The acute effects of four protein meals on insulin, glucose, appetite and energy intake in lean men. Br. J. Nutr., 104: 1241-1248.
24. Sousa, G.T.D., F.S. Lira, J.C. Rosa, E.P. de Oliveira, L.M. Oyama, R.V. Santos and G.D. Pimente, 2012. Dietary whey protein lessens several risk factors for metabolic diseases: A review. Lipids Health Dis., Vol. 11. 10.1186/1476-511X-11-67
25. Hernandez-Ledesma, B., A. Davalos, B. Bartolome and L. Amigo, 2005. Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MS/MS. J. Agric. Food Chem., 53: 588-593.
26. Peng, X., B. Kong, X. Xia and Q. Liu, 2010. Reducing and radical-scavenging activities of whey protein hydrolysates prepared with Alcalase. Int. Dairy J., 20: 360-365.
27. Rajapakse, N., E. Mendis, W.K. Jung, J.Y. Je and S.K. Kim, 2005. Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. Food Res. Int., 38: 175-182.
28. Moure, A., H. Dominguez and J.C. Parajo, 2006. Antioxidant properties of ultrafiltration-recovered soy protein fractions from industrial effluents and their hydrolysates. Process Biochem., 41: 447-456.
29. Qian, Z.J., W.K. Jung and S.K. Kim, 2008. Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, *Rana catesbeiana* Shaw. Bioresour. Technol., 99: 1690-1698.
30. Kume, H., K. Okazaki and H. Sasaki, 2006. Hepatoprotective effects of whey protein on D-galactosamine-induced hepatitis and liver fibrosis in rats. Biosci. Biotechnol. Biochem., 70: 1281-1285.
31. Ferry, A., A. Vignaud, P. Noirez and W. Bertucci, 2000. Respective effects of anabolic/androgenic steroids and physical exercise on isometric contractile properties of regenerating skeletal muscles in the rat. Arch. Physiol. Biochem., 108: 257-261.
32. Joumaa, W.H. and C. Leoty, 2001. Differential effects of nandrolone decanoate in fast and slow rat skeletal muscles. Med. Sci. Sports Exercise, 33: 397-403.
33. Brody, T., 1999. Nutritional Biochemistry. 2nd Edn., Academic Press, San Diego, California, USA., Page: 1006.
34. National Academy, 2003. Dietary Reference Intakes for Energy. Part 1. National Academy Press, London, Washington, DC.
35. Tarnopolsky, M.A., 2004. Protein requirements for endurance athletes. Eur. J. Sport Sci., 4: 1-15.
36. Paget, G.E. and J.M. Barnes, 1964. Inter species dosage Conversion Scheme in Evaluation of Results and Quantitative Application in Different Species. In: Evaluation of Drug Activities: Pharmacometrics, Laurence, D.R. and A.L. Bacharach (Eds.)., Vol. 1, Academic Press, London, New York, pp: 160-162.
37. Bancroft, J.D. and M. Gamble, 2002. Theory and Practice of Histological Techniques. 5th Edn., Churchill Livingstone, London, UK., ISBN-13: 9780443064357, Pages: 796.
38. Pearse, A., 1977. Histochemistry, Theoretical and Applied. 3rd Edn., Vol. 1, Churchill Livingstone, London, UK., Page: 1518.

39. Robinson, D.G., U. Ehlers, R. Herken, B. Herrmann, F. Mayer and F.W. Schurmann, 1987. Methods of Preparation for Electron Microscopy. Springer-Verlag, Berlin Hideberg Germany, Page: 190.
40. Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17: 208-212.
41. Turillazzi, E., G. Perilli, M. Di Paolo, M. Neri, I. Riezzo and V. Fineschi, 2011. Side effects of AAS abuse: An overview. Mini Rev. Med. Chem., 11: 374-389.
42. Al-Kennany, E.R. and E.K. Al-Hamdany, 2014. Pathological effects of anabolic steroid (Sustanon®) on liver of male rats. Iraqi J. Vet. Sci., 28: 31-39.
43. Buttner, A. and D. Thieme, 2010. Side Effects of Anabolic Androgenic Steroids: Pathological Finding and Structure-Activity Relationships. In: Doping in Sports: Biochemical Principles, Effects and Analysis, Thieme, D. and P. Hemmersbach (Eds.)., Vol. 195, Springer-Verlag, Berlin Heidelberg, pp: 459-484.
44. Pertusi, R., R.D. Dickerman and W.J. McConathy, 2001. Evaluation of aminotransferase elevations in a bodybuilder using anabolic steroids: Hepatitis or rhabdomyolysis? J. Am. Osteop. Assoc., 101: 391-394.
45. Boada, L.D., M. Zumbado, S. Torres, A. Lopez, B.N. Diaz-chico, J.J. Cabrera and O.P. Luzardo, 1999. Evaluation of acute and chronic hepatotoxic effects exerted by anabolic-androgenic steroid stanozolol in adult male rats. Arch. Toxicol., 73: 465-472.
46. Hild, S.A., B.J. Attardi, S. Koduri, B.A. Till and J.R. Reel, 2010. Effects of synthetic androgens on liver function using the rabbit as a model. J. Androl., 31: 472-481.
47. Tousson, E., A. Alm-Eldeen and M. El-Moghazy, 2011. p53 and Bcl-2expression in response to boldenone induced liver cells injury. Toxicol. Ind. Health, 27: 711-718.
48. Rone, M.B., J. Fan and V. Papadopoulos, 2009. Cholesterol transport in steroid biosynthesis: Role of protein-protein interactions and implications in disease states. Biochim. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids, 1791: 646-658.
