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# Skeletal Muscle Mitochondrial Impairment in Cirrhosis-Induced Sarcopenia

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## Abstract

Cirrhosis-associated muscle mass loss or sarcopenia is a common complication (17-30% prevalence) in cirrhotic patients. However, the pathogenesis of this complication is poorly understood. Therefore, finding the mechanisms of sarcopenia could lead to the development of therapeutic strategies against this complication. In the current study, rats underwent bile duct ligation (BDL) surgery, and their skeletal muscle (gastrocnemius; GS) was isolated and assessed 28 and 56 days after BDL operation. Significant increase in biomarkers of oxidative stress, including reactive oxygen species (ROS) formation, lipid peroxidation, and increased oxidized glutathione (GSSG) levels were detected in the muscle of cirrhotic animals. Skeletal muscle tissue antioxidant capacity and reduced glutathione (GSH) were also significantly decreased in BDL rats. Moreover, deterioration of several mitochondrial indices, including mitochondrial depolarization, increased mitochondrial permeabilization, depleted ATP reservoirs, and decreased mitochondrial dehydrogenases activity, were evident in the GS isolated from cirrhotic rats. Based on these data, oxidative stress and mitochondrial impairment seem to play as primary mechanisms of cirrhosis-induced sarcopenia.

*Keywords:* Cell death, Cirrhosis, Energy crisis, Mitochondria, Muscle waste, Weakness

## 1. Introduction

Sarcopenia is a common complication in cirrhotic patients (1). Sarcopenia could severely affect patients' quality of life (1, 2). Moreover, this complication directly influences the outcome of other therapeutic interventions such as liver transplantation in cirrhosis (1, 2). It has been found that the survival rate in cirrhotic patients with sarcopenia is lower than their non-sarcopenic counterparts (1, 2). Unfortunately, no specific therapeutic modality has been developed for cirrhosis-associated sarcopenia. The main reason for the lack of therapeutic intervention in cirrhosis-induced sarcopenia is connected with the poor understanding of the mechanism(s) of this complication.

Malnutrition, hormonal and biochemical alterations, as well as circulating endotoxins, could contribute to the pathogenesis of sarcopenia in cirrhosis (3, 4). Perturbation in skeletal muscle protein synthesis and turnover is a hallmark of sarcopenic muscle injury (3, 4). Enhanced proteolysis could lead to the loss of muscle mass in cirrhotic patients (4). Several mediators of the liver-muscle axis, including ammonia, testosterone, growth hormone, and lipopolysaccharides, have been proposed to be involved in the pathogenesis of cirrhosis-associated sarcopenia (4). However, there is no

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investigation on the role of mitochondrial impairment in the pathogenesis of sarcopenia in cirrhotic models.

Previous studies mentioned that bile duct ligation (BDL) could serve as a reliable animal model for investigating cirrhosis-induced sarcopenia (5). Muscle atrophy is adequately induced in this model (5). In the current study, BDL rats were used to evaluate the role of mitochondrial impairment and oxidative stress in cirrhosis-induced sarcopenia.

Finding the mechanism(s) of skeletal muscle injury in cirrhosis could lead to the development of novel therapeutic strategies against this complication. Therefore, the current study was designed to evaluate the role of oxidative stress and mitochondrial impairment in the pathogenesis of cirrhosis-associated sarcopenia. Rats underwent BDL surgery to induce cirrhosis. Then, 28 and 56 days after the BDL operation, the gastrocnemius muscle was isolated and assessed.

## 2. Material and Methods

### *2.1. Chemicals*

Trichloroacetic acid, 2′,7′ dichlorofluorescein diacetate (DCFH-DA), reduced glutathione (GSH), malondialdehyde, oxidized glutathione (GSSG), 3 (N-morpholino) propane sulfonic acid (MOPS), 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), sucrose, D-Mannitol, trypsin, fatty acid-free bovine serum albumin (BSA) fraction V, rhodamine123, Coomassie brilliant blue, 2, 4, 6-tripyridyl-s-triazine (TPTZ), glacial acetic acid, ferric chloride hexahydrate (FeCl3.6H2O), dithiothreitol (DTT), thiobarbituric acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). Iodoacetic acid, ethylenediaminetetraacetic acid (EDTA), di-nitro fluoro benzene, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), meta-phosphoric acid, 2 Amino 2-hydroxymethyl-propane-1, 3-diol-hydrochloride (Tris-HCl), and n-Butanol, were obtained from Merck (Darmstadt, Germany).

### *2.2. Animals*

Male Sprague-Dawley rats (n=18) weighing 200-250 g were obtained from Shiraz University of Medical Sciences, Shiraz, Iran. Animals were housed at the temperature of 23±1 ºC with 40% relative humidity. Rats had free access to tap water and a commercial rodent pellet diet (Royan Feed®, Isfahan, Iran). All procedures were in accordance with the protocol for care and use of laboratory animals and approved by the ethics committee at Shiraz University of Medical Sciences, Shiraz, Iran (94-01-36-10650).

### *2.3. Bile duct ligation surgery for cirrhosis induction and experimental setup*

Animals were anesthetized with a mixture of ketamine (60 mg/kg, i.p) and xylazine (10 mg/kg, i.p). A midline incision (2 cm) through the *linea alba* was made (6-8). The common bile duct was ligated and cut between ligatures. In sham-operated animals, the bile duct was identified and manipulated without ligation (9, 10). The assessment of serum biochemical measurements confirmed the occurrence of cholestasis in BDL rats (11, 12). Rats were allotted into three groups (n = 6 in each group). At days 28 and 56, after BDL surgery, animals were anesthetized (80 mg/kg, thiopental, i.p), and muscle (Gastrocnemius) samples were collected.

### *2.4. Plasma and muscle ammonia levels*

Plasma levels were measured with standard kits (13, 14). GS tissue extract was prepared for assessing the $NH^{4+}$ levels (13, 14). For this purpose, samples (100 mg) of the GS muscle samples were dissected, homogenized, and deproteinized in 3 mL of ice-cooled lysis solution (Trichloroacetic acid, 6%, w/v, 4 °C). After centrifugation (17,000g, 5 min, 4 °C), the supernatant was collected and neutralized with potassium carbonate ($KHCO_3$; 2 mol/L, pH=7). Afterward, brain ammonia content was measured using standard kits (13, 14).

### *2.5. Rotarod test*

Rats underwent four sessions of rotarod performance on a rotarod apparatus to assess muscle function and locomotor activity (15, 16). The speed of the rotarod was 10 rpm. Each rotarod session had three trials for each rat with 10 min interval. The time that rats stayed on the rotating

rod was recorded (15-17).

### *2.6. Reactive oxygen species (ROS) formation in skeletal muscle*

Reactive oxygen species (ROS) formation was estimated using 2′, 7′ dichlorofluorescein diacetate (DCF-DA) (10, 18-22). Briefly, tissue samples (200 mg) were homogenized in 5 mL of ice-cooled Tris-HCl buffer (40 mM, pH=7.4). Then, samples of the tissue homogenate (100 µL) were treated with Tris-HCl buffer (1 mL) and DCF-DA (Final concentration 10 µM) (23-26). The mixture was incubated at 37 ºC (10 min, in the dark). Finally, the fluorescence intensity was assessed using a fluorimeter (FLUOstar Omega®, $\lambda_{excit}$=485 nm, and $\lambda_{emiss}$=525 nm) (9, 10, 27, 28).

### *2.7. Lipid peroxidation in skeletal muscle of cirrhotic animals*

The thiobarbituric acid reactive substances (TBARS) were measured in the GS tissue as an index of lipid peroxidation (10, 29-32). Briefly, 500 µL of tissue homogenate (10% w/v in KCl, 1.15% w: v) was treated with 1 mL of thiobarbituric acid (0.375%, w: v), and 3 mL of phosphoric acid (1% w:v, pH=2) (33-36). Samples were mixed well and heated (100 °C) for 45 minutes. Then, 2 mL of n-butanol was added, mixed well, and centrifuged (10000 g, 20 min) (37-42). Finally, the absorbance of developed color in the n-butanol phase (upper phase) was measured (λ=532 nm, EPOCH® plate reader, BioTek®, USA) (10, 13, 43-46).

### *2.8. Total antioxidant capacity of Gastrocnemius muscle*

Ferric reducing antioxidant power (FRAP) assay for the assessment of the GS total antioxidant capacity in the sham-operated and cirrhotic animals (10, 47-49). Briefly, the working FRAP reagent was freshly-prepared by mixing 10 volumes of 300 mM acetate buffer (pH=3.6), with 1 volume of 10 mM TPTZ (in 40 mM HCl) and 1 volume of 20 mM ferric chloride. Tissue was homogenized in an ice-cooled 250 mM Tris-HCl buffer containing 200 mM sucrose and 5 mM DTT (pH=7.4) (33, 50-54). Afterward, 100 µL of tissue homogenate was added to 900 µL of the FRAP reagent. Samples were incubated in the dark (5 min at 37 ºC). Finally, the absorbance was measured using EPOCH® plate reader (λ=595 nm, BioTek®, USA) (10, 41, 55).

