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Effect of exogenous IGF-1 administration on acetaminophen toxicity induced liver injury

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For liver toxicity, there is no clear protective drug till date. Here, we investigated the protective effects of insulin-like growth factor 1 (IGF-1) on acetaminophen (APAP)-induced liver injury and the molecular processes underlying APAP-induced liver damage involving oxidative stress and endoplasmic reticulum (ER) stress. Forty male Wistar rats were randomly divided into four groups. Group I that had only saline served as the control. Group II received APAP (300 mg/kg body wt.) and saline, Group III & IV received APAP as in Gr. II, plus 1 and 2 mg/kg/day of IGF-1, respectively for three days. Liver histopathology, biochemical analysis and ELISA assays were performed to evaluate the protective effect of IGF-1 against APAP-induced liver injury. Significant cellular damage and necrosis were observed in the liver in the APAP and saline groups. Treatment with IGF-1 resulted in a dose-dependent reduction in cellular damage and necrosis. ALT levels, indicative of liver damage, were significantly decreased in the IGF-1-treated groups. MDA levels, a marker of oxidative stress, were reduced with IGF-1 treatment. GSH levels, an antioxidant, increased with IGF-1 treatment. ATF6 levels were reduced with IGF-1 treatment, while TNF-alpha levels were decreased in a dose-dependent manner. IGF-1 treatment protects against APAP-induced liver injury by reducing cellular damage, oxidative stress and ER stress markers. These findings suggest that IGF-1 may have therapeutic potential in mitigating APAP-induced hepatotoxicity.

Keywords: Hepatotoxicity, Inflammation, Oxidative stress, Paracetamol

Acetaminophen (APAP), considered safe at therapeutic levels, is often used as an antipyretic and analgesic in clinical settings. An APAP overdose may cause severe hepatotoxicity and necrosis[1] in humans and animals. APAP's toxic-metabolic damage causes hepatocyte death and hepatotoxicity by triggering necrosis, apoptosis, and inflammatory cytokines[2].

Although cell necrosis' molecular processes are still unknown, non-covalent bonding, lipid peroxidation and oxidative stress are thought to be the causuative factors[3,4]. Hepatotoxicity caused by APAP's xenobiotic metabolism and N-acetyl-p-benzoquinone (NAPQI) oxidative stress is thought to be the leading cause[5]. After synthesis, GSH and NAPQI interact well. High doses of APAP's harmful metabolite, N-acetyl-p-benzoquinone imine, can deplete GSH stores, causing hepatocellular injury, necrosis and liver failure[6].

APAP overdose, chemotherapy, antiretroviral therapy, and drug-induced liver damage lower hepatic GSH levels, causing NAPQI to bind to intracellular target proteins and cause mitochondrial oxidative stress[7]. APAP metabolism causes liver cell necrosis, DNA damage, membrane disintegration, ATP depletion, and membrane degradation[8]. In addition, APAP-induced liver necrosis produces diverse ROS due to oxidative stress. APAP toxicity causes lipid peroxidation-induced oxidative stress, which is indicated by elevated MDA levels. After an APAP overdose, hepatocytes produce reactive oxygen species (ROS), which are removed by enzymes like glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase. Enzymes convert O_2- to H_2O_2. Examining tissue enzyme systems is crucial to reducing ROS-induced hepatic impairment.

All eukaryotic cells have an endoplasmic reticulum (ER) that synthesizes and processes proteins lipids, and stores calcium. An excess of unfolded or misfolded proteins or a calcium deficiency in the ER causes stress. Due to their high protein synthesis and folding needs, hepatocytes are susceptible to ER stress and perturbations due to their high ER concentration. The biological phenomenon is called

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cellular response. ER stress triggers the Unfolded Protein Response (UPR). The UPR regulates ER equilibrium and cellular viability. If restoration fails, ER stress and the UPR can cause cellular apoptosis. Research shows ER stress, and the UPR are linked to several human diseases, including hepatic disorders[10].

Activating transcription factor 6 (ATF6) is a key regulator of the UPR, a cellular stress response pathway activated by ER stress[11]. ATF6 is transmembrane and located in the ER membrane, is usually inactive. It is released from the ER membrane and translocates to the nucleus to regulate transcription during ER stress[12].

İnsulin-like growth factor 1 (IGF-1) is a peptide hormone that promotes cell growth, development, and proliferation. Liver-produced IGF-1 interacts with a carrier protein in circulation, affecting intracellular signaling pathways. IGF-1 boosts cell growth, differentiation, proliferation, and ER stress resistance[13]. In this study, we have made an attempt to demonstrate the protective and healing effects of IGF-1 by evaluating the ER stress in APAP-induced liver injury as an alternative.

