Original Article

Medical Journal of Viral Hepatitis (MJVH)

Anti-cancer activity of hesperidin against hepatocellular carcinoma-induced in rats through upregulation of RIPK1 expression

Fatima A. Abdelhameid [1], Yara A. Samra [1], Mamdouh M. El-Shishtawy [1,*]

[1] *Department of Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*

Abstract

Background: Receptor-interacting protein kinase-1 (RIPK1) is an important protein in hepatocellular carcinoma (HCC) pathogenesis. Hesperidin (Hes) is a natural product found in citrus species and is believed to have an anti-cancer effect. **Aims:** This research was planned to elucidate the protective influence of Hes on the RIPK1 pathway in thioacetamide (TAA)-induced HCC.

Materials and methods: HCC was induced in male Sprague Dawley rats using TAA and Hes was administered orally. Liver function tests, oxidative stress status, and antioxidant markers were evaluated. Histopathological examination of hepatic tissues was carried out by staining with hematoxylin and eosin stains. The protein level of RIPK1 was quantified by ELISA. **Results:** Hes exerted a hepatoprotective effect by significantly improving liver functions and oxidative stress status. This was supported by the integrity preservation of the liver tissues, which was indicated by histopathological examination. Moreover, Hes significantly increased the hepatic RIPK1 protein level. **Conclusion:** These results reveal that, Hes protected against HCC by decreasing oxidative stress and upregulation of RIPK1 protein level.

Introduction

Hepatocellular carcinoma (HCC) is the seventh most prevalent cancer with an increasing incidence worldwide and the second most common aetiology of cancer deaths [1]. HCC represents the fourth most common cancer in Egypt and the hepatitis C virus is the leading cause [2]. Universally, HCC is the predominant hepatic malignancy, accounting for about 75% of hepatic malignancies. Asia and Africa have the maximum prevalence averages worldwide [3]. However, HCC remains a disease with a poor prognosis.

HCC typically develops following inflammatory hepatic diseases triggered by viral infections, and/or exposure to carcinogens [1]. Cirrhosis of any cause increases the risk of HCC, with an annual incidence between 2 and 4%. This risk varies according to cause, geographic area, sex, age, and degree of liver damage [3].

Keywords: Hepatocellular carcinoma; Hesperidin; Oxidative stress; Receptor interacting protein kinase-1.
Received: 24-09-2022; Accepted: 18-12-2022
* Corresponding author. Email: mshisht@mans.edu.eg

Thioacetamide (TAA), an organosulfur compound, is a potent hepatotoxin. TAA is used in research to induce hepatic lesions mimicking the majority of cases of human liver diseases and eventually leads to HCC by prolonged administration in experimental animal models [4]. TAA is metabolized to TAA sulfoxide and TAA disulfoxide metabolites which exert the hepatotoxic activity of TAA [5]. They bind to cellular macromolecules that are responsible for the change in cell permeability and Ca^{++} uptake. This results in increasing nuclear volume, compromising mitochondrial activity, reactive oxygen species (ROS) generation, impairing antioxidant defence mechanism, and damaging cellular ingredients such as lipids, proteins and DNA eventually leading to hepatic necrosis and hepatocarcinogenicity [6].

Several pathways are involved in HCC pathogenesis. Receptor-interacting protein kinase-1 (RIPK1) is an important pathway in HCC progression. RIPK1 is one of seven members of the RIPK family (RIPK1–RIPK7). RIPK1 contains multiple domains including the kinase domain, death domain, and intermediate domain. It is considered a key player in inflammation, oncogenesis, and cell death. It can adopt opposite functions depending on the cellular context and its post-translational modifications [7].

RIPK1-mediated signalling is triggered by the bounding of tumour necrosis factor (TNF) to the TNF receptor 1 (TNFR1). Consequently, TNFR1 initiates a membrane-associated complex I. In the formed complex, RIPK1 acts as a scaffold through the polyubiquitination process, which results in the activation of nuclear factor-kappa B signalling promoting cell survival. Meanwhile, TNFR1 can lead to apoptosis by forming Complex IIa and Complex IIb and this occurs due to disassembly of the complex I through deubiquitination of RIPK1 [8]. Therefore, the equivalence between RIPK1 pro-survival scaffolding action and its kinase-activity-mediated pro-apoptotic action is critical for hepatocyte apoptosis.