### *2.9. Protein carbonylation*

Protein carbonylation in the skeletal muscle of cirrhotic rats was assessed using dinitrophenylhydrazine (DNPH) (56-58). Briefly, 200 mg of the GS tissue was homogenized in 5 mL of the triton X-100 (0.1%. v:v)-containing phosphate buffer solution (pH=7.5). Tissue homogenate was centrifuged (700 g, 10 min, 4 ºC), and 500 µL of the resulting supernatant was treated with 300 µL of 10 mM DNPH (dissolved in HCl) (59). Samples were then incubated (1 h, 25 ºC, in the dark, with vortexing every 10 minutes) (56, 57, 60-62). Then, 100 µL trichloroacetic acid (20% w:v) was added, tubes were centrifuged (17,000 g, 5 min), and the supernatant was discarded. Afterward, the pellet was washed three times, with ethanol: ethyl acetate (1 mL of 1:1 v:v), and the precipitate was re-dissolved in 6 M guanidine chloride solution (pH=2.3) (35). Finally, samples were centrifuged (17,000 g, 5 min), and absorbance of the supernatant was measured at λ=370 nm (EPOCH® plate reader, BioTek® Instruments, USA) (56-58).

### *2.10. Skeletal muscle mitochondria isolation*

Gastrocnemius was isolated, washed, and minced in an ice-cold buffer medium (70 mM mannitol, 220 mM sucrose, 2 mM HEPES, 0.5 mM EGTA and 0.1% BSA, pH=7.4). Minced tissue was transported into a fresh buffer (5 mL buffer/1g tissue) containing trypsin (0.1% w:v). Samples were incubated on ice (15 min). Then, samples were centrifuged (1000 g, 10 min, 4 °C), and the supernatant was discarded. The pellet was homogenized in fresh mitochondria isolation buffer (5 mL buffer: 1g tissue). Mitochondria were isolated by differential centrifugation method as previously described (63-65). First, unbroken cells and nuclei were pelleted at 1000 g for 10 min at 4 ºC; second, the supernatant was centrifuged at 10000 g for 10 min at 4 ºC to pellet the mitochondria. The second step was repeated at least four times using the fresh isolation buffer medium. Final mitochondrial pellets were suspended in a buffer containing 70 mM mannitol, 2 mM HEPES, 220 mM sucrose, and 0.5

mM EGTA, pH=7.4, except for the mitochondria used to assess mitochondrial depolarization and mitochondrial swelling, which were suspended in depolarization assay buffer (220 mM Sucrose, 68 mM Mannitol, 10 mM KCl, 2 mM $MgCl_2$, 5 mM $KH_2PO_4$, 50 µM EGTA, and 10 mM HEPES, pH=7.2) and swelling assay buffer (125 mM Sucrose, 10 mM HEPES, and 65 mM KCl, pH=7.2) (63, 66). Samples protein content was determined by the Bradford method (67).

### *2.11. Mitochondrial dehydrogenase activity (MTT assay)*

3-(4, 5-dimethylthiazol-2-yl)-2, the 5-diphenyltetrazolium bromide (MTT) was used to estimate mitochondrial dehydrogenase activity in the GS (68-72). Briefly, a mitochondrial suspension (0.5 mg protein/ml) was treated with 40 µL of MTT (0.4% w:v) and incubated at 37 °C (30 min, in the dark). Then, samples were centrifuged (10000 g, 20 min), and the product of purple formazan crystals (pellet) was dissolved in 1 mL dimethyl sulfoxide. Finally, the absorbance at λ=570 nm was assessed using an EPOCH plate reader (Bio-Tek® Instruments, USA) (28, 48, 73).

### *2.12. Mitochondrial depolarization*

The rhodamine 123 uptake by isolated GS mitochondria was used to estimate mitochondrial depolarization (10, 43, 63, 74). Briefly, the mitochondrial fractions (0.5 mg protein/ml) were incubated with 5 µM of rhodamine 123 in the depolarization assay buffer (Final concentration 10 µM) for 10 minutes (21, 75-81). Then, samples were centrifuged (17,000 g, 1 min, 4 ºC), and the fluorescence intensity of the supernatant was measured using a fluorimeter (FLUOstar Omega®, $\lambda_{excitation}$=485 nm and $\lambda_{emission}$=525 nm) (63, 82, 83).

### *2.13. Mitochondrial swelling*

The changes in light scattering at λ=540 nm (25 °C) was used as a method to evaluate mitochondrial swelling (63, 82, 84). Briefly, isolated mitochondria (0.5 mg/mL) were suspended in swelling buffer, and the absorbance was monitored at λ=540 nm (30 min, using an EPOCH plate reader; Bio-Tek® Instruments, USA) (70, 85-87). The decrease in sample absorbance is associated with an increase in mitochondrial swelling (63, 82, 88).

### *2.14. Mitochondrial and muscle tissue reduced (GSH) and oxidized (GSSG) glutathione*

Mitochondrial and tissue glutathione content (oxidized and reduced) was measured using an HPLC method based on a previously reported protocol (89). Briefly, isolated mitochondria (1 mL, 1mg protein/mL) or homogenized GS tissue (1 mL of 10% w:v homogenate) were treated with 100 µL of trichloroacetic acid (50 % w:v). Samples were incubated on ice (15 min) and centrifuged (17,0000 g, 30 min, 4 °C). The supernatant was collected in 5 mL tubes and treated with 300 µL of NaOH: $Na_2CO_3$ (2 M: 2 M solution). Afterward, 100 µL of iodoacetic acid (15% w:v) was added and incubated at 4 °C for one hour. Then, 100 µL of dinitrofluorobenzene was added. Samples were mixed well and incubated in the dark (25 °C, 24 h). Finally, samples were centrifuged (17000 g, 20 min), filtered, and injected (25 µL) to an HPLC apparatus. The HPLC system composed of an NH2 column (Bischoff chromatography, Leonberg, Germany, 25 cm length, 10 µm particle size, 10 Å pore size), and a UV detector (λ=252 nm). A gradient method using the mobile phases A (Water: Methanol; 1:4 v: v) and B (Acetate buffer: Buffer A; l; 1:4 v: v) was used (flow rate of 1 mL/min) (90, 91).

### *2.15. Lipid peroxidation in isolated mitochondria*

Thiobarbituric acid-reactive substances (TBARS) test was used for lipid peroxidation assay in isolated kidney mitochondria (63). As previous studies mentioned, sucrose interferes with the TBARS assay (63). Hence, isolated mitochondria were washed once in an ice-cooled MOPS-KCl buffer (50 mM MOPS and 100 mM KCl, pH=7.4). Afterward, GS isolated mitochondria preparations were re-suspended in MOPS-KCl buffer and used for the lipid peroxidation assay (63). For this purpose, the mitochondrial suspension (1 mL) was added to 2 mL of a mixture containing trichloroacetic acid (15% w/v), thiobarbituric acid (0.375%), HCl (0.24 N), and Trolox (0.5 mM). Samples were heated for 15 min at 100 ºC (63). After centrifugation (17000 g, 10 min), the absor-

bance was measured at λ=532 nm (EPOCH plate reader, BioTek® Instruments, USA) (63).

### 2.16. Mitochondrial ATP content

Based on a previously reported procedure, GS mitochondrial ATP level was assessed using an HPLC (91, 92). Briefly, isolated GS mitochondria (1 mg protein/mL) were treated with 100 µL of ice-cooled perchloric acid (200 mM), incubated on ice (5 min), and centrifuged (30 min, 17,000 g, 4 ºC). Afterward, the supernatant (100 µL) was treated with its equivalent volume of ice-cooled KOH (1 M). Samples were filtered and injected (25 µL) into an HPLC system consisted of an LC-18 column (µ-Bondapak, 15 cm) (93). The mobile phase was composed of tertiary butyl ammonium sulfate (2.3 mM), $KH_2PO_4$ (215 mM), KOH (1 M, 0.4% w:v), and acetonitrile (4 % v:v). The flow rate was 1 mL/min, and the UV detector was set at λ=254 nm (94).

### 2.17. Statistical analysis

Data are represented as mean±SD. Data comparison was performed by the one-way analysis of variance (ANOVA) with Tukey's multiple comparison test as the post hoc. $P<0.05$ was considered a statistically significant difference.

## 3. Results

A significant decrease in muscle mass index was evident in the GS muscle of cirrhotic animals (56 days after BDL surgery) (Figure 1). Animals locomotor activity and muscle function were also significantly declined in cholestatic rats (Figure 1). Moreover, significant changes in muscle tissue, and plasma ammonia levels were evident in the cirrhotic rats (Figure 1).

Significant changes in biomarkers of oxidative stress were evident in the GS muscle isolated from cirrhotic animals (Figure 2). ROS for-