Materials and Methods

Animals

The study employed a sample of 40 male Wistar rats of adult age, with an average weight of 200-210 g. According to Galenty *et al.*[14], the male rat livers exhibited a greater susceptibility to APAP toxicity than their female counterparts. Therefore, male animals were chosen as the subjects for this investigation.

The animals were confined in enclosures and subjected to standardized conditions, including a 12 h light/dark cycle, at 22 ± 2°C. The subjects were provided with a consistent pellet-based diet and unrestricted access to tap water for the research. The Institutional Animal Care and Ethical Committee of the University of Demiroğlu Bilim University approved the study protocol, with an assigned Ethical Number of 2823055200. The chemicals utilized in the experiment were procured from Sigma-Aldrich Inc. unless explicitly stated otherwise.

Experimental design

The current study utilized a sample of 40 male Wistar albino rats. The rats were randomly assigned, resulting in four distinct groups forming. A cohort of 10 rats was subjected to a standard control group and received no pharmacological intervention. A group of 30 rats were administered with a solitary dose of 300 mg/kg APAP (PST) (Parol, Atabay, 10 mg/mL) through the intraperitoneal (i.p.) route. These rats were further divided randomly into three distinct groups. Ten rats were assigned to Group II and received 1 mL/kg/day of 0.9% NaCl saline intraperitoneally for three days. A cohort of 10 rats, designated as Group III & IV, were administered 1 and 2 mg/kg/day of IGF-1 (Genotropin Goquick 5,3 mg/mL, Pfizer) via i.p. injection for three days, respectively. Group I which had only saline, served as the control group.

Upon completion of the study, all animals underwent cervical dislocation as a method of sacrifice while under anesthesia with ketamine (100 mg/kg, Ketasol, Richterpharma AG Austria) and xylazine (50 mg/kg, Rompun, Bayer, Germany). In addition, the participants blood specimens were obtained through the cardiac puncture to conduct biochemical evaluations. Subsequently, liver specimens were procured to conduct histopathological and biochemical evaluations.

Histopathological studies of liver

To facilitate histological and immunohistochemical analyses, the test subjects were subjected to i.p. administration of ketamine (80 mg/kg, alfamine®, Alfasan International BV, Holland) and xylazine (6 mg/kg, alfazyne®, Alfasan International BV, Holland) for anesthesia. Subsequently, the specimens underwent perfusion using a solution of 4% formaldehyde in 0.1 M phosphate buffer saline (PBS) with a volume of 200 mL. Four µm thick and fixed with formalin, liver tissue samples underwent staining with hematoxylin and eosin. The specimens were imaged using an Olympus BX51 microscope with an Olympus C-5050 digital camera.

A computerized image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc. USA) was utilized to perform the morphological evaluation of the liver. The observer examined ten microscopic fields per section at a magnification of 40X without prior knowledge of the study group. The quantification of the percentage of hepatocytes that were damaged was performed.

Quantification of plasma alanine aminotransferase (ALT) concentration

The alanine aminotransferase (ALT) plasma levels were assessed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, which USCN Life Science Inc. specifically provided.

Liver biochemical analysis

Following the sacrification procedure, the liver was expeditiously extracted and preserved at a temperature of −20°C until subjected to biochemical examination. The liver tissue was homogenized using a glass homogenizer in a 5-fold volume of phosphate-buffered saline (pH 7.4) and subsequently underwent centrifugation at 5000×g for 15 min. The supernatant was collected, and the protein concentration in the homogenates was determined using Bradford's method, with bovine serum albumin utilized as the reference standard[15].

ATF6 and TNF-alpha concentrations were quantified in the supernatants using commercially accessible ELISA kits for rats. Duplicate measurements were taken for all animal samples according to the manufacturer's instructions. The Absorbances were measured using a microplate reader (Multiscan Go Laboratory Equipment, NH, U.S.).

Determination of liver lipid peroxidation

The quantification of lipid peroxidation in tissue samples was executed by assessing malondialdehyde (MDA) concentrations as thiobarbituric acid reactive substances (TBARS); the tissue samples were subjected to a process wherein trichloroacetic acid and TBARS reagent were added, followed by mixing and incubation at 100°C for 60 min. Following the cooling process on ice, the samples underwent centrifugation at a rate of 3000 rpm for 20 min. The absorbance of the supernatant was subsequently measured at a wavelength of 535 nanometers. MDA concentrations were determined using the standard calibration curve utilizing tetraethoxypropane and denoted in nmol units per milligram of protein[16].

