https://doi.org/10.35845/kmuj.2024.23629

ORIGINAL ARTICLE

Hepatoprotective role of unacylated ghrelin in different doses: an experimental study

Kumayl Abbas Meghji [1], Tariq Feroz Memon [2], Muhammad Shahab Hanif [3], Muhammad Saqib Baloch [3], Ali Abbas Thalho [4], Naila Noor [1]

1: Department of Physiology, Isra University, Hyderabad, Pakistan
2: Department of Community Medicine and Public Health Sciences, Liaqat University of Medical and Health Sciences, Jamshoro, Pakistan
3: Department of Anatomy, Muhammad Medical College, Mirphurkhas, Pakistan
4: Department of Pharmacology, Isra University, Hyderabad, Pakistan
5: Department of Physiology, Muhammad Medical College, Mirphurkhas, Pakistan

Email✉ : drtariqferoz@gmail.com
Contact #: +92-322-3490040

Date Submitted: March 03, 2024
Date Revised: August 26, 2024
Date Accepted: September 21, 2024

ABSTRACT

OBJECTIVES: To investigate the hepatoprotective effects of Unacylated Ghrelin (UAG) at varying doses in the management of acute liver injury in Wistar albino rats.

METHODS: This quasi-experimental study was conducted at Department of Physiology, Isra University, Hyderabad, Pakistan from March to August 2023. Thirty Wistar albino rats (200-250 grams) were randomly divided into five groups (n=6). Group A served as the control, while liver injury was induced in Groups B, C, D, and E via intraperitoneal injection of 0.1% CCl_4. Groups C, D, and E were subsequently treated with low, medium, and high doses of UAG, respectively. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and superoxide dismutase (SOD) levels were assessed, along with liver histopathology.

RESULTS: Pre-experimental body weights (Mean±SD) for Groups A, B, C, D, and E were 227.33±7.75 g, 229.80±2.08 g, 228.70±5.34 g, 231.33±8.69 g, and 236.38±10.63 g, respectively. The liver index was 4.36±0.28, 6.65±0.37, 5.80±0.17, 5.70±0.08, and 5.06±0.23, respectively, across the Groups. A statistically significant ($p<0.05$) decline was observed in group B compared to Group C, D and E. Moreover, statistically significant ($p<0.05$) rise in ALT, AST, serum IL-6, TNFα, SOD, and MDA levels in Group B compared with the remaining groups.

CONCLUSION: UAG effectively protects the liver from CCl_4-induced injury in rats. Higher doses of UAG reduced liver enzyme levels and improved oxidative stress and inflammation markers, indicating its potential as a therapeutic agent for liver damage. Further research is warranted to explore UAG's therapeutic use for liver disorders.

KEYWORDS: Ghrelin (MeSH); Oxidative Stress (MeSH); Liver Diseases (MeSH); Carbon Tetrachloride (MeSH) Alanine Transaminase (MeSH); Aspartate Aminotransferases (MeSH); Malondialdehyde (MeSH); Interleukin-6 (MeSH); Tumor Necrosis Factor-alpha (MeSH); Superoxide Dismutase (MeSH); Histology (MeSH).

THIS ARTICLE MAY BE CITED AS: Meghji KA, Memon TF, Hanif MS, Baloch MS, Thalho AB, Noor N. Hepatoprotective role of unacylated ghrelin in different doses: an experimental study. Khyber Med Univ J 2024;16(3):249-54. https://doi.org/10.35845/kmuj.2024.23629