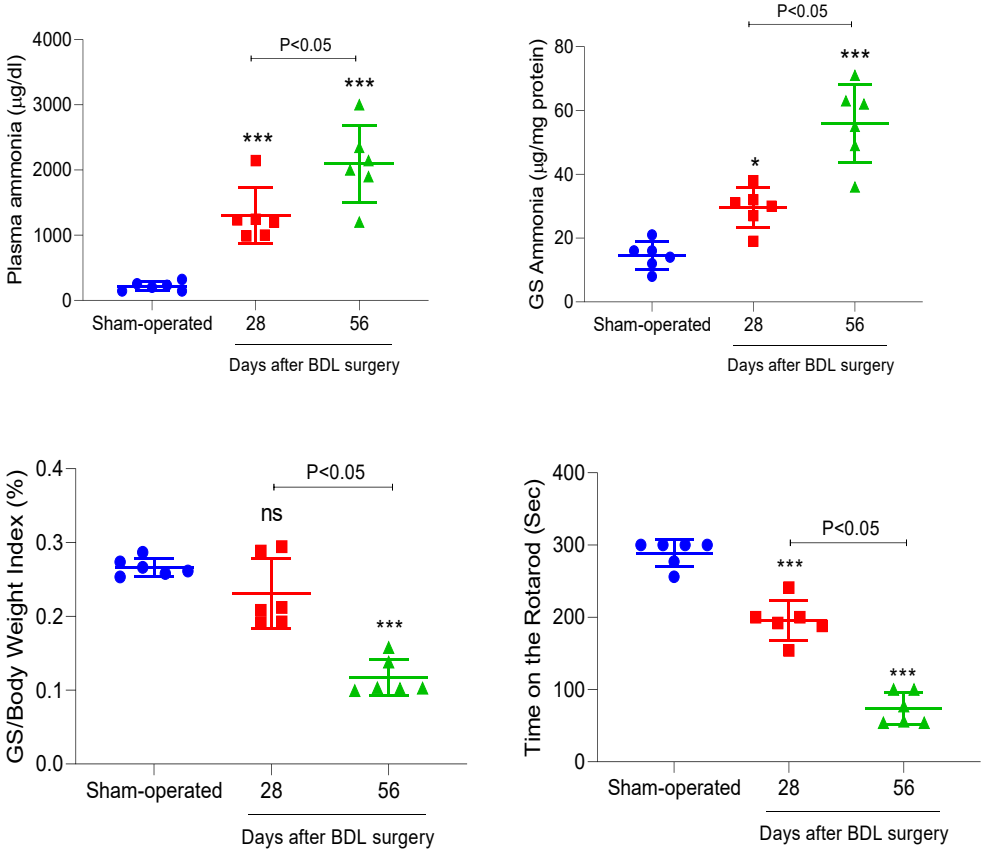


Figure 1. Gastrocnemius muscle (GS) weight index, ammonia level, and rotarod test in bile duct ligated (BDL) rats. Data are given as mean±SD (n=6).
***Indicates significantly different as compared with the sham-operated group ($P<0.05$).
ns: not significant.

mation, lipid peroxidation, increased GSSG levels, and protein carbonylation was detected in GS of cirrhotic rats (Figure 2). On the other hand, GS antioxidant capacity and GSH levels were significantly decreased at 28 and 56 days after the BDL surgery (Figure 2). The development of oxidative stress in the GS tissue of cirrhotic rats was time-dependent in the current study (Figure 2).

Disturbances in mitochondrial function were evident in mitochondria isolated from GS of cirrhotic rats (Figure 3). Significant mitochondrial depolarization, mitochondrial permeabilization, depleted glutathione reservoirs, as well as ATP deprivation were detected in GS mitochondria of cirrhotic animals as assessed 28 and 56 days after BDL operation (Figure 3). It was found that GS mitochondrial function was time-dependently deteriorated in the current model (Figure 3).

Histopathological evaluation of the GS revealed significant muscle atrophy in comparison with the sham-operated group (Figure 4). Muscle atrophy in BDL rats was deteriorated in a time-dependent manner (Figure 4).

## 4. Discussion

Sarcopenia is a frequent but mostly hidden complication of cirrhosis (1, 2). Muscle weakness and atrophy could significantly influence cirrhotic patients' quality of life (1, 2). Lower skeletal muscle mass in cirrhosis also affects the response to

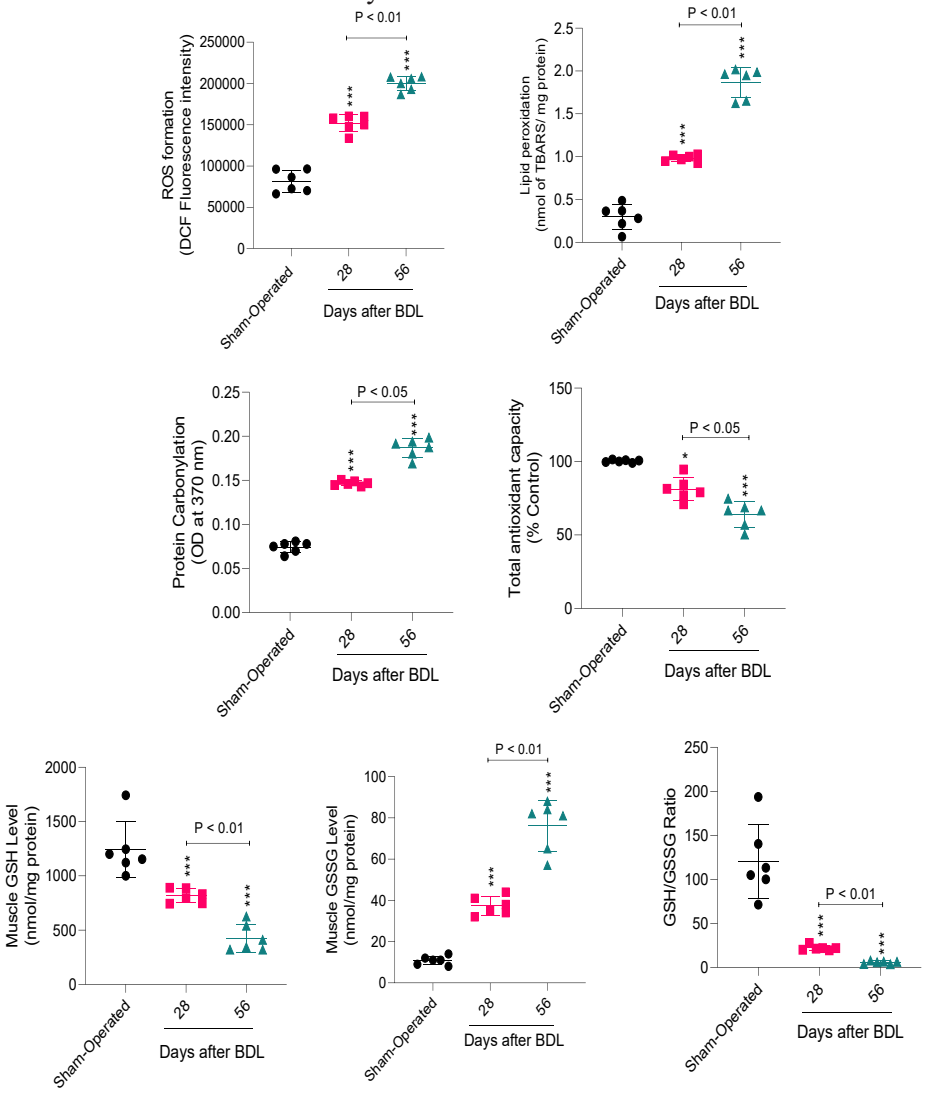


Figure 2. Biomarkers of oxidative stress in the skeletal muscle of cirrhotic rats. BDL: Bile duct ligation. Data are given as mean±SD (n=6).
***Indicates significantly different as compared with the sham-operated group ($P$<0.05).

stressors, such as body infection and surgery (1, 2). Hence, finding the mechanisms of muscle weakness and injury could lead to the development of novel therapeutic strategies in cirrhotic patients. In the current study, it was found that markers of oxidative stress were significantly increased in the GS muscle of cirrhotic rats. Moreover, several mitochondrial indices were impaired in the skeletal muscle during cirrhosis. Based on these data, oxidative stress and mitochondrial dysfunction play a fundamental role in cirrhosis-induced sarcopenia.

The role of several cytotoxic molecules has been highlighted in the mechanism of sarcopenia (1, 2). It has been mentioned that all these agents could act in an interconnected mechanism to induce muscle waste and sarcopenia (1, 2). Ammonia is the primary suspected agent for inducing muscle mass loss and sarcopenia (4). The role of ammonium ion in sarcopenia has been extensively investigated (4). Neurotoxicity is a well-characterized side effect of ammonia (95). Recently, it has been reported that this molecule is also able to induce adverse effects in tissues such as skeletal muscle (4). Following the entry of ammonia in the muscle, it activates a series of signaling mechanisms, which could lead to cytotoxicity and organ injury (4). It has been found that ammonia could induce impaired protein synthesis in the skeletal muscle during cirrhosis (4, 96). Ammonia causes the release of myostatin, which is involved