Treatment of advanced HCC disease is largely ineffective, primarily due to therapy-resistance mechanisms [3]. Cancer prevention, development, progression, and treatment are all correlated with patient nutrition. Higher ingestion of vegetables and fruits in the diet is linked to lesser cancer risk [9]. Hesperidin (Hes) is a flavanone glycoside that is found in abundance in citrus species like lemon, orange, and grapefruit [10]. It exhibits various

biological properties including; antioxidant, radical scavenging, anti-inflammatory, and anticancer effects [11-12].

This work aimed to look into the role of RIPK1 in the development of HCC caused by TAA in rats. Another purpose was to assess the Hes' ability to protect against or prevent HCC via the RIPK1 pathway.

Materials and methods

Animals:

Healthy adult male Sprague Dawley rats (weighing 180–220 g) were purchased from the Egyptian Organization for Biological Products and Vaccines (Giza, Egypt). Rats were kept on a standard diet and temperature setting 12-hour light/dark cycle. All experimental techniques in this work were approved by the "Research Ethics Committee" Faculty of Pharmacy, Mansoura University, Egypt (approval code: 2022-185) and met with the ARRIVE guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

Chemicals and Natural products:

Carboxymethyl cellulose (CMC) (Cairo, Egypt), Hes, and TAA (St. Louis, MO, USA) were purchased.

Experimental study

Three groups of male Sprague–Dawley rats were assembled:

- CMC group (6 rats): Control group received 2 ml of 0.5% CMC, orally, daily for 16 consecutive weeks.
- TAA group (8 rats): Induction of HCC using TAA was achieved as described in previous studies 13-15. Rats in this group received 200 mg/kg of TAA, dissolved in 0.2 ml phosphate-buffered saline (PBS) immediately before use, by i.p. injection twice weekly for 14 consecutive weeks 16.
- TAA+Hes group (8 rats): Received 150 mg/kg/day of Hes, orally as a suspension in 2 ml CMC (0.5%), daily for 2 weeks before TAA injection and continued for 14 consecutive weeks along with TAA 17.

Biological samples collection:

At the end of the 16 weeks study period, rats fasted overnight. Rats were anaesthetized by thiopental (40 mg/kg, i.p.) [18]. Blood samples were gathered and centrifuged to isolate serum. After that, rats were sacrificed and liver specimens were obtained and cut up into two parts. The first part was fixed in a 10% formalin buffered solution for histopathological analysis. The second part was homogenized in ice-cold PBS using an electric homogenizer (Benchmark Scientific, USA). The homogenate was used for measuring oxidative stress biomarkers and RIPK1 protein levels.

Assessment of liver function tests:

Serum was utilized to quantify albumin (Spinreact, Spain), total protein (Spectrum Diagnostics, Egypt), and total bilirubin (Biomed Diagnostics, Egypt) levels using the colourimetric technique after reaction with bromocresol green reagent 19, copper sulfate 20, diazotized sulfanilic acid 21, respectively, according to the instructions of the manufacturer. The absorbance of the sample was measured using a Labomed spectrophotometer (Los Angeles, USA). Alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were measured using the kinetic technique according to the instructions of the manufacturer using the commercially available kit (Agappe Diagnostics, Switzerland). Enzyme activity was evaluated using Robonik prietest kinetic biochemical analyzer (Navi Mumbai, India).

Assessment of oxidative stress status and antioxidant marker

Glutathione (GSH) and malondialdehyde (MDA) concentrations were measured using the spectrophotometric method after reaction with 5, 5`-dithiobis (2-nitrobenzoic acid) [22] and thiobarbituric acid reagent [23], respectively, using the commercially available kit (Biodiagnostic, Egypt).

Assessment of serum alpha-fetoprotein (AFP)

Serum AFP level was measured using a commercially available AFP ELISA Kit (Atlas Medical, Berlin, Germany) and expressed as ng/mL.

Histopathological examination of hepatic tissue

5 µm thick slices were processed and stained with hematoxylin and eosin (H&E) for histopathological analysis of the hepatic structure under the light microscope. Microscopic images of hepatic injury were directed and captured using a digital camera-aided computer system (Nikon digital camera, Tokyo, Japan).