49. Vieira, R.P., R.F. Franca, N.R. Damaceno-Rodrigues, M. Dolhnikoff, E.G. Caldini, C.R.F. Carvalho and W. Ribeiro, 2008. Dose-dependent hepatic response to subchronic administration of nandrolone decanoate. Med. Sci. Sport Exercise, 40: 842-847.
50. Gragera, R., A. Saborido, F. Molano, L. Jimenez, E. Muniz and A. Megias, 1993. Ultrastructural changes induced by anabolic steroids in liver of trained rats. Histol. Histopathol., 8: 449-455.
51. Iredale, J., 2008. Defining therapeutic targets for liver fibrosis: exploiting the biology of inflammation and repair. Pharmacol. Res., 58: 129-136.
52. Lee, G.Y., H. Lee and Y.J. Kim, 2011. Rhabdomyolysis recognized after elevation of liver enzymes following prolonged urologic surgery with lateral decubitus position-A case report. Korean J. Anesthesiol., 61: 341-343.
53. Molano, F., A. Saborido, J. Delgado, M. Moran and A. Megias, 1999. Rat liver lysosomal and mitochondrial activities are modified by anabolic-androgenic steroids. Med. Sci. Sports Exercise, 31: 243-250.
54. Carvalho, M., H. Pontes, F. Remiao, M.L. Bastos and F. Carvalho, 2010. Mechanisms underlying the hepatotoxic effects of ecstasy. Curr. Pharm. Biotechnol., 11: 476-495.
55. Hausmann, R., 2005. Long-Term Effects of Anabolic-Androgenic-Steroid Abuse. Morphological Findings Associated with Fatal Outcome. In: Forensic Pathology Reviews, Tsokos, M. (Ed.)., Vol. 2, Humana Press, Totowa, New Jersey, pp: 273-289.
56. Perez, L.M., P. Milkiewicz, E. Elias, R. Coleman, E.J. Sanchez Pozzi and M.G. Roma, 2006. Oxidative stress induces internalization of the bile salt export pump, Bsep and bile salt secretory failure in isolated rat hepatocyte couplets: A role for protein kinase C and prevention by protein kinase A. Toxicol. Sci., 91: 150-158.
57. Mayol, X., G.E. Neal, R. Davies, A. Romero and J. Domingo, 1992. Ethinyl estradiol-induced cell proliferation in rat liver. Involvement of specific populations of hepatocytes. Carcinogenesis, 13: 2381-2388.
58. Oryan, A., M.H. Eftekhari, M. Ershad, M.R. Panjehshahin and H.R. Tabatabaei, 2011. Hepatoprotective effects of whey protein isolate against acute liver toxicity induced by dimethylnitrosamine in rat. Comparat. Clin. Pathol., 20: 251-257.
59. Peng, X., B. Kong, H. Yu and X. Diao, 2014. Protective effect of whey protein hydrolysates against oxidative stress in D-galactose-induced ageing rats. Int. Dairy J., 34: 80-85.
60. Mansour, D.F., A.A.A. Salama, R.R. Hegazy, E.A. Omara and S.A. Nada, 2017. Whey protein isolate protects against cyclophosphamide-induced acute liver and kidney damage in rats. J. Applied Pharmaceut. Sci., 7: 111-120.
61. Tseng, Y.M., S.K. Lin, J.K. Hsiao, J. Chen, J.H. Lee, S.H. Wu and L.Y. Tsai, 2006. Whey protein concentrate promotes the production of glutathione (GSH) by GSH reductase in the PC12 cell line after acute ethanol exposure. Food Chem. Toxicol., 44: 574-578.
62. Peng, X., Y.L. Xiong and B. Kong, 2009. Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. Food Chem., 113: 196-201.
63. Morr, C.V. and E.Y.W. Ha, 1993. Whey protein concentrates and isolates: Processing and functional properties. Crit. Rev. Food Sci. Nutr., 33: 431-476.
64. Bounous, G., F. Gervais, V. Amer, G. Batist and P. Gold, 1989. The influence of dietary whey protein on tissue glutathione and disease of aging. Clin. Invest. Med., 12: 343-349.
65. Yamaguchi, M., M. Matsuura, K. Kobayashi, H. Sasaki, T. Yajima and T. Kuwata, 2001. Lactoferrin protects against development of hepatitis caused by sensitization of Kupffer cells by lipopolysaccharide. Clin. Diagn. Lab. Immunol., 8: 1234-1239.

66. Hessin, A., R. Hegazy, A. Hassan, N. Yassin and S. Kenawy, 2015. Lactoferrin enhanced apoptosis and protected against thioacetamide-induced liver fibrosis in rats. Open Access Macedonian J. Med. Sci., 3: 195-201.
67. Hegazy, R., A. Salama, D. Mansour and A. Hassan, 2016. Renoprotective effect of lactoferrin against chromium-induced acute kidney injury in rats: Involvement of IL-18 and IGF-1 inhibition. Plos One, Vol. 11. 10.1371/journal.pone.0151486.
68. Mobley, C.B., C.T. Haun, P.A. Roberson, P.W. Mumford and M.A. Romero *et al*., 2017. Effects of whey, soy or leucine supplementation with 12 weeks of resistance training on strength, body composition and skeletal muscle and adipose tissue histological attributes in college-aged males. Nutrients, Vol. 9. 10.3390/nu9090972.
69. Petrella, J.K., J.S. Kim, D.L. Mayhew, J.M. Cross and M.M. Bamman, 2008. Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: A cluster analysis. J. Applied Physiol., 104: 1736-1742.
70. Hulmi, J.J., C.M. Lockwood and J.R. Stout, 2010. Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein. Nutr. Metabol., Vol. 7.