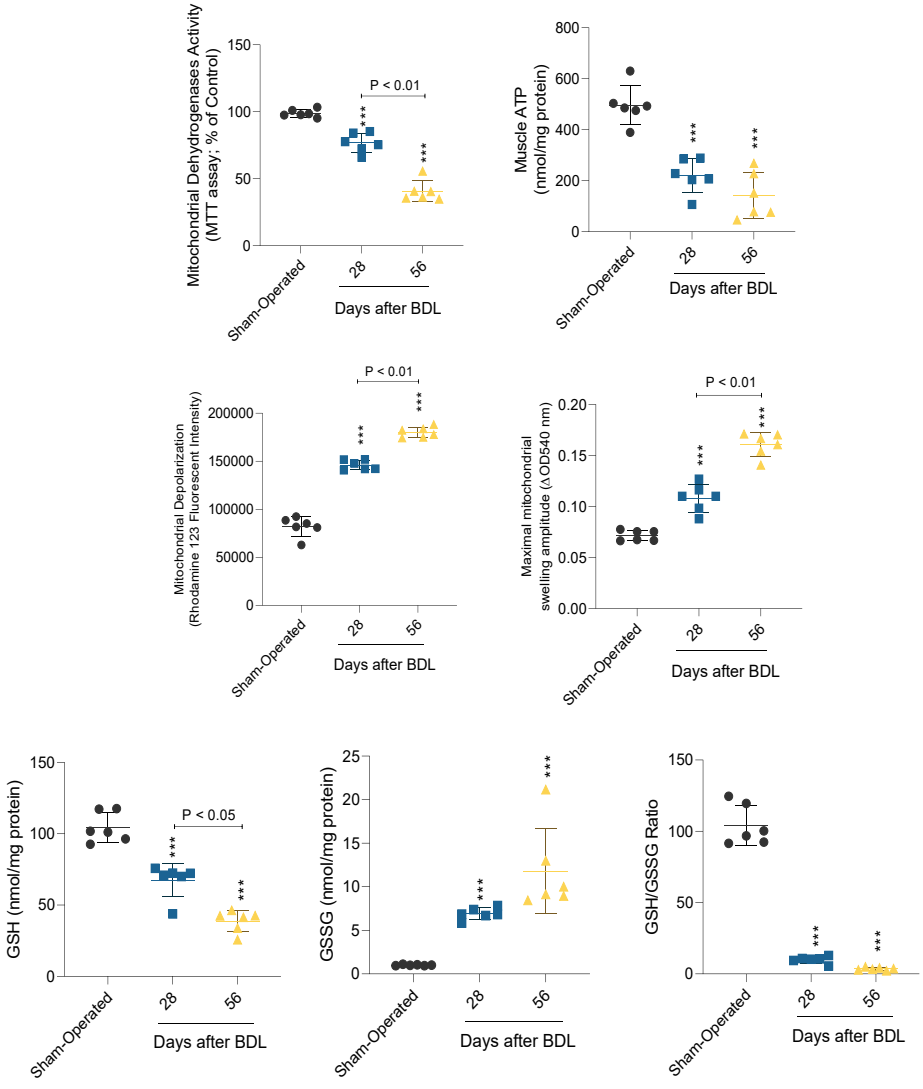


Figure 3. Mitochondrial indices in the skeletal muscle of cirrhotic rats.
Data are represented as mean±SD (n = 6).
***Indicates significantly different as compared with the sham-operated group ($P$<0.05).

in the inhibition of protein synthesis and enhancing proteolysis in skeletal muscle (4). It has been found that the expression and levels of myostatin are significantly increased in the skeletal muscle of cirrhotic patients (4). In the current study, we found that critical mitochondrial indices such as mitochondrial depolarization, mitochondrial permeabilization and swelling, tissue ATP levels, and mitochondrial dehydrogenases activity were significantly decreased in the GS of cirrhotic rats.

Ammonia is able to disrupt mitochondrial function in different tissues such as the brain (95). It has been repeatedly mentioned that ammonia cause mitochondrial impairment in neural tissue (14, 97-101). Ammonia could also inhibit critical enzymes involved in energy (ATP) metabolism in the skeletal muscle (4). Our data from the current study revealed that similar perturbation could occur in the skeletal muscle of cirrhotic animals.

Ammonia also could activate the myostatin protein (102, 103). It has been found that myostatin could decrease cellular ATP levels through different pathways (102, 103). It has been well-known that high skeletal muscle ammonia levels could significantly decrease α-ketoglutarate (104). This phenomenon is known as "cataplerosis" (104). Increase muscle ammonia could lead to the activation of a protein named "hypoxia-inducible factor-alpha; HIF-α" (105). Activation of HIF-α could lead to catastrophic events such as increased myostatin levels, decreasing acetyl-CoA, and finally, skeletal muscle energy crisis (106). In the current study, we found that skeletal muscle energy (ATP) levels were significantly depleted in the GS of cirrhotic animals (Figure 3). Although it has not been evaluated in the current study, a part of ATP depletion in the muscle of cirrhotic rats could be connected with the HIF-α pathway.

Cellular mitochondria are the primary sources of intracellular reactive oxygen species (ROS) (107). Therefore, oxidative stress and mitochondrial impairment are two firmly-interconnected events (107, 108). In the current study, we found that markers of oxidative stress were significantly increased in the GS of cirrhotic rats (Figure

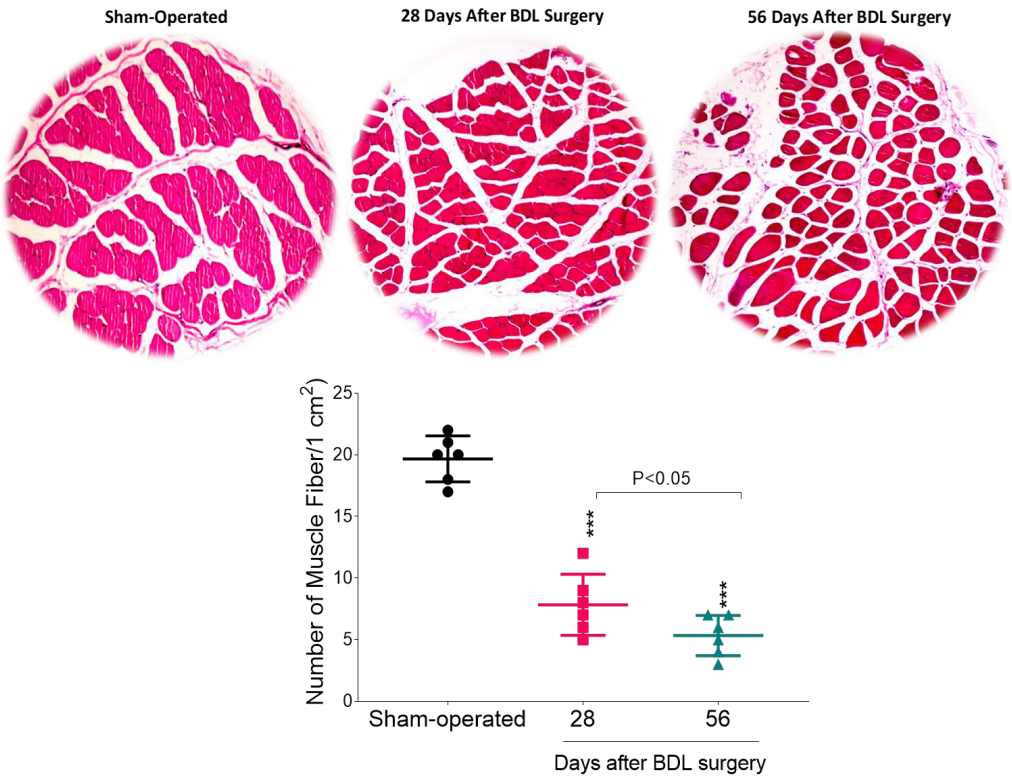


Figure 4. Skeletal muscle histopathological alterations in cirrhotic rats. Significant muscle atrophy was detected in cirrhotic animals at different time intervals post-bile duct ligation (BDL) surgery.
Data are represented as mean±SD (n=6).
***Indicates significantly different as compared with the sham-operated group ($P$<0.05).

2). Although more investigations are warranted to be precisely revealed, cellular mitochondria could act as a significant source of ROS in the GS tissue during cholestasis.

Several therapeutic options have been suggested for managing cirrhosis-associated sarcopenia (105, 109). Nutritional supplementation is the first approach for preventing skeletal muscle loss in cirrhotic patients (109, 110). Therefore, adequate calorie and protein intake have been recommended in cirrhosis (109, 110). Although nutritional supplementation could enhance muscle strength, there are some discrepancies about the effects of this strategy on cirrhotic patients' survival (110). It has been reported that nutritional supplementation might increase muscle mass and it's capacity in ammonia removal, glucose disposal, and insulin response (110). However, the contractile function of the skeletal muscle might not back to its average level (110). Protein supplementation and using ammonia lowering agents are the other recommended strategies for blunting cirrhosis-induced sarcopenia (4). However, the benefits of these strategies in improving cirrhotic patients' quality of life and survival have been questioned (4). Mild exercise also has been recommended for the cirrhotic patient to blunt muscle mass loss (111-113). The mechanism of exercise in improving cirrhotic patients' muscle strength might be associate with enhanced mitochondrial biogenesis in these patients (114). Mitochondria biogenesis could improve muscle energy (ATP) and power in cirrhotic patients (114).

Based on the data obtained from the current study, mitochondrial impairment and energy crisis could play a pivotal role in the pathogenesis of cirrhosis-associated sarcopenia. Therefore, mitochondria-targeted therapies could serve as a viable option in enhancing muscle strength and the quality of life in cirrhotic patients. Several safe and clinically-applicable agents have been developed, which could robustly improve mitochondrial function. Our previous studies mentioned the positive effects of several amino acids, antioxidant molecules, and peptides on mitochondrial function and energy metabolism in various experimental models (8, 20, 47, 52, 70, 115-118). These safe agents might find therapeutic value in enhancing muscle strength during cirrhosis. Obviously, much more investigations are needed for identifying the precise mechanism(s) of muscle weakness in cirrhotic patients and evaluating antioxidants and mitochondria protecting agents as therapeutic options.

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## Conflict of Interest

None declared.

## References

1. Montano-Loza AJ. Clinical relevance of sarcopenia in patients with cirrhosis. *World J Gastroenterol*. 2014 Jul 7;20(25):8061-71. doi: 10.3748/wjg.v20.i25.8061. PMID: 25009378; PMCID: PMC4081677.

2. Montano-Loza AJ, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, Sawyer MB. Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol*. 2012 Feb;10(2):166-73, 173.e1. doi: 10.1016/j.cgh.2011.08.028. Epub 2011 Sep 3. PMID: 21893129.

3. Hanai T, Shiraki M, Nishimura K, Ohnishi S, Imai K, Suetsugu A, Takai K, Shimizu M, Moriwaki H. Sarcopenia impairs prognosis of patients with liver cirrhosis. *Nutrition*. 2015 Jan;31(1):193-9. doi: 10.1016/j.nut.2014.07.005. Epub 2014 Jul 30. PMID: 25441595.