ELISA assay of hepatic contents of RIPK1

A commercially available rat ELISA kit was utilized to evaluate the hepatic content of RIPK1 (Bioassay Technology Laboratory, China) expressed as ng/100 mg tissue as instructed by the manufacturer. The produced colour intensity was determined using BioTek ELISA Reader (Winooski, USA) and sample concentration was calculated using the standard curve.

Statistical analysis

Mean ± standard error of the mean (S.E.M.) was employed to express the data. Analysis of variance (ANOVA), then, Tukey's post-hoc test was employed to compare groups. Statistics and graphing were conducted by Graph Pad Prism 8.01, 2018 (Graph Pad Software, San Diego, CA, USA). The threshold for statistical significance was established at $p < 0.05$.

Results

Hepatoprotective effect of Hes

The TAA group displayed a significant decline in serum albumin and total protein levels and a significant rise in serum ALT and AST activities as well as total bilirubin levels in comparison with the control group ($P < 0.001$). Furthermore, pretreatment with Hes significantly enhanced the markers of liver function as compared to the TAA group revealed by a significant rise in serum albumin and total protein levels and a significant decline in serum ALT and

AST activities, and total bilirubin level (P < 0.001) **(Table 1)**.

Effect of Hes on hepatic oxidative stress status and antioxidant marker

The TAA group exerted marked oxidative impairment demonstrated by a significant decline in hepatic GSH level and a significant rise in hepatic MDA content (P < 0.001), as compared to the control group. However, rats pretreated with Hes prevented oxidative impairment through a significant rise in hepatic GSH level with a significant decline in hepatic MDA level (P < 0.001), as compared to the TAA group **(Fig. 1A, B)**.

Effect of Hes on TAA-induced macroscopic and microscopic features of hepatic injury

The gross evaluation of the livers in the control group revealed glistening, uniform, and reddish-brown surfaces of the livers. While livers from the TAA group indicated distinct nodular surfaces with yellow areas reflecting fat accumulation in tumour cells. On the other hand, the macroscopic features of HCC were barely observed in the livers of pretreated groups with Hes **(Fig. 2A)**. H&E-stained hepatic sections showed normal hepatic structure in the control group. On the contrary, the TAA group revealed a loss of normal hepatic architecture with multiple nodules and thick necro-inflammatory zones. Some nodules developed into well-differentiated HCC. Some other nodules showed micro- or macro-vesicular steatosis and ballooning degeneration. In contrast, the pretreated group with Hes showed partial improvement of hepatic architecture with smaller necro-inflammatory zones, mild focal micro- or macro-vesicular steatosis, and ballooning degeneration **(Fig. 2B)**.

Effect of Hes on TAA-induced elevation of AFP

The TAA group exerted a significant rise in AFP level compared to the control group (P < 0.001). While rats pretreated with Hes displayed a significant decline in AFP level compared to the TAA group (P < 0.001) **(Fig. 3)**.

Effect of Hes on protein expression of hepatic RIPK1

Data from ELISA analysis displayed a significant decline in hepatic RIPK1 protein content in the TAA group in comparison with the control group. However, the data displayed a significant increase in hepatic RIPK1 content in rats pretreated with Hes as compared to the TAA group

(P < 0.001) **(Fig. 4)**.

Table 1. Effect of Hes on liver function markers

Group	Albumin (g/dl)	Total protein (g/dl)	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dl)
CMC	4.242 ± 0.209	6.400 ± 0.072	82.17 ± 3.609	219.3 ± 2.765	0.5580 ± 0.023
TAA	2.626 ± 0.058 ###	4.371 ± 0.268 ###	191.2 ± 13.41 ###	459.3 ± 22.57 ###	0.9936 ± 0.01 ###
TAA+Hes	4.217 ± 0.117 ***	7.486 ± 0.444 ***	117.2 ± 9.311 ***	276.2 ± 21.37 ***	0.6084 ± 0.009 ***

ALT: alanine aminotransferase, **AST:** aspartate aminotransferase, **CMC:** carboxymethyl cellulose, **Hes:** hesperidin, **TAA:** thioacetamide. Six to eight rats in each group, Values: (Mean ± SEM). **###:** significant difference with CMC group (P < 0.001); *****:** significant difference with TAA group (P < 0.001)

