International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.439.446

Research Article
Metformin and Resveratrol Suppress Oxidative Stress/Hypoxia Axis Associated with the Amelioration of Thioacetamide-Induced Liver Injury

[1]Mohammed Attieh Alzahrani, [2]Hasnaa Ali Ebrahim, [3]Aida Sanad Alqarni and [4]Fahaid Hassan Al-Hashem

[1]Department of Internal Medicine, College of Medicine, King Khalid University, Abha 62529, Saudi Arabia
[2]Department of Basic Medical Sciences, College of Medicine, Princess Nourah bint Abdulrahman University, P.O. Box. 84428, Riyadh 11671, Saudi Arabia
[3]Department of Medical Surgical Nursing, College of Nursing, King Khalid University, Abha 61421, Saudi Arabia
[4]Department of Physiology, College of Medicine, King Khalid University, Abha 62529, Saudi Arabia

Abstract

Background and Objective: Chronic liver disease is a serious and often life-threatening condition. The hypothesis is that a combination of metformin and resveratrol could effectively mitigate hepatic injury induced by the hepatotoxic chemical, thioacetamide (TAA), by inhibiting liver tissue levels of the oxidative stress (ROS)/Hypoxia-Inducible Factor 1-alpha (HIF-1α) axis were tested. **Materials and Methods:** To this end, chronic liver injury was induced in rats by TAA (200 mg kg^{-1}) injections for 8 weeks starting at week 2 (model group). Another group was treated with metformin (200 mg kg^{-1}) plus resveratrol (20 mg kg^{-1}) for 2 weeks before TAA injections and continued receiving these agents and TAA until being sacrificed at the end of the experiment, at week 10 (protective group). **Results:** The model group (TAA) exhibited extensive liver tissue damage associated with a significant ($p<0.0001$) increase in hepatic tissue levels of ROS/HIF-1α, as well as blood and tissue levels of chronic liver injury biomarkers. However, treatment with Met+Res significantly ($p<0.0001$) effectively inhibited all these parameters. Additionally, a significant ($p<0.0001$) correlation between hepatocyte damage and tissue and blood levels of ROS, HIF-1α, ALT, AST and CRP was observed. **Conclusion:** The TAA-induced chronic liver injury is associated with an increase in the ROS/HIF-1α axis and biomarkers of hepatic damage, which can be protected for a period of ten weeks by treatment with metformin plus resveratrol. These findings suggest that this combination may have therapeutic potential in humans.

Key words: Oxidative stress, Hypoxia-Inducible Factor 1-alpha (HIF-1α), chronic liver injury, alpha-Smooth Muscle Actin (α-SMA), thioacetamide, rat model

Citation: Alzahrani, M.A., H.A. Ebrahim, A.S. Alqarni and F.H. Al-Hashem, 2023. Metformin and resveratrol suppress oxidative stress/hypoxia axis associated with the amelioration of thioacetamide-induced liver injury. Int. J. Pharmacol., 19: 439-446.

Corresponding Author: Fahaid Hassan Al-Hashem, Department of Physiology, College of Medicine, King Khalid University, Abha 62529, Saudi Arabia
Tel: 966-500142929

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

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

INTRODUCTION

Hepatic fibrosis is the initial stage of liver scarring and is considered a complication of chronic liver injury that can progress to further tissue scarring (cirrhosis) and ultimately liver failure[1]. Industrial toxins such as Carbon Tetrachloride (CCl_4) and TAA are well-known chemicals that can harm both humans and animals. For example, acute liver injury has been reported in patients exposed to CCl_4[2] and a single oral dose of CCl_4 (2 g kg^{-1}) caused liver damage in pregnant and lactating rats[3]. Additionally, a single injection of TAA (300 mg kg^{-1}) caused acute liver injury in rats after one week, associated with oxidative stress and inflammation[4]. The TAA injections for six weeks induced hepatotoxicity and liver fibrosis[5]. Furthermore, both CCl_4 and TAA induced liver cirrhosis and hepatocellular carcinoma in mice and rats[6].

Oxidative stress, as demonstrated by increased tissue levels of reactive oxygen species (ROS), has been linked to acute and chronic liver injury caused by Nonalcoholic Fatty Liver Disease (NAFLD)[7], alcoholic liver disease[8], hepatitis viruses[9] and cholestasis[10]. The transcription factor HIF-1α is upregulated in response to ROS during acute and chronic liver injuries, such as ischemia-reperfusion animal models that induce acute liver injury, metabolic disorders, viral infection and alcoholic liver disease[11]. Additionally, inhibition of HIF-1α by the herbal medicine compound paeoniflorin was associated with protection against CCl_4-induced hepatic stellate cells (HSC), α-SMA and liver fibrosis[12].