Determination of liver glutathione (GSH) levels

The spectrophotometric measurement method proposed by Ellman was employed to determine the concentration of GSH in liver samples[17]. The present methodology involves the interaction between thiols and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), forming a chromophoric anion exhibiting a maximum absorbance peak at 412 nm. The levels of GSH were determined by utilizing the standard calibration curve and subsequently denoted in units of nmol/mg of protein[16].

Statistical analysis

The data are displayed as mean values accompanied by the standard error of the mean (SEM). The statistical software used for data analysis was SPSS version 15.0 for the Windows operating system. The data underwent analysis using a non-parametric test, specifically the Mann-Whitney U test. Statistical significance was determined for *P* values equal to or less than 0.05.

Results

Histopathological slices results

In the normal group (Gr. I) liver, when examined at 40X magnification, the sinusoids (S) and hepatocytes (H) exhibited a normal and intact morphology, indicating a healthy liver without any evidence of cellular damage or necrosis. Liver sections from the APAP and saline group (Gr. II) showed significant cellular damage indicated by asterisks and necrosis indicated by arrows. These changes were predominantly observed in the centrilobular area. The APAP and 1 mg/kg/day IGF-1 group (Gr. III) showed no evidence of cellular damage or necrosis in the centrilobular area. In the APAP and 2 mg/kg/day IGF-1 group (Gr. IV), no cellular changes or necrosis were observed in the centrilobular area (Fig. 1).

Biochemical results

In the normal group, ALT (alanine amino-transferase) levels, an enzyme indicating liver damage, were 45.7 ± 3.9 U/L. However, ALT levels increased significantly to 248.5 ± 12.2 U/L in the group treated with APAP and saline ($P < 0.01$).

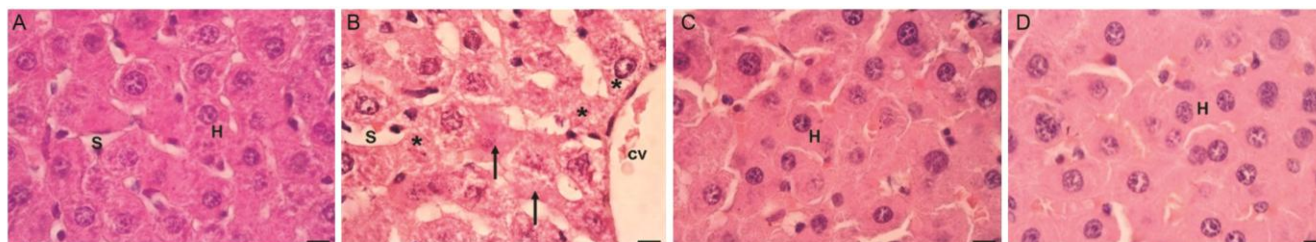

Fig. 1 — The histopathology of sections from rat liver stained with Hematoxylin and Eosin (H&E) at 40X magnification. (A) Normal group liver, S: Sinusoid, H: Hepatocyte; (B) Acetaminophen and saline group liver, have a cellular injury (asterisk) and necrosis (arrow) in centrilobular area of the liver; and (C & D) Acetaminophen and 1 and 2 mg/kg/day IGF-1 group liver, no any cellular injury and necrosis in the centrilobular area of the liver.

Table 1 — Biochemical results of liver tissue and plasma in 1 and 2 mg/kg/day

	Control group (Saline)	APAP (PST) and saline	APAP and IGF-1	
			1 mg/kg/day	2 mg/kg/day
ALT (U/L)	45.7±3.9	248.5±12.2**	165.4±7.3$^{\#\#}$	112.1±6.8$^{\#\#}$
Liver MDA Level (nmol/mg protein)	1.24±0.33	6.5±0.19*	4.1±0.11$^{\#}$	2.2±0.25$^{\#}$
Liver GSH Level (nmol/mg protein)	5.3±0.45	2.4±0.27*	3.9±0.16$^{\#}$	4.1±0.13$^{\#\#}$
Liver ATF6 Level (pg/mg protein)	3.4±0.08	13.1±0.1*	10.9±1.1	6.06±0.2$^{\#}$
Liver TNF-alpha Level (ng/g protein)	96.8±7.05	214.5±6.6**	164.3±3.5$^{\#\#}$	132.3±9.1$^{\#\#}$
Damaged hepatocytes (percent)	4.5±0.2	69.2±3.1**	13.7±2.03$^{\#\#}$	10.8±5.4$^{\#\#}$

[Data are expressed as mean ± SEM. **P* <0.05 (different from control), (*P* <0.05), (*P* <0.01) (different from PST + Saline)]

Treatment with APAP and 1 mg/kg/day IGF-1 led to a significant decrease in ALT levels to 165.4 ± 7.3 U/L (*P* <0.01), while a further decrease was observed in the group treated with APAP and 2 mg/kg/day IGF-1, with ALT levels measuring 112.1 ± 6.8 U/L (*P* <0.01) (Table 1).