4. Dasarathy S, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. *J Hepatol*. 2016 Dec;65(6):1232-1244. doi: 10.1016/j.jhep.2016.07.040. Epub 2016 Aug 8. PMID: 27515775; PMCID: PMC5116259.

5. Bosoi CR, Oliveira MM, Ochoa-Sanchez R, Tremblay M, Ten Have GA, Deutz NE, Rose CF, Bemeur C. The bile duct ligated rat: A relevant model to study muscle mass loss in cirrhosis.

*Metab Brain Dis.* 2017 Apr;32(2):513-518. doi: 10.1007/s11011-016-9937-4. Epub 2016 Dec 15. PMID: 27981407.
6. Siavashpour A, Khalvati B, Azarpira N, Mohammadi H, Niknahad H, Heidari R. Poly (ADP-Ribose) polymerase-1 (PARP-1) overactivity plays a pathogenic role in bile acids-induced nephrotoxicity in cholestatic rats. *Toxicol Lett*. 2020;330:144-58.10.1016/j.toxlet.2020.05.012
7. Moezi L, Heidari R, Amirghofran Z, Nekooeian AA, Monabati A, Dehpour AR. Enhanced anti-ulcer effect of pioglitazone on gastric ulcers in cirrhotic rats: the role of nitric oxide and IL-1β. *Pharmacol* Rep. 2013;65(1):134-43. doi: 10.1016/s1734-1140(13)70971-x. PMID: 23563031.
8. Jamshidzadeh A, Heidari R, Abasvali M, Zarei M, Ommati MM, Abdoli N, Khodaei F, Yeganeh Y, Jafari F, Zarei A, Latifpour Z, Mardani E, Azarpira N, Asadi B, Najibi A. Taurine treatment preserves brain and liver mitochondrial function in a rat model of fulminant hepatic failure and hyperammonemia. *Biomed Pharmacother.* 2017 Feb;86:514-520. doi: 10.1016/j.biopha.2016.11.095.
9. Heidari R, Ghanbarinejad V, Mohammadi H, Ahmadi A, Esfandiari A, Azarpira N, Niknahad H. Dithiothreitol supplementation mitigates hepatic and renal injury in bile duct ligated mice: Potential application in the treatment of cholestasis-associated complications. *Biomed Pharmacother*. 2018 Mar;99:1022-1032. doi: 10.1016/j.biopha.2018.01.018. Epub 2018 Jan 5. PMID: 29307496.
10. Heidari R, Niknahad H. The Role and Study of Mitochondrial Impairment and Oxidative Stress in Cholestasis. *Methods Mol Biol*. 2019;1981:117-132. doi: 10.1007/978-1-4939-9420-5_8. PMID: 31016651.
11. Jamshidzadeh A, Heidari R, Mohammadi-Samani S, Azarpira N, Najbi A, Jahani P, et al. A comparison between the nephrotoxic profile of gentamicin and gentamicin nanoparticles in mice. *J Biochem Mol Toxicol*. 2015;29(2):57-62.10.1002/jbt.21667
12. Ommati MM, Farshad O, Azarpira N, Shafaghat M, Niknahad H, Heidari R. Betaine alleviates cholestasis-associated renal injury by mitigating oxidative stress and enhancing mitochondrial function. *Biologia*. 2020.10.2478/s11756-020-00576-x
13. Jamshidzadeh A, Heidari R, Latifpour Z, Ommati MM, Abdoli N, Mousavi S, Azarpira N, Zarei A, Zarei M, Asadi B, Abasvali M, Yeganeh Y, Jafari F, Saeedi A, Najibi A, Mardani E. Carnosine ameliorates liver fibrosis and hyperammonemia in cirrhotic rats. *Clin Res Hepatol Gastroenterol.* 2017 Sep;41(4):424-434. doi: 10.1016/j.clinre.2016.12.010. Epub 2017 Mar 7. PMID: 28283328.
14. Heidari R, Jamshidzadeh A, Niknahad H, Mardani E, Ommati MM, Azarpira N, Khodaei F, Zarei A, Ayarzadeh M, Mousavi S, Abdoli N, Yeganeh BS, Saeedi A, Najibi A. Effect of taurine on chronic and acute liver injury: Focus on blood and brain ammonia. *Toxicol Rep*. 2016 Apr 13;3:870-879. doi: 10.1016/j.toxrep.2016.04.002.
15. Carter RJ, Morton J, Dunnett SB. Motor coordination and balance in rodents. Curr Protoc Neurosci. 2001 Aug;Chapter 8:Unit 8.12. doi: 10.1002/0471142301.ns0812s15.
16. Heidari R, Jamshidzadeh A, Ghanbarinejad V, Ommati MM, Niknahad H. Taurine supplementation abates cirrhosis-associated locomotor dysfunction. *Clin Exp Hepatol.* 2018;4(2):72-82. doi:10.5114/ceh.2018.75956
17. Metz GA, Merkler D, Dietz V, Schwab ME, Fouad K. Efficient testing of motor function in spinal cord injured rats. *Brain Res.* 2000 Nov 17;883(2):165-77. doi: 10.1016/s0006-8993(00)02778-5. PMID: 11074045.
18. Heidari R, Babaei H, Eghbal MA. Cytoprotective effects of taurine against toxicity induced by isoniazid and hydrazine in isolated rat hepatocytes. *Arh Hig Rada Toksikol.* 2013 Jun;64(2):15-24. doi: 10.2478/10004-1254-64-2013-2297. PMID: 23819928.
19. Abdoli N, Heidari R, Azarmi Y, Eghbal MA. Mechanisms of the statins cytotoxicity in freshly isolated rat hepatocytes. *J Biochem Mol Toxicol.* 2013 Jun;27(6):287-94. doi: 10.1002/jbt.21485. Epub 2013 Apr 23. PMID: 23761184.
20. Heidari R, Niknahad H, Sadeghi A, Mohammadi H, Ghanbarinejad V, Ommati MM, Hosseini A, Azarpira N, Khodaei F, Farshad O, Rashidi E, Siavashpour A, Najibi A, Ahmadi A, Jamshidzadeh A. Betaine treatment protects liver through regulating mitochondrial function and counteracting oxidative stress in acute and chronic animal models of hepatic injury. *Biomed Phar-*

*macother.* 2018 Jul;103:75-86. doi: 10.1016/j.biopha.2018.04.010. Epub 2018 Apr 7. PMID: 29635131.
21. Niknahad AM, Ommati MM, Farshad O, Moezi L, Heidari R. Manganese-induced nephrotoxicity is mediated through oxidative stress and mitochondrial impairment. *Journal of Renal and Hepatic Disorders*. 2020;4(2):1-10.10.15586/jrenhep.2020.66
22. Jamshidzadeh A, Heidari R, Abazari F, Ramezani M, Khodaei F, Ommati MM, et al. Antimalarial drugs-induced hepatic injury in rats and the protective role of carnosine. *Pharm Sci.* 2016;22(3):170-80.10.15171/PS.2016.27
23. Ommati MM, Farshad O, Ghanbarinejad V, Mohammadi HR, Khadijeh M, Negar A, Zahra M, Ilkhaninasab F, Moezi L, Heidari R. The Nephroprotective Role of Carnosine Against Ifosfamide-Induced Renal Injury and Electrolytes Imbalance is Mediated Via the Regulation of Mitochondrial Function and Alleviation of Oxidative Stress. *Drug Res (Stuttg).* 2020 Jan;70(1):49-56. doi: 10.1055/a-1017-5085.
24. Ommati MM, Amjadinia A, Mousavi K, Azarpira N, Jamshidzadeh A, Heidari R. N-acetyl cysteine treatment mitigates biomarkers of oxidative stress in different tissues of bile duct ligated rats. *Stress*. 2020 Jun 22:1-16. doi: 10.1080/10253890.2020.1777970.
25. Ommati MM, Manthari RK, Tikka C, Niu R, Sun Z, Sabouri S, Zamiri MJ, Ahmadi HN, Ghaffari H, Heidari R, Wang J. Arsenic-induced autophagic alterations and mitochondrial impairments in HPG-S axis of mature male mice offspring (F1-generation): A persistent toxicity study. *Toxicol Lett.* 2020 Jun 15;326:83-98. doi: 10.1016/j.toxlet.2020.02.013.
26. Heidari R, Mohammadi H, Ahmadi A, Ghanbarinejad V, Kasra F, Khosravi A. Protective effect of glycine and tri-methyl glycine (betaine) against heavy metals-induced oxidative stress in liver-derived post-nuclear supernatant (PNS). *Trends in Pharmaceutical Sciences.* 2018;4(2):113-24.
27. Heidari R, Esmailie N, Azarpira N, Najibi A, Niknahad H. Effect of Thiol-reducing Agents and Antioxidants on Sulfasalazine-induced Hepatic Injury in Normotermic Recirculating Isolated Perfused Rat Liver. *Toxicol Res.* 2016;32(2):133-140. doi:10.5487/TR.2016.32.2.133
28. Ommati MM, Heidari R, Ghanbarinejad V, Abdoli N, Niknahad H. Taurine treatment provides neuroprotection in a mouse model of manganism. *Biol Trace Elem Res*. 2019;190(2):384-95.10.1007/s12011-018-1552-2
29. Heidari R, Babaei H, Eghbal MA. Amodiaquine-Heidari R, Babaei H, Eghbal MA. Amodiaquine-induced toxicity in isolated rat hepatocytes and the cytoprotective effects of taurine and/or N-acetyl cysteine. *Res Pharm Sci.* 2014 Mar-Apr;9(2):97-105.
30. Niknahad H, Heidari R, Mokhtebaz T, Mansouri S, Dehshahri S, Abdoli N, et al. Evaluating the effects of different fractions obtained from Gundelia tournefortii extract against carbon tetrachloride-induced liver injury in rats. *Trends in Pharmaceutical Sciences.* 2016;2(1):25-34.
31. Heidari R, Sadeghi N, Azarpira N, Niknahad H. Sulfasalazine-induced hepatic injury in an ex vivo model of isolated perfused rat liver and the protective role of taurine. *Pharm Sci.* 2015;21(4):211-9. doi: 10.15171/PS.2015.39
32. Eghbal MA, Anoush M, Ghoreyshi A, Heidari R. The cytoprotective effects of Allium cepa methanolic extract in freshly isolated hepatocytes. *Trends in Pharmaceutical Sciences*. 2019;5(4):207-16. doi:10.30476/tips.2020.84848.1036
33. Ommati MM, Farshad O, Niknahad H, Mousavi K, Moein M, Azarpira N, et al. Oral administration of thiol-reducing agents mitigates gut barrier disintegrity and bacterial lipopolysaccharide translocation in a rat model of biliary obstruction. *Current Research in Pharmacology and Drug Discovery*. 2020;1:10-8.10. doi:1016/j.crphar.2020.06.001
34. Ommati MM, Farshad O, Mousavi K, Jamshidzadeh A, Azmoon M, Heidari S, et al. Betaine supplementation mitigates intestinal damage and decreases serum bacterial endotoxin in cirrhotic rats. *PharmaNutrition*. 2020;12:100179. doi:10.1016/j.phanu.2020.100179
35. Heidari R, Ahmadi F, Rahimi HR, Azarpira N, Hosseinzadeh M, Najibi A, Niknahad H. Exacerbated liver injury of antithyroid drugs in endotoxin-treated mice. *Drug Chem Toxicol.* 2019 Nov;42(6):615-623. doi: 10.1080/01480545.2018.1459668. Epub 2018 May 3. PMID: 29722569.
36. Jamshidzadeh A, Heidari R, Razmjou