A combination of the antidiabetic drug metformin with atorvastatin can protect the liver against injuries induced by NAFLD and diabetes[13] and metformin inhibits ROS-induced apoptosis of hepatocytes[14]. Furthermore, metformin inhibited HIF-1α expression induced by cobalt chloride in HSC (hepatic stellate cells)[15]. The polyphenolic antioxidant compound resveratrol protected against liver injuries induced by hepatic steatosis, hepatic toxicity induced by cyclophosphamide and liver fibrosis[16-18]. Therefore, these reports suggested that the ROS/HIF-1α axis may be involved in TAA-induced liver injury and could be more effective in being protected by a combination of metformin and resveratrol than a single agent.

MATERIALS AND METHODS

Study area: This work was performed at the Research Centre, College of Medicine, King Khalid University, Abha, Saudi Arabia from February to June, 2021.

Experimental design: Albino male rats weighing 170-200 g were taken from the animal house (King Saud University, Riyadh, Saudi Arabia) and housed in a clean environment with controlled room temperature and light exposure and provided with free access to water and food. All experimental procedures were conducted under protocol number H-01-R-059 issued by the Research Ethics Committee of Princess Nourah Bint Abdulrahman University. After acclimatization, the rats were divided into three groups (n = 8 per group): The control group, which received no treatment, the model group (TAA), which received intraperitoneal injections of 200 mg kg^{-1} TAA twice a week for eight weeks[19] and the treated group (Met+Res+TAA), which received a daily combination of Met (200 mg kg^{-1}) and Res (20 mg kg^{-1}) throughout the ten weeks experiment, along with TAA injections starting on day 15. At the end of the ten weeks, all rats were anesthetized, blood was collected and the rats were euthanized to obtain organ tissue specimens.

Histological examination: Formalin-fixed liver specimens were processed and stained with Hematoxylin and Eosin (H&E) as described by Al-Hashem *et al.*[5] and examined under a light microscope (Olympus, Tokyo, Japan) to assess hepatocyte damage.

α-SMA immunohistochemistry: The α-SMA, a marker of chronic liver injury, was assessed in all rats using immunohistochemical staining with anti-α-SMA (Dako, cat#M0851), following a previously reported protocol[5]. The primary antibody was added after antigen retrieval, followed by incubating tissue sections with the secondary antibody.

HIF-1α western blotting analysis: Protein extracts (20 µg per sample) from liver tissues were subjected to Western blotting as reported by Al-Ani *et al.*[20]. Immunoassay membranes were incubated with anti-HIF-1α antibody purchased from Thermo Fisher Scientific, MA, USA for 15 hrs at 4°C. Protein bands were visualized using an enhanced chemiluminescence (ECL) kit obtained from Amersham-Pharmacia, UK and the relative expression of the targeted proteins was obtained by comparison to β-actin.

Determination of MDA, SOD, ALT, AST and hsCRP levels: Hepatic tissue levels of malondialdehyde (MDA) and superoxide dismutase (SOD) were determined using ELISA kits purchased from Cayman Chemical, Ann Arbor, Michigan, USA. Enzymatic kits purchased from Randox Laboratories, Crumlin,

UK, were used to assess blood levels of alanine aminotransferase and aspartate aminotransferase[21]. The CRP blood levels were assessed using an ELISA kit obtained from Assaypro, Saint Charles, Missouri, USA. All measurements were performed according to the manufacturer's instructions.

Statistical analysis and morphometry: Data analyses were conducted using SPSS version 10.0 (SPSS, Inc., Chicago, Ill., USA). Statistical comparisons were made using One-way ANOVA followed by Tukey's *post hoc* Test. Pearson correlation was used to assess the significance between two different parameters. A $p \leq 0.05$ was considered statistically significant. Morphometry was performed by measuring the percentage areas of hepatocyte damage and α-SMA positive immunostaining in 10 different fields using an image analyzer (Leica Qwin 500 C). Data were analyzed using ANOVA as described.