INTRODUCTION

The liver plays a pivotal role in maintaining homeostasis and detoxifying harmful substances, making it essential for survival. However, it is highly susceptible to damage from various chemicals and toxins.[1] Hepatic tissues can be harmed by exogenous compounds like carbon tetrachloride (CCl_4), foreign chemicals, and elevated cholesterol, leading to varying degrees of liver injury.[2] Ghrelin, the only known natural ligand for the growth hormone secretagogue receptor (GHSR), exists in both acylated and unacylated forms and is involved in numerous biological processes.[3] The receptor for ghrelin, GHSR1a, is expressed in various organs, including the gastrointestinal tract (liver and pancreas), cardiovascular system (heart), nervous system (hypothalamus, pituitary, cerebral cortex), reproductive system (breast, testes, ovaries), thyroid, and adrenal glands.[4] Unacylated Ghrelin (UAG) is an incarnation of the stomach ghrelin that accounts for 80-90% of the circulating Ghrelin.[5] UAG has demonstrated hepatoprotective properties by preventing apoptosis and enhancing hepatocyte regeneration. With its anti-oxidative properties, it minimizes the impact of oxidative stress that results from the production of free radicals (reactive oxygen species) after acute liver injuries by suppressing the silent information regulator 2 related enzyme 1 (sirtuin1, SIRT1) signaling process. Moreover, the anti-inflammatory properties of UAG reduces the inflammatory response linked to liver damage by lowering the production of cytokines including TNF-α and IL-6.[7] Several studies have also alluded that exogenous administration of UAG lowers the acylated Ghrelin/ Unacylated Ghrelin circulatory ratio.[7-9]

While UAG's anti-inflammatory, antioxidant, and immune-modulatory effects have been documented, and its therapeutic potential for treating acute liver injury is recognized, there remains a lack of comprehensive data regarding its dose-dependent hepatoprotective effects. This study was designed to explore the hepatoprotective properties of exogenous UAG in different doses in in the management of acute live injury in animal models.

METHODS

The quasi-experimental study was conducted by the Department of

Physiology, Isra University, Hyderabad, Pakistan from March to August 2023. Thirty male Wistar albino rats between 200-250 g, were purchased from the Animal Husbandry of Sindh Agricultural University, TandoJam, Sindh, Pakistan.

The rats were housed under controlled environmental conditions, maintaining an optimal temperature of 22±2°C and humidity at 55±10%, with a regulated 12:12-hour light-dark cycle. After a one-week acclimatization period, the experimental procedures commenced.

The study was approved by the Ethical Review Committee of Isra University (ERB letter # IU/RR-10-IRC-23/N/2023/287) and adhered to the international guidelines for the Care and Use of Laboratory Animals.[10]

The thirty rats were randomly divided into five groups (n=6). Group A served as the control, Group B was subjected to liver injury induction, and Groups C, D, and E received varying doses of UAG. Specifically, Group C was administered 50 µg/kg of UAG, Group D received 100 µg/kg, and Group E was given 200 µg/kg, all through intraperitoneal injections (NJPetide, Nanjing, China) for three consecutive days. Three hours after the final UAG injection, all rats, except those in the control group, were injected intraperitoneally with 0.1% CCl_4 dissolved in corn oil to induce liver injury. The control group received only corn oil at a volume of 0.1 mL per 10 g of body weight.

The rats were weighed, and samples were collected 24 hours after liver injury induction. All animals were sacrificed by cervical dislocation, and blood samples were obtained via cardiac puncture. The collected serum was stored at -20°C in sealed containers. Following the manufacturer's instructions, hepatic markers, including serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as oxidative stress markers like malondialdehyde (MDA) and superoxide dismutase (SOD), were analyzed using commercial colorimetric kits. Moreover, the inflammatory markers such as IL-6 and TNF-α levels were measured using Solarbio ELISA kits (SEKR-0005-48T and SEKR-0009-48T, Beijing, China). After blood sample collection, the liver was excised from each rat by dissecting the abdominal cavity, weighed, and the liver index was calculated using the following standard

$$Liver\ index\ \frac{Liver\ weight\ of\ rat}{Body\ weight\ of\ rat} X\ 100$$

A small section of the liver was excised and prepared in a 10% homogenate ice saline solution, then submerged in 10% paraformaldehyde to create paraffin sections. These sections were stored at -80°C. The paraffin-embedded sections were sliced and stained with Hematoxylin and Eosin (H&E) for histopathological examination. The analysis was performed using a light microscope (Olympus CX31) at 100x magnification. Data were analyzed using SPSS version 24. All quantitative variables were expressed as mean ± standard deviation. One-way ANOVA followed by Post-hoc Tukey's test was used to assess significant differences between and within groups. A p-value of <0.05 was considered statistically significant.