M, et al. An in vivo and in vitro investigation on hepatoprotective effects of Pimpinella anisum seed essential oil and extracts against carbon tetrachloride-induced toxicity. *Iran J Basic Med Sci.* 2015;18(2):205-211.
37. Heidari R, Ahmadi A, Ommati MM, Niknahad H. Methylene Blue Improves Mitochondrial Function in The Liver of Cholestatic Rats. *Trends in Pharmaceutical Sciences*. 2020;6(2):73-86. doi:10.30476/tips.2020.85961.1043
38. Heidari R, Jamshidzadeh A, Keshavarz N, Azarpira N. Mitigation of Methimazole-Induced Hepatic Injury by Taurine in Mice. *Sci Pharm.* 2014;83(1):143-158. Published 2014 Sep 30. doi:10.3797/scipharm.1408-04
39. Heidari R, Taheri V, Rahimi HR, Shirazi Yeganeh B, Niknahad H, Najibi A. Sulfasalazine-induced renal injury in rats and the protective role of thiol-reductants. *Ren Fail.* 2016;38(1):137-41. doi: 10.3109/0886022X.2015.1096731. Epub 2015 Oct 19. PMID: 26479898.
40. Karamikhah R, Jamshidzadeh A, Azarpira N, Saeidi A, Heidari R. Propylthiouracil-induced liver injury in mice and the protective role of taurine. *Pharm Sci.* 2016;21(2):94-101.10.15171/PS.2015.23
41. Heidari R, Jamshidzadeh A, Ommati MM, Rashidi E, Khodaei F, Sadeghi A, et al. Ammonia-induced mitochondrial impairment is intensified by manganese co-exposure: relevance to the management of subclinical hepatic encephalopathy and cirrhosis-associated brain injury. *Clin Exp Hepatol.* 2019;5(2):109-17. doi:10.5114/ceh.2019.85071
42. Mobasher MA, Jamshidzadeh A, Heidari R, Ghahiri G, Mobasher N. Hepatoprotective effects of Artemia salina L. extract against carbon tetrachloride-induced toxicity. T*rends in Pharmaceutical Sciences*. 2016;2(4):259-64.
43. Heidari R, Babaei H, Eghbal M. Mechanisms of methimazole cytotoxicity in isolated rat hepatocytes. *Drug Chem Toxicol.* 2013 Oct;36(4):403-11. doi: 10.3109/01480545.2012.749272. Epub 2012 Dec 21. PMID: 23256569.
44. Jamshidzadeh A, Heidari R, Golzar T, Derakhshanfar A. Effect of Eisenia foetida Extract against Cisplatin-Induced Kidney Injury in Rats. *J Diet Suppl.* 2016;13(5):551-9. doi: 10.3109/19390211.2015.1124163. Epub 2016 Feb 11. PMID: 26864051.
45. Heidari R, Rasti M, Shirazi Yeganeh B, Niknahad H, Saeedi A, Najibi A. Sulfasalazine-induced renal and hepatic injury in rats and the protective role of taurine. *Bioimpacts*. 2016;6(1):3-8. doi: 10.15171/bi.2016.01. Epub 2016 Mar 28. PMID: 27340618; PMCID: PMC4916549.
46. Najafi N, Jamshidzadeh A, Fallahzadeh H, Omidi M, Abdoli N, Najibi A, et al. Valproic acid-induced hepatotoxicity and the protective role of thiol reductants. *Trends in Pharmaceutical Sciences.* 2017;3(2):63-70.
47. Ommati MM, Jamshidzadeh A, Heidari R, Sun Z, Zamiri MJ, Khodaei F, Mousapour S, Ahmadi F, Javanmard N, Shirazi Yeganeh B. Carnosine and Histidine Supplementation Blunt Lead-Induced Reproductive Toxicity through Antioxidative and Mitochondria-Dependent Mechanisms. *Biol Trace Elem Res.* 2019 Jan;187(1):151-162. doi: 10.1007/s12011-018-1358-2. Epub 2018 May 16. PMID: 29767280.
48. Heidari R, Behnamrad S, Khodami Z, Ommati MM, Azarpira N, Vazin A. The nephroprotective properties of taurine in colistin-treated mice is mediated through the regulation of mitochondrial function and mitigation of oxidative stress. *Biomed Pharmacother*. 2019 Jan;109:103-111. doi: 10.1016/j.biopha.2018.10.093.
49. Ommati MM, Heidari R, Jamshidzadeh A, Zamiri MJ, Sun Z, Sabouri S, Wang J, Ahmadi F, Javanmard N, Seifi K, Mousapour S, Yeganeh BS. Dual effects of sulfasalazine on rat sperm characteristics, spermatogenesis, and steroidogenesis in two experimental models. *Toxicol Lett.* 2018 Mar 1;284:46-55. doi: 10.1016/j.toxlet.2017.11.034.
50. Heidari R, Moezi L, Asadi B, Ommati MM, Azarpira N. Hepatoprotective effect of boldine in a bile duct ligated rat model of cholestasis/cirrhosis. *PharmaNutrition.* 2017;5(3):109-17.10.1016/j.phanu.2017.07.001
51. Ommati MM, Jamshidzadeh A, Niknahad H, Mohammadi H, Sabouri S, Heidari R, et al. N-acetylcysteine treatment blunts liver failure-associated impairment of locomotor activity. *PharmaNutrition.* 2017;5(4):141-7. doi: 10.1016/j.phanu.2017.10.003
52. Heidari R, Ghanbarinejad V, Mohammadi H, Ahmadi A, Ommati MM, Abdoli N, Aghaei F, Esfandiari A, Azarpira N, Niknahad H. Mitochondria protection as a mechanism underlying the