The study found a significant increase in liver malondialdehyde (MDA) levels, an established oxidative stress marker, in both the APAP and saline groups compared to the normal group. The mean MDA levels were 6.5 ± 0.19 nmol/mg protein and 1.24 ± 0.33 nmol/mg protein, respectively, with a statistically significant difference (*P* <0.05). The administration of IGF-1 led to a reduction in MDA levels in a dose-dependent manner. The Gr. IV that received a dosage of 2 mg/kg/day of IGF-1 after APAP, demonstrated the lowest levels of MDA at 2.2 ± 0.25 nmol/mg protein, with statistical significance at *P* <0.05 (Table 1).

Similar trends were observed in liver glutathione (GSH) levels, an important antioxidant. The PST and the saline group showed significantly decreased GSH levels compared to the normal group (2.4±0.27 nmol/mg protein versus 5.3 ± 0.45 nmol/mg protein, (*P* <0.05). However, treatment with IGF-1 caused a dose-dependent increase in GSH levels, and the group receiving 2 mg/kg/day IGF-1 showed the highest levels with 4.1 ± 0.13 nmol/mg protein (*P* <0.01) (Table 1).

The present study observed a significant increase in the levels of liver-activating transcription factor 6 (ATF6), a key player in the ER stress response, in both the PST and saline groups as compared to the normal group (13.1 ± 0.1 pg/mg protein *vs*. 3.4 ± 0.08 pg/mg protein, *P* <0.05). Administration of both doses of IGF-1 reduced ATF6 levels, although this reduction did not attain statistical significance (Table 1).

The study found that there was a significant increase in liver tumor necrosis factor-alpha (TNF-alpha) levels, which is a pro-inflammatory cytokine, in both the APAP and saline group (Gr. II) when compared to the control group (214.5 ± 6.6 ng/g protein *vs*. 96.8 ± 7.05 ng/g protein, ***P* <0.01). Treatment with IGF-1 caused a dose-dependent decrease in TNF-alpha levels, with the group receiving 2 mg/kg/day IGF-1 showing the lowest levels at 132.3 ± 9.1 ng/g protein (Table 1).

Histopathological findings in kidney tissue

The percentage of damaged hepatocytes was significantly higher in the PST and saline group compared to the normal group (69.2 ± 3.1% *vs*. 4.5 ± 0.2%, (*P* <0.01). Treatment with both IGF-1 doses significantly reduced the percentage of damaged hepatocytes, with the group receiving 2 mg/kg/day IGF-1 showing the lowest percentage of 10.8 ± 5.4% (*P* <0.01) (Table 1).

Discussion

This study investigated the effects of IGF-1 treatment on APAP-induced liver injury, oxidative stress, inflammatory response, and hepatocellular damage. The findings from the experiment show that IGF-1, especially at a dose of 2 mg/kg/day, effectively attenuates the adverse effects induced by APAP, providing essential insights into the potential therapeutic application of IGF-1 in liver-related disorders.

Tripathi *et al*.[18] revealed that the liver and serum IGF-1 levels were highest at 250 mg/kg and correlated with other doses. In previous studies, doses of 100 and 250 mg/kg of APAP were reported to be moderately toxic.[19]. The present investigation employed a dosage of 300 mg/kg APAP. Our findings indicate that IGF-1 exhibits a dose-dependent impact on mitigating hepatocyte injury and elevating IGF-1 levels.

Previously, IGF-1 was regarded as a growth factor that circulates in the body and is primarily synthesized by the liver. It is responsible for facilitating the impact of growth factors on the growth of the body. Nevertheless, subsequent research has

revealed that IGF-1 is also significantly expressed in various tissues. This event suggests that the autocrine/paracrine effects of locally expressed IGF-1 may play a vital role in regulating tissue growth[20]. The exogenous administration of IGF-1 to individuals with cancer is contraindicated in clinical settings due to its potential to worsen the progression of the disease.

Furthermore, Zhao *et al*.[21] have demonstrated that IGF-1 mitigates the antineoplastic efficacy of cisplatin. Regrettably, the results do not reflect real clinical correlation, and the systemic administration of IGF-1 would probably worsen the condition of cancer patients. The study conducted by Sakai *et al*.[20] demonstrated that exogenous administration of IGF-1 may have the potential as a therapeutic intervention for mitigating or reversing the deleterious effects of cisplatin-induced muscle atrophy, specifically about skeletal muscle mass. This study presents the therapeutic potential of exogenous administration of IGF-1 in mitigating the hepatotoxicity induced by APAP. At this juncture, we can discern the anabolic effects of IGF-1 through its paracrine and autocrine capabilities.