RESULTS

TAA induces chronic liver injury: The disease was modeled in rats to investigate the working hypothesis. A marked increase in the blood levels of biomarkers of liver injury, ALT (Fig. 1a) and AST (Fig. 1b) were observed in the model group 8 weeks following the TAA injections compared to the control rats. In addition, immunohistochemistry of the marker that is known to be increased in chronic liver diseases (α-SMA) revealed weak positive immunostaining in the control rats (Fig. 1c) compared with strong α-SMA positive immunostaining cells (Fig. 1d) in the TAA group. These data supported the establishment of a chronic liver injury animal model.

TAA-induced ROS/HIF-1α axis is inhibited by metformin plus resveratrol: The ROS and HIF-1α were augmented in hepatic injury and ROS is located upstream of HIF-1α in cell signaling. To evaluate the extent of protection provided by Met+Res to rats injected with the hepatotoxic compound TAA for a duration of eight weeks, hepatic levels of MDA, SOD and HIF-1α were assessed in all rats. As shown in Fig. 2, TAA caused a significant ($p<0.0001$) increase in MDA (Fig. 2a), SOD (Fig. 2b) and HIF-1α (Fig. 2c and inset), which were substantially inhibited by Met+Res. In addition, Met+Res significantly ($p<0.0001$) ameliorated blood levels of biomarkers of liver injury: hsCRP (Fig. 2d), ALT (Fig. 2e) and AST (Fig. 2f), to levels comparable to the control group.

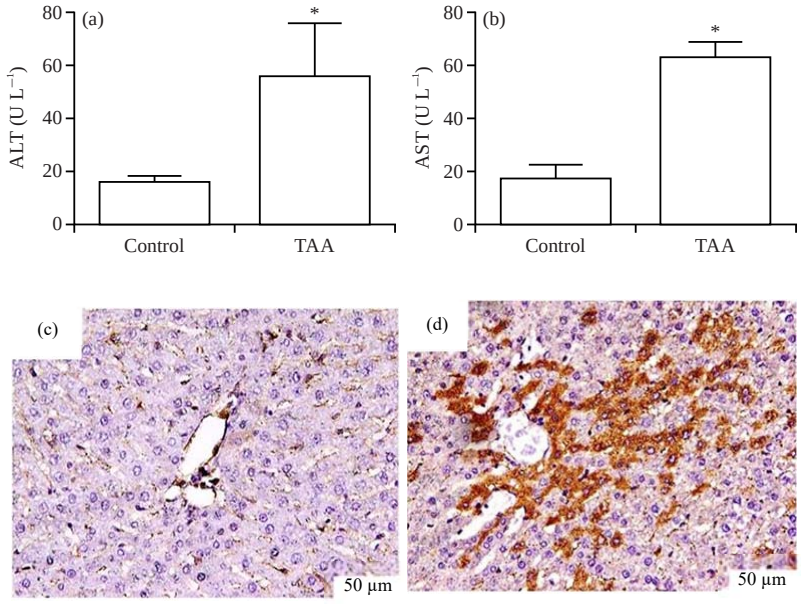

Fig. 1(a-d): Induction of chronic liver injury by TAA, (a) ALT, (b) AST blood levels were determined at week 10 in the model group (TAA) and the control rats (control) and (c) and (d) α-SMA immunohistochemistry liver tissue images (×200) taken from the control and the model groups at week 10 are depicted using light microscopy

*p<0.0001 vs control, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase and TAA: Thioacetamide