hepatoprotective effects of glycine in cholestatic mice. *Biomed Pharmacother*. 2018 Jan;97:1086-1095. doi: 10.1016/j.biopha.2017.10.166.
53. Emadi E, Abdoli N, Ghanbarinejad V, Mohammadi HR, Mousavi Mobarakeh K, Azarpira N, Mahboubi Z, Niknahad H, Heidari R. The potential role of mitochondrial impairment in the pathogenesis of imatinib-induced renal injury. *Heliyon.* 2019 Jun 22;5(6):e01996. doi: 10.1016/j.heliyon.2019.e01996.
54. Jamshidzadeh A, Abdoli N, Niknahad H, Azarpira N, Mousavi S, Mardani E, et al. Carnosine supplementation mitigates brain tissue markers of oxidative stress in a rat model of fulminant hepatic failure. *Trends in Pharmaceutical Sciences.* 2017;3(3):149-60.10.1111/tips.v3i3.149
55. Niknahad H, Hosseini H, Gozashtegan F, Ebrahimi F, Azarpira N, Abdoli N, et al. The hepatoprotective role of thiol reductants against mitoxantrone-induced liver injury. T*rends in Pharmaceutical Sciences*. 2017;3(2):113-22.
56. Zhang Y-T, Zheng Q-S, Pan J, Zheng R-L. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. *Basic Clin Pharmacol Toxicol*. 2004;95(2):53-8.10.1111/j.1742-7843.2004.950202.x
57. Khodaei F, Rashedinia M, Heidari R, Rezaei M, Khoshnoud MJ. Ellagic acid improves muscle dysfunction in cuprizone-induced demyelinated mice via mitochondrial Sirt3 regulation. *Life Sci.* 2019;237:116954. doi:10.1016/j.lfs.2019.116954
58. Niknahad H, Heidari R, Firuzi R, Abazari F, Ramezani M, Azarpira N, Hosseinzadeh M, Najibi A, Saeedi A. Concurrent Inflammation Augments Antimalarial Drugs-Induced Liver Injury in Rats. *Adv Pharm Bull.* 2016 Dec;6(4):617-625. doi: 10.15171/apb.2016.076.
59. Mohammadi H, Ommati MM, Farshad O, Jamshidzadeh A, Nikbakht MR, Niknahad H, et al. Taurine and isolated mitochondria: A concentration-response study. *Trends in Pharmaceutical Sciences.* 2019;5(4):197-206. doi:10.30476/tips.2020.84851.1037
60. Heidari R, Niknahad H, Jamshidzadeh A, Azarpira N, Bazyari M, Najibi A. Carbonyl traps as potential protective agents against methimazole-induced liver injury. *J Biochem Mol Toxicol.* 2015 Apr;29(4):173-81. doi: 10.1002/jbt.21682. Epub 2014 Dec 24. Erratum in: J Biochem Mol Toxicol. 2015 Aug;29(8):398. Dosage error in article text. PMID: 25545158.
61. Ommati MM, Farshad O, Mousavi K, Taghavi R, Farajvajari S, Azarpira N, et al. Agmatine alleviates hepatic and renal injury in a rat model of obstructive jaundice. *PharmaNutrition.* 2020:100212.10.1016/j.phanu.2020.100212
62. Heidari R, Mohammadi H, Ghanbarinejad V, Ahmadi A, Ommati MM, Niknahad H, Jamshidzadeh A, Azarpira N, Abdoli N. Proline supplementation mitigates the early stage of liver injury in bile duct ligated rats. *J Basic Clin Physiol Pharmacol*. 2018 Dec 19;30(1):91-101. doi: 10.1515/jbcpp-2017-0221. PMID: 30205645.
63. Caro AA, Adlong LW, Crocker SJ, Gardner MW, Luikart EF, Gron LU. Effect of garlic-derived organosulfur compounds on mitochondrial function and integrity in isolated mouse liver mitochondria. *Toxicol Lett.* 2012;214(2):166-174. doi:10.1016/j.toxlet.2012.08.017
64. Seifi K, Rezaei M, Yansari AT, Riazi GH, Zamiri MJ, Heidari R. Saturated fatty acids may ameliorate environmental heat stress in broiler birds by affecting mitochondrial energetics and related genes. *J Therm Biol.* 2018;78:1-9. doi:10.1016/j.jtherbio.2018.08.018
65. Niknahad H, Jamshidzadeh A, Heidari R, Abdoli N, Mehdi M, Ommati FJ, et al. The postulated hepatotoxic metabolite of methimazole causes mitochondrial dysfunction and energy metabolism disturbances in liver. *Pharm Sci.* 2016;22(4):217-26.
66. Heidari R, Mandegani L, Ghanbarinejad V, Siavashpour A, Ommati MM, Azarpira N, et al. Mitochondrial dysfunction as a mechanism involved in the pathogenesis of cirrhosis-associated cholemic nephropathy. *Biomed Pharmacother.* 2019;109:271-80.10.1016/j.biopha.2018.10.104
67. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976 May 7;72:248-54. doi: 10.1006/abio.1976.9999. PMID: 942051.
68. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983 Dec 16;65(1-2):55-63. doi: 10.1016/0022-1759(83)90303-4. PMID: 6606682.
69. Niknahad H, Heidari R, Alzuhairi AM,

Najibi A. Mitochondrial dysfunction as a mechanism for pioglitazone-induced injury toward HepG2 cell line. *Pharm Sci*. 2015;20(4):169-74.
70. Ommati MM, Farshad O, Jamshidzadeh A, Heidari R. Taurine enhances skeletal muscle mitochondrial function in a rat model of resistance training. *PharmaNutrition.* 2019;9:100161. doi:10.1016/j.phanu.2019.100161
71. Niknahad H, Heidari R, Mohammadzadeh R, Ommati MM, Khodaei F, Azarpira N, et al. Sulfasalazine induces mitochondrial dysfunction and renal injury. *Ren Fail.* 2017 Nov;39(1):745-753. doi: 10.1080/0886022X.2017.1399908.
72. Ommati MM, Niknahad H, Farshad O, Azarpira N, Heidari R. In vitro and in vivo evidence on the role of mitochondrial impairment as a mechanism of lithium-induced nephrotoxicity. *Biol Trace Elem Res*. 2020:In-Press. doi:10.1007/s12011-020-02302-9
73. Mohammadi H, Sayad A, Mohammadi M, Niknahad H, Heidari R. N-acetyl cysteine treatment preserves mitochondrial indices of functionality in the brain of hyperammonemic mice. *Clin Exp Hepatol*. 2020;6(2):106-115. doi:10.5114/ceh.2020.95814
74. Heidari R, Babaei H, Eghbal MA. Ameliorative effects of taurine against methimazole-induced cytotoxicity in isolated rat hepatocytes. *Sci Pharm.* 2012 Oct-Dec;80(4):987-99. doi: 10.3797/scipharm.1205-16.
75. Ahmadi N, Ghanbarinejad V, Ommati MM, Jamshidzadeh A, Heidari R. Taurine prevents mitochondrial membrane permeabilization and swelling upon interaction with manganese: Implication in the treatment of cirrhosis-associated central nervous system complications. *J Biochem Mol Toxicol.* 2018 Nov;32(11):e22216. doi: 10.1002/jbt.22216.
76. Heidari R, Babaei H, Eghbal MA. Cytoprotective Effects of Organosulfur Compounds against Methimazole Induced Toxicity in Isolated Rat Hepatocytes. *Adv Pharm Bull.* 2013;3(1):135-142. doi:10.5681/apb.2013.023
77. Nafisi S, Heidari R, Ghaffarzadeh M, Ziaee M, Hamzeiy H, Garjani A, et al. Cytoprotective effects of silafibrate, a newly-synthesised siliconated derivative of clofibrate, against acetaminophen-induced toxicity in isolated rat hepatocytes. *Arh Hig Rada Toksikol.* 2014 Jun;65(2):169-78. doi: 10.2478/10004-1254-65-2014-2434.
78. Ommati MM, Heidari R, Zamiri MJ, Sabouri S, Zaker L, Farshad O, et al. The Footprints of Oxidative Stress and Mitochondrial Impairment in Arsenic Trioxide-Induced Testosterone Release Suppression in Pubertal and Mature F1-Male Balb/c Mice via the Downregulation of 3β-HSD, 17β-HSD, and CYP11a Expression. *Biol Trace Elem Res.* 2020 May;195(1):125-134. doi: 10.1007/s12011-019-01815-2.
79. Ommati MM, Farshad O, Niknahad H, Arabnezhad MR, Azarpira N, Mohammadi HR, et al. Cholestasis-associated reproductive toxicity in male and female rats: The fundamental role of mitochondrial impairment and oxidative stress. *Toxicol Lett.* 2019;316:60-72.10.1016/j.toxlet.2019.09.009
80. Khodaei F, Khoshnoud MJ, Heidaryfar S, Heidari R, Baseri MHK, Azarpira N, et al. The effect of ellagic acid on spinal cord and sciatica function in a mice model of multiple sclerosis. *J Biochem Mol Toxicol.* In Press. doi:10.1002/jbt.22564
81. Heidari R, Abdoli N, Ommati MM, Jamshidzadeh A, Niknahad H. Mitochondrial impairment induced by chenodeoxycholic acid: The protective effect of taurine and carnosine supplementation. *Trends in Pharmaceutical Sciences.* 2018;4(2).10.1111/tips.v4i2.182
82. Niknahad H, Jamshidzadeh A, Heidari R, Hosseini Z, Mobini K, Khodaei F, et al. Paradoxical effect of methimazole on liver mitochondria: In vitro and in vivo. *Toxicol Lett*. 2016;259:108-15.10.1016/j.toxlet.2016.08.003
83. Heidari R, Mousavi K, Amin S, Ommati MM, Niknahad H. N-acetylcysteine treatment protects intestinal mitochondria in a surgical stress model. *Trends in Pharmaceutical Sciences.* 2020;6(2):87-96. doi:10.30476/tips.2020.85960.1042
84. Heidari R, Arabnezhad MR, Ommati MM, Azarpira N, Ghodsimanesh E, Niknahad H. Boldine supplementation regulates mitochondrial function and oxidative stress in a rat model of hepatotoxicity. *Pharm Sci.* 2019;25(1):1-10.doi:10.15171/PS.2019.1
85. Ommati MM, Farshad O, Mousavi K, Khalili M, Jamshidzadeh A, Heidari R. Chlorogenic acid supplementation improves skeletal muscle mitochondrial function in a rat model of resistance training. *Biologia*. 2020;75:1221-30. doi: 10.2478/