One of the critical findings of this study was the significant decrease in ALT levels, an enzyme that indicates liver damage, following treatment with IGF-1. The group receiving 2 mg/kg/day IGF-1 showed the most significant decrease in ALT levels. This result suggests that IGF-1 treatment protects against APAP-induced liver injury. These findings are consistent with previous studies showing the hepatoprotective effects of IGF-1 in various experimental liver injury models[18].

It is widely recognized that superoxide dismutase (SOD) confers protective properties against Reactive oxygen species (ROS), while malondialdehyde (MDA) serves as a significant indicator of lipid peroxidation[22,23]. The administration of APAP results in hepatotoxicity, which is associated with an elevatedlevels of ROS and the onset of oxidative stress[24]. The pathogenesis of liver diseases is significantly influenced by oxidative stress, marked by elevated MDA levels and reduced GSH levels[25]. The results of our investigation indicate that the administration of IGF-1 resulted in a reduction of MDA levels dependent on the dose administered and an increase in GSH levels that was also dose-dependent. The cohort administered with a dose of 2 mg/kg/day of IGF-1 demonstrated the least amount of MDA levels and the most significant amount of GSH levels, which suggests the robust antioxidant characteristics of IGF-1. The present findings are in line with prior research demonstrating the antioxidative properties of IGF-1 across various experimental paradigms[26]. The ability of IGF-1 to counteract oxidative stress is of significant clinical interest as it suggests the potential to prevent or reduce liver injury associated with oxidative stress-related disorders.

According to Prisco *et al*.[27], ATF6 is among the sensor proteins that respond to ER. The findings of our investigation indicate the activation of ATF6 in our APAP toxicity model, which is in line with previous studies that have reported the activation of the UPR in instances of moderate or severe APAP toxicity. Numerous studies on human liver specimens and animal disease models have established the importance of ER stress and UPR signaling pathways in the pathogenesis of diverse liver ailments, including drug-induced liver injury. Recently, there has been a growing interest in exploring the potential of ER stress, UPR proteins, and genes as viable therapeutic targets for treating liver diseases[28-30]. In a study by Brown & Dauber[31] on acetaminophen overdose, they demonstrated that a sublethal dose of acetaminophen activates ATF6. In our study, the liver ATF6 levels increased in the acetaminophene+ saline group and decreased with IGF-1 treatment.

The production of IGF-1 occurs in numerous tissues, including hepatocytes, which are responsible for approximately 70% of the overall blood IGF-1 secretion into the extracellular fluid, as documented by Sukhanov *et al*.[32].

Inflammation is an important component of liver injury and chronic disease progression. Our study revealed that treatment with IGF-1 led to a dose-dependent decrease in TNF-alpha levels, a pro-inflammatory cytokine. The Gr. III animals (2 mg/kg/day IGF-1) showed the lowest TNF-alpha levels, indicating the anti-inflammatory potential of IGF-1. The results presented in this study align with prior research that has demonstrated the anti-inflammatory properties of IGF-1 in different inflammation and liver injury models[33,34]. The ability IGF-1 to modulate the inflammatory response suggests its potential as a therapeutic agent for inflammatory liver disorders.

Upon entering the nucleus, ATF6 selectively adheres to distinct DNA sequences recognized as ER stress response elements (ERSE), thereby instigating the transcription of diverse genes that play a role in reinstating ER homeostasis. The ER stress response mediated by ATF6 has been implicated in the pathogenesis of liver diseases[34]. Our study demonstrated that APAP administration activated the stress response by elevating ATF6 levels. Treatment with IGF-1 led to a decrease in ATF6 levels. However, the difference was not statistically significant at a dose of 1 mg/kg/day but was significant at 2 mg/kg/day. This result suggests that IGF-1 may have a dose-dependent modulatory effect on the ER stress response. However, further research is needed to fully elucidate its effect in the context of APAP-induced liver injury.

In parenchymal liver disease, IGF-I levels decrease because the liver makes less of it. The level of IGF-I is strongly linked to measures of liver function, such as serum levels of aspartate aminotransferase, bilirubin, alkaline phosphatases, and albumin[35]. It is known that IGF-1 decreases in patients with chronic liver disease in whom IGF-1 is used to monitor liver damage[36].