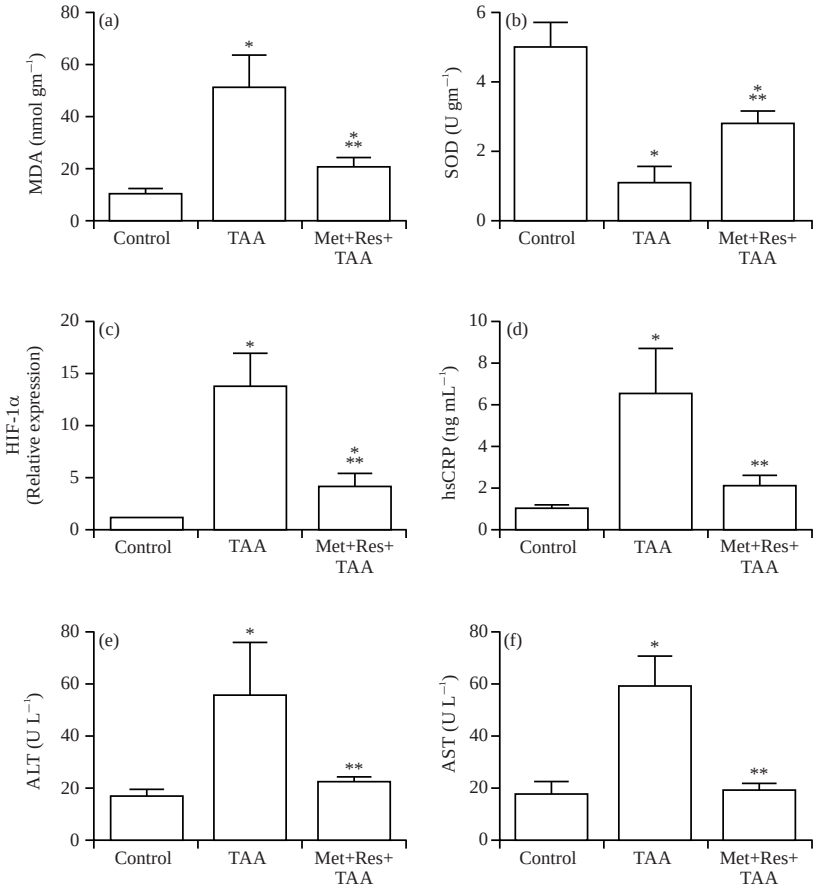

Fig. 2(a-f): Metformin (Met) plus resveratrol (Res) inhibit ROS, HIF-1α and biomarkers of liver injury induced by TAA, ELISA analyses of liver tissue levels of, (a) MDA, (b) SOD, as well as relative protein expression of liver, (c) HIF-1α, were determined at week 10 in all rats, blood levels of (d) hsCRP, (e) ALT and (f) AST were evaluated in all rats at week 10

*p<0.0001 vs control, **p<0.0001 vs TAA, MDA: Malondialdehyde, SOD: Superoxide dismutase, HIF-1α: Hypoxia-inducible factor-1α, hsCRP: High sensitivity C-reactive protein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase and TAA: Thioacetamide

TAA-induced hepatocyte injury is inhibited by metformin plus resveratrol: Given the above results showing that Met+Res had substantially protected hepatic tissue against the ROS/HIF-1α axis and biomarkers of liver injury enzymes induced by TAA, the extent of liver tissue protection by a combination of these agents was assessed at week 10. Compared to normal liver tissue architecture in the control group (Fig. 3a), liver sections of TAA-injected rats prepared for basic histology staining (H&E) revealed hepatic cell damage, vacuolated cytoplasm (arrowheads) and dark pyknotic nuclei (P), as well as dilated congested blood vessels (arrows) and infiltration of inflammatory cells (Fig. 3b). The Met+Res treatment markedly preserved liver tissue and protected against TAA-induced liver injury (Fig. 3c). Furthermore, quantification of hepatocyte damage (Fig. 3d) demonstrated effective protection of hepatocytes by Met+Res.

Correlation between the score of hepatocyte damage and the parameters associated with chronic liver injury induction: Correlation between the hepatocyte damage score and the tissue and blood levels of MDA, SOD, HIF-1α, hsCRP, ALT and AST was determined. This also endorses that the role of Met+Res is an effective agent in liver injury. Figure 4a-f displayed a significant ($p<0.0001$) link between hepatocyte damage score and these parameters: MDA ($r = 0.911$), SOD ($r = -0.823$), HIF-1α ($r = 0.946$), hsCRP ($r = 0.877$), ALT ($r = 0.783$) and AST ($r = 0.923$).