s11756-020-00429-7
86. Jamshidzadeh A, Niknahad H, Heidari R, Azadbakht M, Khodaei F, Arabnezhad MR, et al. Propylthiouracil-induced mitochondrial dysfunction in liver and its relevance to drug-induced hepatotoxicity. *Pharm Sci.* 2017;23(2):95-102. doi:10.15171/PS.2017.15
87. Heidari R, Jafari F, Khodaei F, Shirazi Yeganeh B, Niknahad H. Mechanism of valproic acid-induced Fanconi syndrome involves mitochondrial dysfunction and oxidative stress in rat kidney. *Nephrology (Carlton).* 2018 Apr;23(4):351-361. doi: 10.1111/nep.13012. PMID: 28141910.
88. Ommati MM, Azarpira N, Khodaei F, Niknahad H, Gozashtegan V, Heidari R. Methylene blue treatment enhances mitochondrial function and locomotor activity in a C57BL/6 mouse model of multiple sclerosis. *Trends in Pharmaceutical Sciences.* 2020;6(1):29-42.10.30476/tips.2020.85962.1044
89. Vazin A, Heidari R, Khodami Z. Curcumin Supplementation Alleviates Polymyxin E-Induced Nephrotoxicity. *J Exp Pharmacol.* 2020;12:129-136. Published 2020 Jun 4. doi:10.2147/JEP.S255861
90. Kaplowitz, N. Hepatotoxicology. Edited by R.G. Meeks, S.D. Harrison and R.J. Bull1991:640 pp. Boca Raton: CRC Press 1991. $95. Hepatology, 16: 1308-1308. doi:10.1002/hep.1840160532
91. Heidari R, Ahmadi A, Mohammadi H, Ommati MM, Azarpira N, Niknahad H. Mitochondrial dysfunction and oxidative stress are involved in the mechanism of methotrexate-induced renal injury and electrolytes imbalance. *Biomed Pharmacother.* 2018 Nov;107:834-840. doi: 10.1016/j.biopha.2018.08.050. Epub 2018 Aug 22. PMID: 30142545.
92. Volonté MG, Yuln G, Quiroga P, Consolini AE. Development of an HPLC method for determination of metabolic compounds in myocardial tissue. *J Pharm Biomed*. 2004;35(3):647-53. doi:10.1016/j.jpba.2004.02.002
93. Ommati MM, Heidari R, Manthari RK, Tikka Chiranjeevi S, Niu R, Sun Z, et al. Paternal exposure to arsenic resulted in oxidative stress, autophagy, and mitochondrial impairments in the HPG axis of pubertal male offspring. *Chemosphere*. 2019 Dec;236:124325. doi: 10.1016/j.chemosphere.2019.07.056. Epub 2019 Jul 15. PMID: 31326754.
94. Seifi K, Rezaei M, Yansari AT, Zamiri MJ, Riazi GH, Heidari R. Short chain fatty acids may improve hepatic mitochondrial energy efficiency in heat stressed-broilers. *J Therm Biol.* 2020;89:102520.10.1016/j.jtherbio.2020.102520
95. Heidari R. Brain mitochondria as potential therapeutic targets for managing hepatic encephalopathy. *Life Sci*. 2019 Feb 1;218:65-80. doi: 10.1016/j.lfs.2018.12.030. Epub 2018 Dec 19. PMID: 30578865.
96. Qiu J, Tsien C, Thapalaya S, Narayanan A, Weihl CC, Ching JK, Eghtesad B, Singh K, Fu X, Dubyak G, McDonald C, Almasan A, Hazen SL, Naga Prasad SV, Dasarathy S. Hyperammonemia-mediated autophagy in skeletal muscle contributes to sarcopenia of cirrhosis. *Am J Physiol Endocrinol Metab*. 2012 Oct 15;303(8):E983-93. doi: 10.1152/ajpendo.00183.2012. Epub 2012 Aug 14. PMID: 22895779; PMCID: PMC3469607.
97. Niknahad H, Jamshidzadeh A, Heidari R, Zarei M, Ommati MM. Ammonia-induced mitochondrial dysfunction and energy metabolism disturbances in isolated brain and liver mitochondria, and the effect of taurine administration: relevance to hepatic encephalopathy treatment. *Clin Exp Hepatol.* 2017;3(3):141-151. doi:10.5114/ceh.2017.68833
98. Heidari R, Jamshidzadeh A, Ommati MM, Rashidi E, Khodaei F, Sadeghi A, et al. Ammonia-induced mitochondrial impairment is intensified by manganese co-exposure: relevance to the management of subclinical hepatic encephalopathy and cirrhosis-associated brain injury. *Clin Exp Hepatol*. 2019 May;5(2):109-117. doi: 10.5114/ceh.2019.85071. Epub 2019 May 13. PMID: 31501786; PMCID: PMC6728860.
99. Ommati MM, Heidari R, Ghanbarinejad V, Aminian A, Abdoli N, Niknahad H. The neuroprotective properties of carnosine in a mouse model of manganism is mediated via mitochondria regulating and antioxidative mechanisms. *Nutritional Neuroscience*. 2020;23(9):731-43. doi:10.1080/1028415X.2018.1552399
100. Jamshidzadeh A, Abdoli N, Niknahad H, Azarpira N, Mardani E, Mousavi S, et al. Taurine alleviates brain tissue markers of oxidative stress in a rat model of hepatic encephalopathy. *Trends in Pharmaceutical Sciences.* 2017;3(3):181-92.
101. Jamshidzadeh A, Niknahad H, Heidari R,

Zarei M, Ommati MM, Khodaei F. Carnosine protects brain mitochondria under hyperammonemic conditions: Relevance to hepatic encephalopathy treatment. *PharmaNutrition*. 2017;5(2):58-63.10.1016/j.phanu.2017.02.004
102. Qiu J, Thapaliya S, Runkana A, Yang Y, Tsien C, Mohan ML, et al. Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-κB-mediated mechanism. *Proc Natl Acad Sci U S A*. 2013 Nov 5;110(45):18162-7. doi: 10.1073/pnas.1317049110.
103. Dasarathy S. Myostatin and beyond in cirrhosis: all roads lead to sarcopenia. *J Cachexia Sarcopenia Muscle.* 2017 Dec;8(6):864-869. doi: 10.1002/jcsm.12262.
104. Holeček M. Branched-chain amino acid supplementation in treatment of liver cirrhosis: Updated views on how to attenuate their harmful effects on cataplerosis and ammonia formation. *Nutrition*. 2017 Sep;41:80-85. doi: 10.1016/j.nut.2017.04.003.
105. Sakuma K, Yamaguchi A. Novel intriguing strategies attenuating to sarcopenia. *J Aging Res.* 2012;2012:251217. doi: 10.1155/2012/251217. Epub 2012 Feb 20. PMID: 22500226; PMCID: PMC3303581.
106. Lum JJ, Bui T, Gruber M, et al. The transcription factor HIF-1alpha plays a critical role in the growth factor-dependent regulation of both aerobic and anaerobic glycolysis. *Genes Dev.* 2007;21(9):1037-1049. doi:10.1101/gad.1529107
107. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol.* 2004 Oct;287(4):C817-33. doi: 10.1152/ajpcell.00139.2004. PMID: 15355853.
108. Heidari R. The footprints of mitochondrial impairment and cellular energy crisis in the pathogenesis of xenobiotics-induced nephrotoxicity, serum electrolytes imbalance, and Fanconi's syndrome: A comprehensive review. *Toxicology*. 2019 Jul 1;423:1-31. doi: 10.1016/j.tox.2019.05.002. Epub 2019 May 13. PMID: 31095988.
109. Naseer M, Turse EP, Syed A, Dailey FE, Zatreh M, Tahan V. Interventions to improve sarcopenia in cirrhosis: A systematic review. *World J Clin Cases.* 2019 Jan 26;7(2):156-170. doi: 10.12998/wjcc.v7.i2.156.
110. Dasarathy S. Cause and management of muscle wasting in chronic liver disease. *Curr Opin Gastroenterol.* 2016;32(3):159-165. doi:10.1097/MOG.0000000000000261
111. Zenith L, Meena N, Ramadi A, Yavari M, Harvey A, Carbonneau M, et al. Eight weeks of exercise training increases aerobic capacity and muscle mass and reduces fatigue in patients with cirrhosis. *Clin Gastroenterol Hepatol.* 2014 Nov;12(11):1920-6.e2. doi: 10.1016/j.cgh.2014.04.016.
112. Jones JC, Coombes JS, Macdonald GA. Exercise capacity and muscle strength in patients with cirrhosis. *Liver Transpl*. 2012 Feb;18(2):146-51. doi: 10.1002/lt.22472.
113. Tandon P, Ismond KP, Riess K, Duarte-Rojo A, Al-Judaibi B, Dunn MA, et al. Exercise in cirrhosis: Translating evidence and experience to practice. *J Hepatol.* 2018 Nov;69(5):1164-1177. doi: 10.1016/j.jhep.2018.06.017. Epub 2018 Jun 30. PMID: 29964066.
114. Ebadi M, Bhanji RA, Mazurak VC, Montano-Loza AJ. Sarcopenia in cirrhosis: from pathogenesis to interventions. *J Gastroenterol.* 2019;54(10):845-859. doi:10.1007/s00535-019-01605-6
115. Heidari R, Ghanbarinejad V, Ommati MM, Jamshidzadeh A, Niknahad H. Mitochondria protecting amino acids: Application against a wide range of mitochondria-linked complications. *PharmaNutrition.* 2018;6(4):180-90.10.1016/j.phanu.2018.09.001
116. Shafiekhani M, Ommati MM, Azarpira N, Heidari R, Salarian AA. Glycine supplementation mitigates lead-induced renal injury in mice. *J Exp Pharmacol.* 2019;11:15-22. Published 2019 Feb 18. doi:10.2147/JEP.S190846
117. Ommati MM, Shi X, Li H, Zamiri MJ, Farshad O, Jamshidzadeh A, et al. The mechanisms of arsenic-induced ovotoxicity, ultrastructural alterations, and autophagic related paths: An enduring developmental study in folliculogenesis of mice. *Ecotoxicol Environ Saf.* 2020;204:110973. doi:10.1016/j.ecoenv.2020.110973
118. Ghanbarinejad V, Ahmadi A, Niknahad H, Ommati MM, Heidari R. Carnosine mitigates manganese mitotoxicity in an in vitro model of isolated brain mitochondria. *Adv Pharm Bull.* 2019;9(2):294-301.10.15171/apb.2019.034