In experimental studies, the replacement of low IGF-1 was tested in cirrhotic rats, and it was observed that IGF-1 decreased fibrinogenesis (via GH/IGF-1) and slowed down the cirrhotic process of the liver in addition to anti-inflammatory and antioxidant effects[37].

In APAP intoxication, no drug has been included in the treatment protocol in emergency services, except for n-acetylcysteine (NAC)[38]. Despite its ease of use and low cost, the usefulness or use of NAC is controversial, although an alternative form of treatment has yet to be included in the guidelines[39,40]. Studies could not offer an excellent alternative to NAC except for the experimental testing of some antioxidant drugs in APAP intoxication[38]. To the best of our knowledge, this study is the first in the literature on using IGF-1 in APAP intoxication. However, the fact that ATF6 is the protein most affected by the first damage to hepatocytes and that the liver's response to ER stress at the start of the injury causes it to rise and that this rise is quickly and significantly reversed when IGF-1 is given, suggests that IGF-1 may be able to stop the damage, even though it is still an expensive drug in the early stages of toxicity. This study showed that exogen IGF-1 application has a curative effect on APAP intoxication at cellular, histological, and biochemical levels.

Since we evaluated MDA and ATF6 values at the liver level with these results, it may be doubtful whether the values of exogenously administered IGF-1 in the blood are the same with its passage to the tissues. In order to eliminate this doubt, studies have proven that IGF-1 in the liver is not different from IGF-1 in plasma[18,40,41]. This knowledge strengthens our hand that early intervention and follow-up can be made by observing the IGF-1 level, especially in the emergency department where the patient is first checked. More experimental and comparative data on these results are needed.

Conclusion

This study has demonstrated that the insulin-like growth factor 1 (IGF-1) treatment reduces acetaminophen (APAP) induced liver injury by reducing ER stress. We have shown that the decreased levels of inflammatory and oxidative markers in tissue and plasma. This result is promising for initiating life-saving treatment in the emergency department. Future studies should examine the long term benefits, optimal dose, and side effects of IGF-1 therapy for clinical safety and efficacy.

Conflicts of Interest

Authors declare no competing interests.

References

1 Kocabey H, Bekdas M, Cetinkaya A, Duzcu SE, Alisik M, Dincel GK & Turel I, *Abelmoschus esculentus* (L.) Moench seed extract alleviates acute acetaminophen induced liver damage in rats. *Indian J Exp Biol*, 61 (2023) 852. DOI: https:// doi.org/10.56042/ijeb.v61i11.1326.

2 El-Gendy ZA, El-Batran SA, Youssef S, Ramadan A, Hotaby WE, Bakeer RM & Ahmed RF, Hepatoprotective effect of Omega-3 PUFAs against acute paracetamol-induced hepatic injury confirmed by FTIR. *Hum Exp Toxicol*, 40 (2021) 526.

3 Tran M, Wu J, Wang L & Shin DJ, A Potential Role for SerpinA3N in Acetaminophen-Induced Hepatotoxicity. *Mol Pharmacol*, 99 (2021) 277.

4 da Silva SB, Feitosa SGD, de L Alves SM, Santos RCA, Dos Anjos JV & Araújo AV, A Concise and Useful Guide to Understand How Alpha1 Adrenoceptor Antagonists Work. *Mini Rev Med Chem*, 22 (2022) 2383.

5 Esh CJ, Chrismas BCR, Mauger AR & Taylor L, Pharmacological hypotheses: Is acetaminophen selective in its cyclooxygenase inhibition? *Pharmacol Res Perspect*, 9 (2021) e00835.

6 Tujios S, Stravitz RT & Lee WM, Management of Acute Liver Failure: Update 2022. *Semin liver dis*, 42 (2022) 362.

7 Kim HY, Yoon HS, Heo AJ, Jung EJ, Ji CH, Mun SR, Lee MJ, Kwon YT & Park JW, Mitophagy and endoplasmic reticulum-phagy accelerated by a p62 ZZ ligand alleviates paracetamol-induced hepatotoxicity. *Br J Pharmacol*, 180 (2023) 1247.

8 Jaeschke H, Adelusi OB, Akakpo JY, Nguyen NT, Sanchez-Guerrero G, Umbaugh DS, Ding WX & Ramachandran A, Recommendations for the use of the acetaminophen hepatotoxicity model for mechanistic studies and how to avoid common pitfalls. *Acta Pharm Sin B*, 11 (2021) 3740.

9 Dua TK, Palai S, Roy A & Paul P, Protective effect of probiotics against acetaminophen induced nephrotoxicity. *Mol Biol Rep*, 49 (2022) 8139.