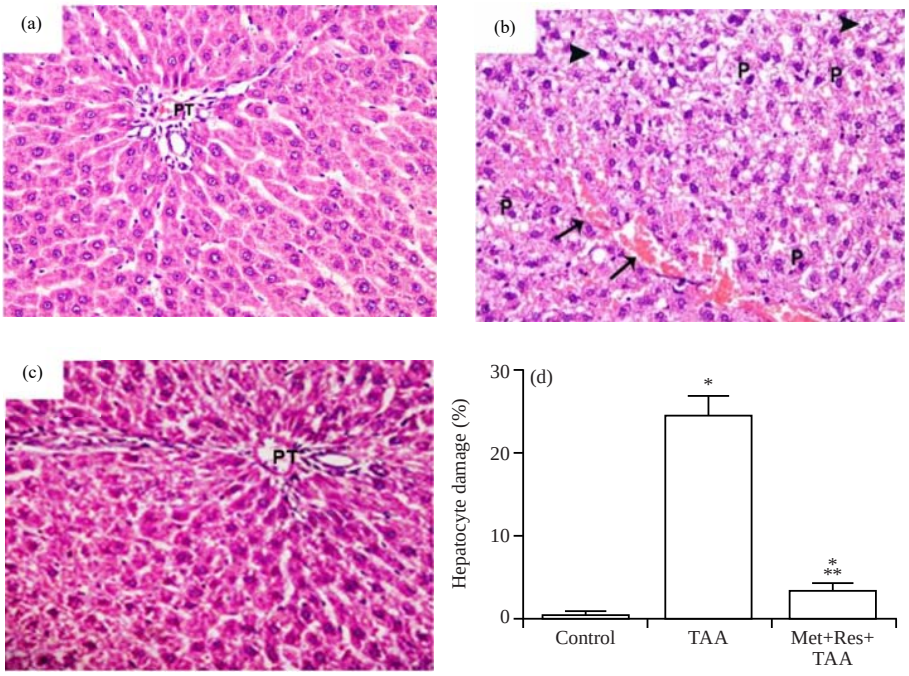

Fig. 3(a-d): Inhibition of TAA-induced liver tissue damage by metformin (Met) plus resveratrol (Res), H&E stained images of liver tissues from the (a) Control, (b) TAA, (c) Met+Res+TAA groups of rats were obtained at week 10 and (d) Degree of hepatocyte damage in rats from these groups is illustrated in histograms

*p = 0.011 vs control, **p<0.0001 vs TAA, TAA: Thioacetamide and PT: Portal tracts

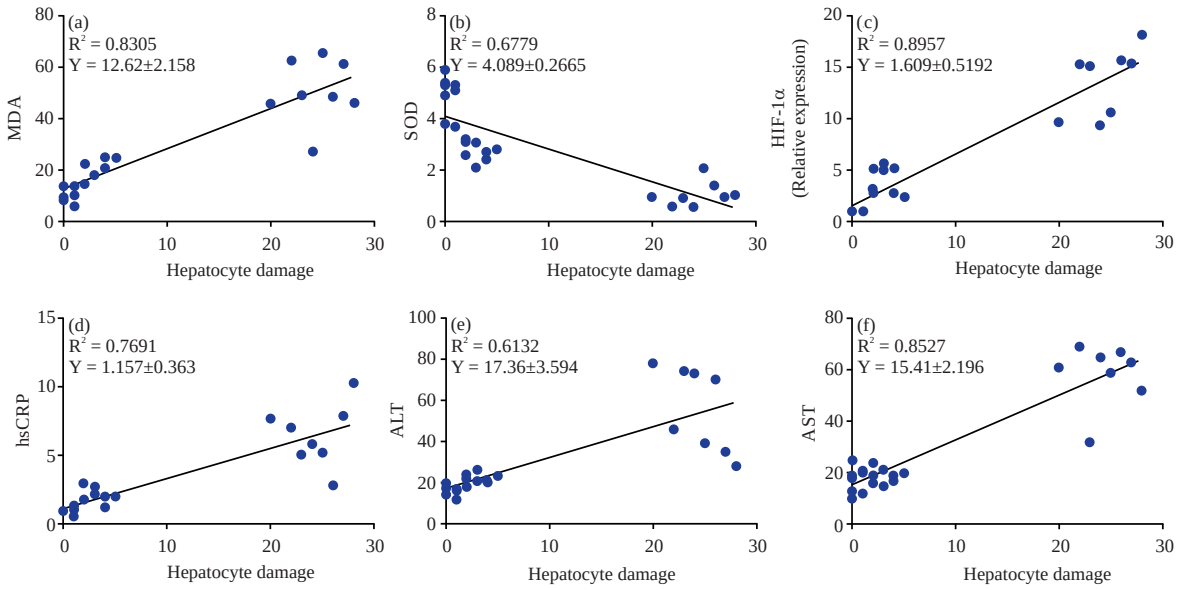

Fig. 4(a-f): Hepatocyte damage score correlates with ROS, HIF-1α and biomarkers of liver injury. The degree of hepatocyte damage was assessed in all rats 10 weeks after the beginning of the experiment and a significant (p<0.0001) correlation was noticed between hepatocyte damage versus, (a) MDA, (b) SOD, (c) HIF-1α, (d) hsCRP, (e) ALT and (f) AST