RESULTS

The pre-experimental body weight (Mean±SD) for Groups A, B, C, D, and E was 227.33±7.75 gm, 229.80±2.08 gm, 228.70±5.34 gm, 231.33±8.69 gm, and 236.38±10.63 gm, respectively. A significant difference in post-experimental body weight was observed across all groups. Group A showed an increase in weight (249.0±23.78 gm), while Groups B (192.0±9.21 gm), C (219.10±2.14 gm), D (226.63±7.56 gm), and E (233.66±11.57 gm) experienced weight reductions, as illustrated in Figure 1. The difference between the groups was statistically significant, with a p-value of <0.05.

The distribution and post-hoc analysis of hepatic and inflammatory markers are summarized in Table I. A statistically significant increase (p<0.05) in ALT, AST, serum IL-6, and TNF-α levels was observed in group B. Although Groups C, D, and E also showed an increase in these markers, the elevation was less pronounced compared to Group B, with Group E demonstrating the most favorable outcomes (p<0.05) (Table I).

Regarding the liver index of all study animals, significant differences (p<0.05) were observed among the groups. Rats in Group B exhibited a markedly increased liver index compared to all other groups. Although a rise in liver index was noted in the experimental groups, it was less pronounced than in Group B. Among the experimental groups, Group E demonstrated the most favorable results (Table II).

Table III presents the oxidative stress markers distribution in all study groups. An increase in MDA level and a decrease in levels of SOD was observed in Group B compared with other groups. Whereas, post-induction treatment with UAG in the higher dose group (E)