10 Ajoolabady A, Kaplowitz N, Lebeaupin C, Kroemer G, Kaufman RJ, Malhi H & Ren J, Endoplasmic reticulum stress in liver diseases. *Hepatology*, 77 (2023) 619.

11 Saaoud F, Lu Y, Xu K, Shao Y, Praticò D, Vazquez-Padron RI, Wang H & Yang X, Protein-rich foods, sea foods, and gut microbiota amplify immune responses in chronic diseases and cancers -Targeting PERK as a novel therapeutic strategy for chronic inflammatory diseases, neurodegenerative disorders, and cancer. *Pharmacol Ther*, 255 (2024) 108604.

12 Chen Q, Fang W, Shen Y, Xu D, Chen Q, Cui K, Mai K & Ai Q, Suppression of cideb under endoplasmic reticulum stress exacerbated hepatic inflammation by inducing hepatic steatosis and oxidative stress. *Free Radic Biol Med*, 185 (2022) 67.

13 Li H, Yu X, Meng F, Zhao Z, Guan S & Wang L, Ferulic Acid Supplementation Increases Lifespan and Stress Resistance via Insulin/IGF-1 Signaling Pathway in *C. elegans*. *Int J Mol Sci*, 22 (2021) 4279.

14 Galanty A, Grudzińska M, Paździora W & Paśko P, Erucic Acid-Both Sides of the Story: A Concise Review on Its Beneficial and Toxic Properties. *Molecules*, 28 (2023) 1924.

15 Kayalı A, Bora ES, Acar H & Erbaş O, Evaluation of the Reparative Effect of Sinomenine in an Acetaminophen-Induced Liver Injury Model. *Curr Issues Mol Biol*, 46 (2024) 923.

16 Obata T, Glutaminergic tonic action potentiate MPP^+-induced hydroxyl radical production in rat striatum. *Neurosci Lett*, 705 (2019) 51.

17 Ellman GL, Reprint of: Tissue Sulfhydryl Groups. *Arch Biochem Biophys*, 726 (2022) 109245.

18 Tripathi SS, Singh S, Garg G, Kumar R, Verma AK, Singh AK, Bissoyi A & Rizvi SI, Metformin ameliorates acetaminophen-induced sub-acute toxicity via antioxidant property. *Drug Chem Toxicol*, 45 (2022) 52.

19 Assis JB, Cogliati B, Esteves E, Capurro ML, Fonseca DM & Sá-Nunes A, Aedes aegypti mosquito saliva ameliorates acetaminophen-induced liver injury in mice. *PLoS One*, 16 (2021) e0245788.

20 Sakai H, Asami M, Naito H, Kitora S, Suzuki Y, Miyauchi Y, Tachinooka R, Yoshida S, Kon R, Ikarashi N, Chiba Y & Kamei J, Exogenous insulin-like growth factor 1 attenuates cisplatin-induced muscle atrophy in mice. *J Cachexia Sarcopenia Muscle*, 12 (2021) 1570.

21 Zhao S, Yu N, Wang H, Wan Z, Diao C, Chen Y, Liu T, Yang Y, Gao F, Bai C, Cao K & Cai J, Long non-coding RNA PANDAR promoted radiation and cisplatin-induced DNA damage repair through ATR/CHK1 in NSCLC. *J Gene Med*, 25 (2023) e3565

22 Haywood NJ, Luk C, Bridge KI, Drozd M, Makava N, Skromna A, Maccannell A, Ozber CH, Warmke N, Wilkinson CG, Watt NT, Koch-Paszkowski J, Teh I, Boyle JH, Smart S, Schneider JE, Yuldasheva NY, Roberts LD, Beech DJ, Sukumar P, Wheatcroft SB & Cubbon RM, Kearney MT, Endothelial IGF-1 receptor mediates crosstalk with the gut wall to regulate microbiota in obesity. *EMBO Rep*, 22 (2021) e50767.

23 Gazia C, Lenci I, Manzia TM, Martina M, Tisone G, Angelico R, Abenavoli L, Grassi G, Signorello A & Baiocchi L, Current Strategies to Minimize Ischemia-Reperfusion Injury in Liver Transplantation: A Systematic Review. *Rev Recent Clin Trials*, 16 (2021) 372.

24 Maity J, Dey T, Banerjee A, Chattopadhyay A, Das AR & Bandyopadhyay D, Melatonin ameliorates myocardial infarction in obese diabetic individuals: The possible involvement of macrophage apoptotic factors. *J Pineal Res*, 74 (2023) e12847.

25 Salmond RJ, Unleashing T cell responses to cancer through removal of intracellular checkpoints. *Immunol Cell Biol*, 100 (2022) 18.