MDA: Malondialdehyde, SOD: Superoxide dismutase, HIF-1α: Hypoxia-inducible factor-1α, hsCRP: High sensitivity C-reactive protein, ALT: Alanine aminotransferase and AST: Aspartate aminotransferase

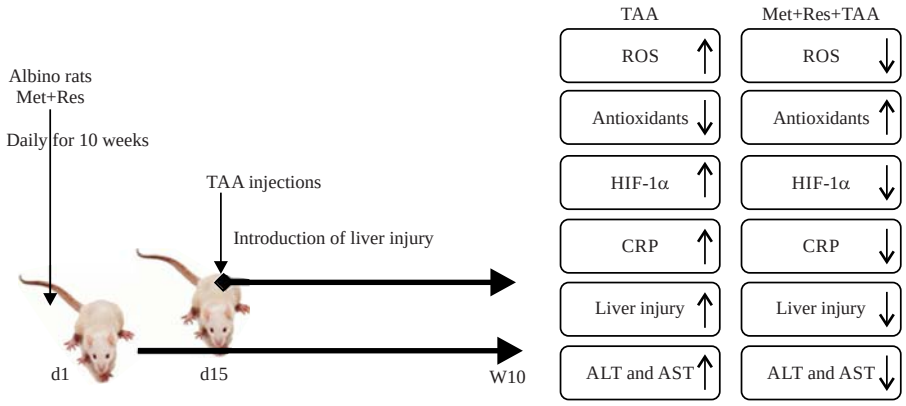

Fig. 5: Proposed model for TAA-induced chronic liver injury is inhibited by Met+Res

TAA: Thioacetamide, Met: Metformin, Res: Resveratrol, ROS: Reactive oxygen species, HIF-1α: Hypoxia-inducible factor-1α, CRP: C-reactive protein, ALT: Alanine aminotransferase and AST: Aspartate aminotransferase

Finally, the proposed model for TAA-induced chronic liver injury is inhibited by Met+Res. The TAA as shown in Fig. 5.

DISCUSSION

In this study, an association between hepatocyte damage and the ROS/HIF-1α axis in a rat model of chronic liver injury induced by the hepatotoxic agent TAA was established, as well as an association with biomarkers of chronic liver disease. Additionally, effective protection of these parameters and liver tissue in rats for a period of 8 weeks using a combination of metformin and resveratrol was observed (Fig. 5). Current findings supported the hypothesis that the ROS/HIF-1α axis is upregulated in chronic liver injury and that metformin plus resveratrol can effectively inhibit this axis, as well as biomarkers of chronic liver injury. These results further substantiate our previous findings on the beneficial effects of either metformin or resveratrol on hepatotoxicity induced by TAA via different cell signaling pathways[5,22] that provided a relatively better protection when using a combination of Met+Res over using a single agent.

The industrial toxicant, TAA is a well-known hepatotoxic compound that can induce tissue oxidative stress, inflammation, organ injury, fibrosis and liver cirrhosis depending on the dose and exposure time of the body[22,23]. Figure 1-3 indicated the induction of hepatic ROS, CRP, α-SMA and liver injury by TAA, which is consistent with previous reports. Furthermore, previous reports have demonstrated the augmentation of the ROS/HIF-1α axis in various diseases, (i) in a mouse model of CCl_4-induced liver fibrosis[15]. The HIF-1α upregulation is viewed here to reduce the deleterious effects of ROS on mitochondria, (ii) in renal fibrosis, ROS increased HIF-1α levels[24]. The HIF-1α upregulation is viewed here to cause kidney damage and (iii) in pulmonary artery smooth muscle cells, ROS-activated HIF-1α promoter[25]. All these reports were consistent with current data demonstrating the upregulation of this axis in our model of chronic liver injury (Fig. 2 and 3). Moreover, inhibition of ROS and HIF-1α by metformin and resveratrol[5,26] corroborate the current study findings presented in this work. However, one of the limitations of this study is the lacking of a fourth animal group treated with the potent antioxidant N-acetylcysteine (NAC) that increases the level of the endogenous antioxidant, glutathione. This would permit us to compare between the inhibitory effects of Met+Res and NAC.

CONCLUSION

This study demonstrated the augmentation of the ROS/HIF-1α axis, as well as biomarkers of chronic liver disease, in a rat model of chronic liver injury induced by TAA. Results also showed that this axis and these biomarkers were effectively protected for a period of 8 weeks by a combination of metformin and resveratrol. Thus, may offer therapeutic potential in humans with chronic liver disease since this type of disease can lead to progressive liver failure, leaving liver transplantation as the only viable treatment option.