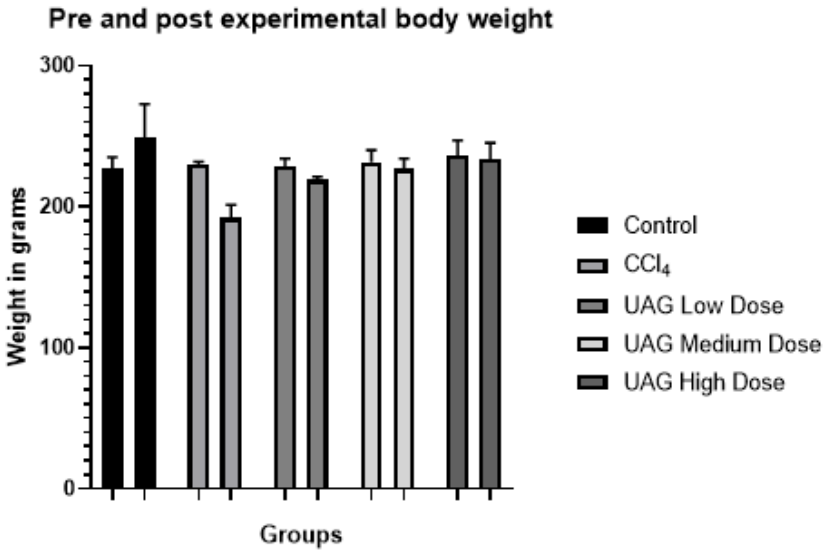

Figure 1: Distribution of pre and post-experimental body weight among groups

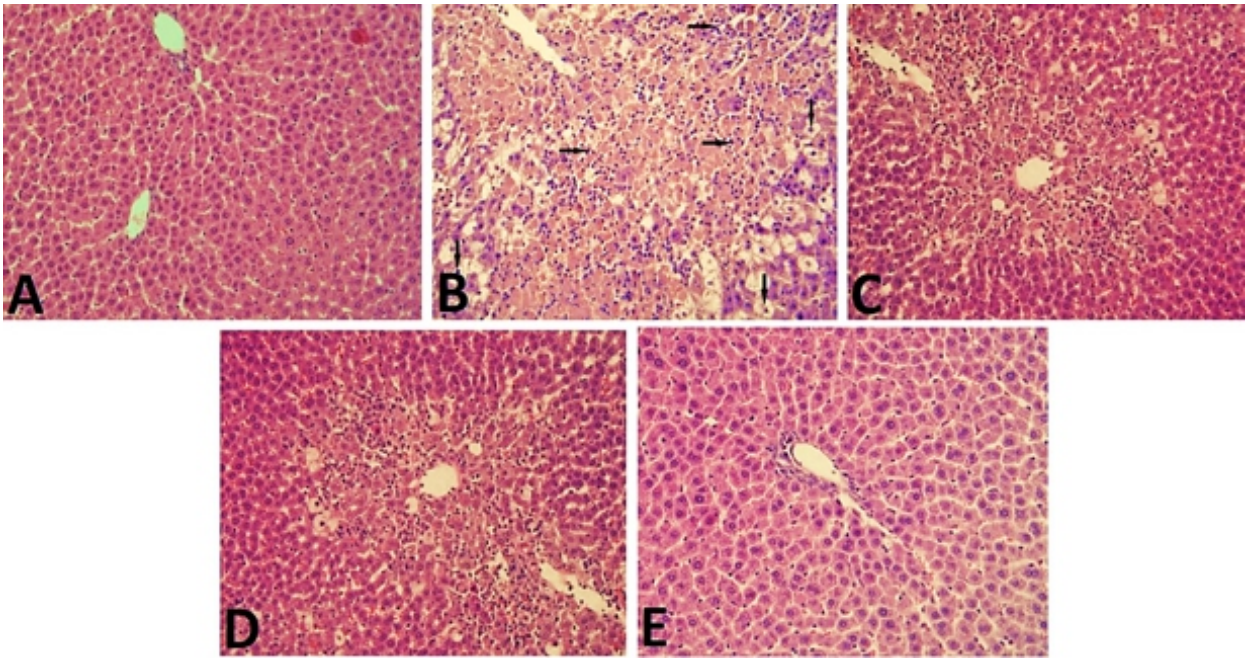

Figure 2: Evaluation of hepatic architecture in experimental animals (100x magnification)

showed a significant improvement in MDA and SOD levels in comparison with both treatment groups (C and D).

Figure 2 illustrates the histopathological changes across the study groups. Group A (control) displayed normal hepatic architecture. In contrast, Group B (CCl_4-induced liver injury) showed significant pathological alterations, including fatty degeneration, lymphocyte infiltration, and extensive necrosis of the liver parenchyma. Groups C (UAG low dose) and D (UAG medium dose) also displayed similar histopathological changes, though lymphocytic infiltration and necrosis were less pronounced compared to Group B. Group E (UAG high dose) demonstrated near-normal hepatic architecture with preserved liver parenchyma and minimal lymphocytic infiltration.

DISCUSSION

The physiological effects of ghrelin's acylated form have been extensively studied since its identification as a gut hormone. In contrast, its unacylated form, previously considered inactive, has not received the same level of attention.[11,12] However, recent research suggests that UAG has significant physiological and pathological roles that may complement or counteract the effects of acylated Ghrelin.[13,15] This study aimed to evaluate the hepatoprotective properties of UAG in an acute liver injury model using Wistar albino rats.

Carbon tetrachloride (CCl_4), a potent liver toxin, is commonly used in animal models to induce liver damage. It is metabolized by the cytochrome P450 enzyme system, producing reactive free radicals that cause oxidative stress and hepatocyte injury.[11,16] In this study, Groups B, C, D, and E were subjected to liver injury through intra-peritoneal injection of 0.1% CCl_4, while Groups C, D, and E received UAG in varying doses to assess its protective effects.

Significant changes in body weights were observed pre- and post-experiment in all groups, with statistically significant differences between Groups B, C, D, and E compared to the control Group A. These findings align with previous studies by Gong Y, et al.,[11] and Rossetti A, et al.,[17] which also reported significant body weight variations in response to similar experimental conditions.

In the present study, a statistically significant increase in ALT and AST levels was observed in Group B following intra-peritoneal CCl_4 induction, compared to the other study groups. Conversely, the administration of UAG in different doses resulted in a substantial and statistically significant reduction in ALT and AST levels, with the most pronounced effects seen in Group E (high-dose UAG). These results are consistent with findings from Gong Y, et al. [11] and Tuero C, et al.,[18] who also reported the beneficial effects of UAG on elevated liver enzyme levels.