26 Abdellatif M, Trummer-Herbst V, Heberle AM, Humnig A, Pendl T, Durand S, Cerrato G, Hofer SJ, Islam M, Voglhuber J, Ramos Pittol JM, Kepp O, Hoefler G, Schmidt A, Rainer PP, Scherr D, von Lewinski D, Bisping E, McMullen JR, Diwan A, Eisenberg T, Madeo F, Thedieck K, Kroemer G & Sedej S, Fine-Tuning Cardiac Insulin-Like Growth Factor 1 Receptor Signaling to Promote Health and Longevity. *Circulation*, 145 (2022) 1853.

27 Prisco V, Prisco L & Donnarumma B, Aberrant behavior and psychiatric disorders in Möbius sequence: focus on a polymorphic syndrome. *Minerva Pediatr* (Torino), 74 (2022) 609.

28 Perera LA, Ron D. AMPylation and Endoplasmic Reticulum Protein Folding Homeostasis. *Cold Spring Harb Perspect Biol*, 15 (2023) a041265.

29 Sun S, Wang C, Zhao P, Kline GM, Grandjean JMD, Jiang X, Labaudiniere R, Wiseman RL, Kelly JW & Balch WE, Capturing the conversion of the pathogenic alpha-1-antitrypsin fold by ATF6 enhanced proteostasis. *Cell Chem Biol*, 30 (2023) 22.

30 Zhao T, Zheng T, Yu H, Hu BH, Hu B, Ma P, Yang Y, Yang N, Hu J, Cao T, Chen G, Yan B, Peshoff M, Hatzoglou M, Geng R, Li B & Zheng QY, Autophagy impairment as a key feature for acetaminophen-induced ototoxicity. *Cell Death Dis*, 12 (2021) 3.

31 Brown DD & Dauber A, Growth Hormone and Insulin-Like Growth Factor Dysregulation in Pediatric Chronic Kidney Disease. *Horm Res Paediatr*, 94 (2021) 105.

32 Sukhanov S, Higashi Y, Yoshida T, Danchuk S, Alfortish M, Goodchild T, Scarborough A, Sharp T, Jenkins JS, Garcia D, Ivey J, Tharp DL, Schumacher J, Rozenbaum Z, Kolls JK, Bowles D, Lefer D & Delafontaine P, Insulin-like growth factor 1 reduces coronary atherosclerosis in pigs with familial hypercholesterolemia. *JCI Insight*, 8 (2023) e165713.

33 Janssen JAMJL & Smith TJ, Lessons Learned from Targeting IGF-I Receptor in Thyroid-Associated Ophthalmopathy. *Cells*, 10 (2021) 383.

34 Kasprzak A, Autophagy and the Insulin-like Growth Factor (IGF) System in Colonic Cells: Implications for Colorectal Neoplasia. *Int J Mol Sci*, 24 (2023) 3665.

35 Chaves FM, Wasinski F, Tavares MR, Mansano NS, Frazao R, Gusmao DO, Quaresma PGF, Pedroso JAB, Elias CF, List EO, Kopchick JJ, Szawka RE & Donato J, Effects of the Isolated and Combined Ablation of Growth Hormone and IGF-1 Receptors in Somatostatin Neurons. *Endocrinology*, 163 (2022) bqac045.

36 González L, Díaz ME, Miquet JG, Sotelo AI & Dominici FP, Growth Hormone Modulation of Hepatic Epidermal Growth Factor Receptor Signaling. *Trends Endocrinol Metab*, 32 (2021) 403.

37 Kim JD, [Acute Liver Failure: Current Updates and Management]. Korean J *Gastroenterol*, 81 (2023) 17.

38 Chomchai S, Mekavuthikul P, Phuditshinnapatra J & Chomchai C, Sensitivity of dose-estimations for acute acetaminophen overdose in predicting hepatotoxicity risk using the Rumack-Matthew Nomogram. *Pharmacol Res Perspect*, 10 (2022) e00920.

39 BinMowyna MN & AlFaris NA, Kaempferol suppresses acetaminophen-induced liver damage by upregulation/activation of SIRT1. *Pharm Biol*, 59 (2021) 146.

40 Takahashi Y, Nonalcoholic fatty liver disease and adult growth hormone deficiency: An under-recognized association? *Best Pract Res Clin Endocrinol Metab*, 37 (2023) 101816.

41 Cristin L, Montini A, Martinino A, Scarano Pereira JP, Giovinazzo F & Agnes S, The Role of Growth Hormone and Insulin Growth Factor 1 in the Development of Non-Alcoholic Steato-Hepatitis: A Systematic Review. *Cells*, 12 (2023) 517.