SIGNIFICANCE STATEMENT

A combination of metformin and resveratrol was used in this study to assess the degree of protection provided by these agents against TAA-induced chronic liver injury

associated with the augmentation of the hepatic ROS/HIF-1α axis known to be involved in liver pathology. The data revealed effective protection that brought the values of liver injury biomarkers to levels comparable to the control group. Further work will assess the beneficial effects of giving Met+Res post the induction of chronic liver injury.

ACKNOWLEDGMENTS

This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R171), Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia and by the Research Deanship of King Khalid University, Abha, Saudi Arabia, Grant number No. GRP/71/44. We are also grateful to Dr. Mariam Al-Ani from Face Studio Clinic, 90 Hagley Road, Edgbaston, Birmingham, B16 8LU, UK for proofreading the manuscript.

REFERENCES

1. Bzowej, N., D.R. Nelson, N.A. Terrault, G.T. Everson, L.L. Teng, A. Prabhakar and M.R. Charlton, 2011. PHOENIX: A randomized controlled trial of peginterferon alfa-2a plus ribavirin as a prophylactic treatment after liver transplantation for hepatitis C virus. Liver Transpl., 17: 528-538.
2. Teschke, R., 2018. Liver injury by carbon tetrachloride intoxication in 16 patients treated with forced ventilation to accelerate toxin removal via the lungs: A clinical report. Toxics, Vol. 6. 10.3390/toxics6020025.
3. Mochizuki, M., S. Shimizu, Y. Urasoko, K. Umeshita and T. Kamata *et al*., 2009. Carbon tetrachloride-induced hepatotoxicity in pregnant and lactating rats. J. Toxicol. Sci., 34: 175-181.
4. Luo, M., L. Dong, J. Li, Y. Wang and B. Shang, 2015. Protective effects of pentoxifylline on acute liver injury induced by thioacetamide in rats. Int. J. Clin. Exp. Pathol., 8: 8990-8996.
5. Al Hashem, F., S. Al Humayed, S.N. Amin, S.S. Kamar and S.S. Mansy *et al*., 2019. Metformin inhibits mTOR-HIF 1α axis and profibrogenic and inflammatory biomarkers in thioacetamide-induced hepatic tissue alterations. J. Cell. Physiol., 234: 9328-9337.
6. de Minicis, S., M. Marzioni, A. Benedetti and G. Svegliati-Baroni, 2013. New insights in hepatocellular carcinoma: From bench to bedside. Ann. Transl. Med., Vol. 1.
7. Bovi, A.P.D., F. Marciano, C. Mandato, M.A. Siano, M. Savoia and P. Vajro, 2021. Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. Front. Med., Vol. 8. 10.3389/fmed.2021.595371.
8. Tan, H.K., E. Yates, K. Lilly and A.D. Dhanda, 2020. Oxidative stress in alcohol-related liver disease. World J. Hepatol., 12: 332-349.
9. Ivanov, A.V., V.T. Valuev-Elliston, D.A. Tyurina, O.N. Ivanova, S.N. Kochetkov, B. Bartosch and M.G. Isaguliants, 2017. Oxidative stress, a trigger of hepatitis C and B virus-induced liver carcinogenesis. Oncotarget, 8: 3895-3932.
10. Eldeen, A.M.S., B. Al Ani, H.A. Ebrahim, L. Rashed and A.M. Badr *et al*., 2021. Resveratrol suppresses cholestasis induced liver injury and fibrosis in rats associated with the inhibition of TGFβ1–Smad3–miR21 axis and profibrogenic and hepatic injury biomarkers. Clin. Exp. Pharmacol. Physiol., 48: 1402-1411.
11. Ju, C., S.P. Colgan and H.K. Eltzschig, 2016. Hypoxia-inducible factors as molecular targets for liver diseases. J. Mol. Med., 94: 613-627.
12. Zhao, Y., X. Ma, J. Wang, Y. Zhu and R. Li *et al*., 2014. Paeoniflorin alleviates liver fibrosis by inhibiting HIF-1α through mTOR-dependent pathway. Fitoterapia, 99: 318-327.
13. Matafome, P., T. Louro, L. Rodrigues, J. Crisostomo and E. Nunes *et al*., 2011. Metformin and atorvastatin combination further protect the liver in type 2 diabetes with hyperlipidaemia. Diabetes Metab. Res. Rev., 27: 54-62.
14. de la Rosa, L.C., T.E. Vrenken, M. Buist-Homan, K.N. Faber and H. Moshage, 2015. Metformin protects primary rat hepatocytes against oxidative stress-induced apoptosis. Pharmacol. Res. Perspect., Vol. 3. 10.1002/prp2.125.
15. Li, Z., Q. Ding, L.P. Ling, Y. Wu, D.X. Meng, X. Li and C.Q. Zhang, 2018. Metformin attenuates motility, contraction, and fibrogenic response of hepatic stellate cells *in vivo* and *in vitro* by activating AMP-activated protein kinase. World J. Gastroenterol., 24: 819-832.
16. El-Sheikh, A.A., M.A. Morsy and A.M. Okasha, 2017. Inhibition of NF-κB/TNF-α pathway may be involved in the protective effect of resveratrol against cyclophosphamide-induced multi-organ toxicity. Immunopharmacol. Immunotoxicol., 39: 180-187.
17. Trepiana, J., I. Milton-Laskibar, S. Gómez-Zorita, I. Eseberri, M. González, A. Fernández-Quintela and M. Portillo, 2018. Involvement of 5'AMP-activated protein kinase (AMPK) in the effects of resveratrol on liver steatosis. Int. J. Mol. Sci., Vol. 19. 10.3390/ijms19113473.
18. Yu, B., S.y. Qin, B.l. Hu, Q.y. Qin, H.x. Jiang and W. Luo, 2019. Resveratrol improves CCL4-induced liver fibrosis in mouse by upregulating endogenous IL-10 to reprogramme macrophages phenotype from M(LPS) to M(IL-4). Biomed. Pharmacother., Vol. 117. 10.1016/j.biopha.2019.109110.
19. Wallace, M.C., K. Hamesch, M. Lunova, Y. Kim, R. Weiskirchen, P. Strnad and S.L. Friedman, 2015. Standard operating procedures in experimental liver research: Thioacetamide model in mice and rats. Lab. Anim., 49: 21-29.