After the induction of CCl_4, a statistically significant increase ($p<0.05$) in serum IL-6, TNF-α, and MDA levels, along with a decrease in SOD, was observed in Group B (induction group) compared to the other groups. Conversely, the administration of UAG demonstrated notable hepatoprotective effects, attributed to its ability to mitigate oxidative stress and inflammation induced by CCl_4. This was particularly evident in the high-dose UAG Group (Group E), where a statistically significant ($p<0.05$) improvement was observed. CCl_4 induces acute liver damage through oxidative stress, which creates an imbalance between pro-oxidants and antioxidants. Superoxide dismutase (SOD), an enzyme that neutralizes free radicals, serves as a measure of hepatic antioxidant capacity. Malondialdehyde (MDA), a byproduct of lipid peroxidation, indirectly indicates the extent of liver damage caused by oxidative stress. Elevated MDA levels correlate with increased liver cell damage and subsequent necrosis. Additionally, inflammatory markers

Table I: Post-hoc analysis of hepatic and inflammatory markers in all groups

Group	Group A	Group B	Group C	Group D	Group E	p-value
ALT (U/L)	26.12±1.2^{bcd}	168.3±9.3^{acde}	147.0±8.8^{abde}	76.16±3.5^{abce}	34.5±3.0^{bcd}	0.000*
AST (U/L)	27.66±1.8^{bcd}	90.33±3.7 ^{acde}	77.83±2.1^{abde}	48.33±1.6 ^{abce}	28.33±1.9 ^{bcd}	0.000*
Serum IL-6 (pg/ml)	108.33±8.1^{bcde}	189.83±9.1^{acde}	172.16±7.0^{abde}	157.83±1.9^{abce}	142.33±5.0^{abcd}	0.000*
TNFα (pg/ml)	104.5±4.5 ^{bcde}	289.66±9.8^{acde}	205±7.7^{abe}	204.33±7.9^{abe}	128.66±6.8^{abcd}	0.000*

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; IL-6: Serum Interleukin-6; TNFα: Tumor Necrosis Factor Alpha*ANOVA (statistically significant); data presented as mean± SD

Table II: Post-hoc analysis of liver index of variations between all groups

Group	Group A	Group B	Group C	Group D	Group E	p-value
Liver Index	4.36±0.28^{bcded}	6.65±0.37^{acde}	5.80±0.17^{abde}	5.70±0.08^{abce}	5.06±0.23^{abcd}	0.000*

*ANOVA (statistically significant); data presented as mean± SD

Table III: Post-hoc analysis of oxidative stress markers distribution in all study groups

Group	Group A	Group B	Group C	Group D	Group E	p-value
MDA (Nmol/mL)	1.38±0.1^{bcde}	3.21±0.3^{acde}	2.61±0.1^{abde}	1.90±0.1^{abc}	1.8±0.06^{ab}	0.000*
SOD (U/mL)	113.8±9.9^{bcd}	45.6±3.7^{ade}	55.5±3.4^{ade}	80.0±4.7^{abce}	110.5±8.8^{bcd}	0.000*

MDA=Malondialdehyde; SOD=superoxide dismutase; *ANOVA (statistically significant); data presented as mean± SD

such as IL-6 and TNF-α are elevated in response to the liver injury.[11]

Numerous liver disorders are caused by the strong inflammatory response and hepatocyte death that results from the effects of TNF-α.[19] Moreover, serum IL-6 contributes to the body's immunological response by encouraging inflammation and exacerbating the oxidative stress response.[20] Gong Y, et al.,[11] Raghay K, et al.,[21] and Bianchi E, et al.[22] demonstrated the similar effects of serum IL-6, TNF-α, SOD, and MDA levels and their effects on the liver and other body cells. They further reported the protective effects of UAG against these altered levels resulting from the CCl_4 and other inducers.

Histological findings in this study revealed that CCl_4 induction led to significant damage to the cell membrane, increasing permeability and causing hepatocyte injury in Group B (induction group). The liver's architecture became disorganized, showing necrosis, cellular breakdown, and inflammatory infiltration. In contrast, treatment with UAG resulted in notable repair of liver damage, indicating a potential dose-dependent hepatoprotective effect.

To the best of our knowledge, this study is the first to explore the intervention effect of UAG on acute liver injury in this context. Exogenous UAG appears to exert hepatoprotective effects by reducing liver oxidative stress and modulating the inflammatory response. The findings expand the understanding of UAG's pharmacological role and may serve as a foundation for future research on UAG's potential in managing liver disorders. However, this study represents only an initial exploration of UAG's pharmacological activities. Further research is needed to fully elucidate its therapeutic potential and underlying mechanisms.

CONCLUSION

This study demonstrated that UAG exhibits significant hepatoprotective effects in acute liver injury induced by CCl_4 in Wistar albino rats. UAG treatment, particularly at higher doses, effectively mitigated liver damage as evidenced by the significant reduction in liver enzyme levels (ALT and AST) and improvement in oxidative and inflammatory markers. The study highlights the potential of UAG as a therapeutic agent in managing acute liver injury, suggesting its beneficial impact in reducing oxidative stress and inflammation associated with liver damage. These findings support further investigation into UAG's pharmacological properties and its potential applications in liver-related disorders.

REFERENCES

1. Juanola A, Tiwari N, Solé C, Adebayo D, Wong F, Ginès P. Organ dysfunction and failure in liver disease. Liver Int 2023 May 24. https://doi.org/10.1111/liv.15622
2. Munir F, Khan MKA. Hepatotoxicity induced by carbon tetrachloride in experimental model: hepatotoxicity induced by carbon tetrachloride. Pak BioMed J 2023:10-5.
3. Müller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, et al. Ghrelin. Mol Metab 2015;4(6):437-60.https://doi.org/10.1016/j.molmet.2015.03.005
4. Ringuet MT, Furness JB, Furness SGB. G protein-coupled receptor interactions and modification of signalling involving the ghrelin receptor, GHSR1a. J Neuroendocrinol 2022;34(9):e13077. https://doi.org/10.1111/jne.13077
5. Ezquerro S, Mocha F, Frühbeck G, Guzmán-Ruiz R, Valentí V, Mugueta C, et al. Ghrelin reduces TNF-α–induced human hepatocyte apoptosis, autophagy, and pyroptosis: role in obesity-associated NAFLD. J Clin Endocrinol Metab 2019;104(1):21-37.https://doi.org/10.1210/jc.2018-01171
6. Quiñones M, Fernø J, Al-Massadi O. Ghrelin and liver disease. Rev Endocr Metab Disord 2020;21:45-56. https://doi.org/10.1007/s11154-019-09528-6

7. Lewiński A, Karbownik-Lewińska M, Wieczorek-Szukała K, Stasiak M, Stawerska R. Contribution of ghrelin to the pathogenesis of growth hormone deficiency. Int J Mol Sci 2021;22(16):9066. https://doi.org/10.3390/ijms22169066

8. Hornsby AK, Buntwal L, Carisi MC, Santos VV, Johnston F, Roberts LD, et al. Unacylated-ghrelin impairs hippocampal neurogenesis and memory in mice and is altered in parkinson's dementia in humans. Cell Rep Med 2020;1(7). https://doi.org/10.1016/j.xcrm.2020.100120

9. Jabeen A, Ahsin S, Nasar Abbas ZS, Kiyani H. Ghrelin: A potent nephro-protective agent against nicotine induced kidney damage in mice. Pak J Med Health Sci 2023;17(01):171. https://doi.org/10.53350/pjmhs2023171171

10. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory animals. guide for the care and use of laboratory animals. 8th edition. Washington (DC): National Academies Press (US); 2011. [Accessed on: January 10, 2023]. Available from URL: https://www.ncbi.nlm.nih.gov/books/NBK54050.https://doi.org/10.17226/12910