20. Al-Ani, B., P.W. Hewett, M.J. Cudmore, T. Fujisawa and M. Saifeddine *et al*., 2010. Activation of proteinase-activated receptor 2 stimulates soluble vascular endothelial growth factor receptor 1 release via epidermal growth factor receptor transactivation in endothelial cells. Hypertension, 55: 689-697.
21. Zhou, Z., Y. Zhou, Z. Huang, M. Wang and J. Jiang *et al*., 2023. Notopterol improves cognitive dysfunction and depression-like behavior via inhibiting STAT3/NF-κB pathway mediated inflammation in glioma-bearing mice. Int. Immunopharmacol., Vol. 118. 10.1016/j.intimp.2023.110041.
22. Ebrahim, H.A., S.S. Kamar, M.A. Haidara, N.S.A. Latif and M.A. Ellatif *et al*., 2022. Association of resveratrol with the suppression of TNF-α/NF-kB/iNOS/HIF-1α axis-mediated fibrosis and systemic hypertension in thioacetamide-induced liver injury. Naunyn-Schmiedeberg's Arch Pharm., 395: 1087-1095.
23. Bruck, R., M. Ashkenazi, S. Weiss, I. Goldiner and H. Shapiro *et al*., 2007. Prevention of liver cirrhosis in rats by curcumin. Liver Int., 27: 373-383.
24. Zhang, H., R. Xu and Z. Wang, 2021. Contribution of oxidative stress to HIF-1-mediated profibrotic changes during the kidney damage. Oxid. Med. Cell. Longevity, Vol. 2021. 10.1155/2021/6114132.
25. Bonello, S., C. Zähringer, R.S. BelAiba, T. Djordjevic and J. Hess *et al*., 2007. Reactive oxygen species activate the HIF-1α promoter via a functional NFκB site. Arterioscler. Thromb. Vasc. Biol., 27: 755-761.
26. Dawood, A.F., S. Al Humayed, M.A. Momenah, M. El-Sherbiny and H. Ashour *et al*., 2022. MiR-155 dysregulation is associated with the augmentation of ROS/p53 axis of fibrosis in thioacetamide-induced hepatotoxicity and is protected by resveratrol. Diagnostics, Vol. 12. 10.3390/diagnostics12071762.