11. Gong Y, Qiu B, Zheng H, Li X, Wang Y, Wu M, et al. Unacylated ghrelin attenuates acute liver injury and hyperlipidemia via its anti-inflammatory and anti-oxidative activities. Iran J Basic Med Sci 2024;27(1):49. https://doi.org/10.22038/IJBMS.2023.70831.15388

12. Kiyani HP, Ahsin S, Imran M, Ashraf H. Effect of ghrelin in alleviating nicotine induced oxidative stress in balb/C mice. Pak J Physiol 2022;18(3):3-6. https://doi.org/10.69656/pjp.v18i3.1451

13. Alharbi S. Exogenous administration of unacylated ghrelin attenuates hepatic steatosis in high-fat diet-fed rats by modulating glucose homeostasis, lipogenesis, oxidative stress, and endoplasmic reticulum stress. Biomed Pharmacother 2022;151:113095. https://doi.org/10.1016/j.biopha.2022.113095

14. Au CC, Docanto MM, Zahid H, Raffaelli F-M, Ferrero RL, Furness JB, et al. Des-acyl ghrelin inhibits the capacity of macrophages to stimulate the expression of aromatase in breast adipose stromal cells. The J Steroid Biochem Mol Biol 2017;170:49-53. https://doi.org/10.1016/j.jsbmb.2016.07.005

15. Ugwu FN, Yu AP, Sin TK, Tam BT, Lai CW, Wong S, et al. Protective effect of unacylated ghrelin on compression-induced skeletal muscle injury mediated by SIRT1-signaling. Front Physiol 2017;8:962. https://doi.org/10.3389/fphys.2017.00962

16. Ramos-Tovar E, Muriel P. Molecular mechanisms that link oxidative stress, inflammation, and fibrosis in the liver. Antioxidants 2020;9(12):1279. https://doi.org/10.3390/antiox9121279

17. Rossetti A, Togliatto G, Rolo AP, Teodoro JS, Granata R, Ghigo E, et al. Unacylated ghrelin prevents mitochondrial dysfunction in a model of ischemia/reperfusion liver injury. Cell Death Discov 2017;3(1):1-11. https://doi.org/10.1038/cddiscovery.2017.77

18. Tuero C, Becerril S, Ezquerro S, Neira G, Frühbeck G, Rodríguez A. Molecular and cellular mechanisms underlying the hepatoprotective role of ghrelin against NAFLD progression. J Physiol Biochem 2023;79(4):833-49. https://doi.org/10.1007/s13105-022-00933-1

19. Jing Z-T, Liu W, Xue C-R, Wu S-X, Chen W-N, Lin X-J, et al. AKT activator SC79 protects hepatocytes from TNF-α-mediated apoptosis and alleviates d-Gal/LPS-induced liver injury. Am J Physiol-Gastrointest Liver Physiol 2019;316(3):G387-G96. https://doi.org/10.1152/ajpgi.00350.2018

20. Guo Y, Gao S, Jiang Z, Huang J, He X, Jin R, et al. Calcium-sensing receptor (CaSR) agonist R568 inhibits small intestinal motility of mice through neural and non-neural mechanisms. Food Function 2021;12(23):11926-37. https://doi.org/10.1039/d1fo01988k

21. Raghay K, Akki R, Bensaid D, Errami M. Ghrelin as an anti-inflammatory and protective agent in ischemia/reperfusion injury. Peptides 2020;124:170226. https://doi.org/10.1016/j.peptides.2019.170226

22. Bianchi E, Boekelheide K, Sigman M, Hall SJ, Hwang K. Ghrelin modulates testicular damage in a cryptorchid mouse model. PLoS One 2017;12(5):e0177995. https://doi.org/10.1371/journal.pone.0177995

AUTHORS' CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

KAM: Conception and study design, acquisition of data, drafting the manuscript, approval of the final version to be published

TFM: Acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

MSH & MSB: Acquisition of data, drafting the manuscript, approval of the final version to be published

AAT & NN: Analysis and interpretation of data, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors declared no specific grant for this research from any funding agency in the public, commercial or non-profit sectors

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

KMUJ web address: www.kmuj.kmu.edu.pk
Email address: kmuj@kmu.edu.pk