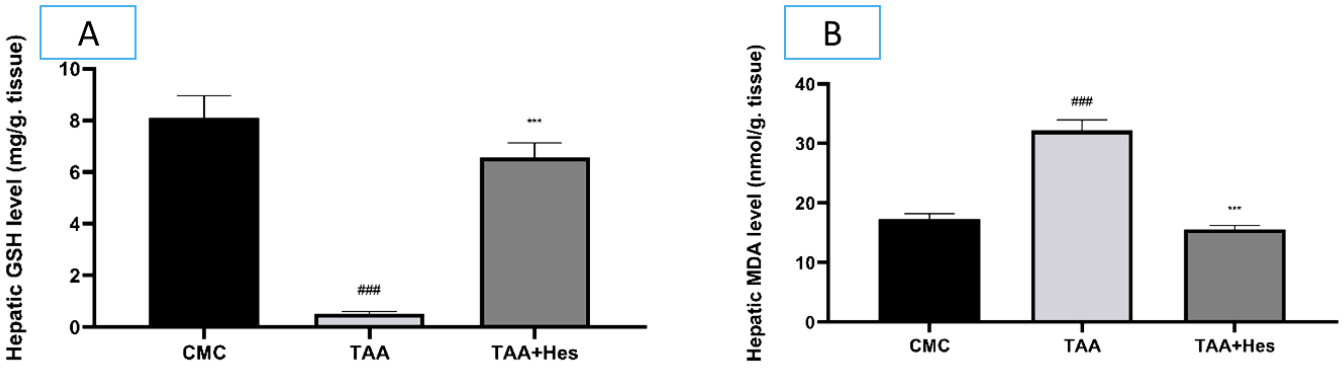

Figure 1: Effect of Hes on hepatic oxidative stress status. Oxidative stress status was evaluated by determining hepatic contents of (A) glutathione (GSH) and (B) malondialdehyde (MDA). CMC: carboxymethyl cellulose, Hes: hesperidin, TAA: thioacetamide. Six to eight rats in each group, Values: (Mean ± SEM); ###: significant difference with CMC group (P < 0.001);***: significant difference with TAA group (P < 0.001)

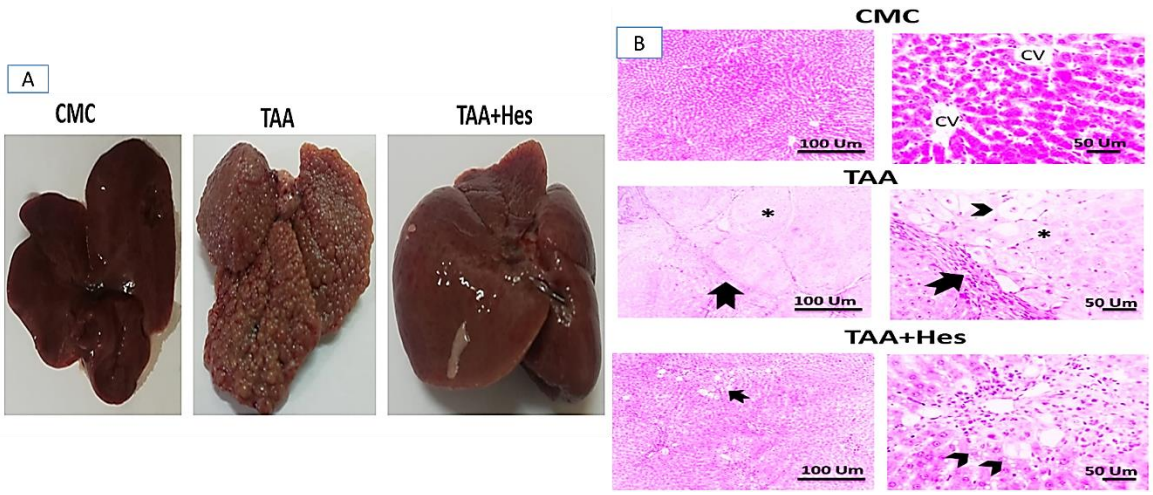

Figure 2: Effect of Hes on TAA-induced macroscopic and microscopic features of hepatic injury. (A) Images of rat livers. (B) Microscopic analysis of hepatic slides with Hematoxylin and Eosin stain. ***** represents tumor nodules. (Arrow) represents necroinflammatory zones. (Arrowhead) represents micro- or macro-vesicular steatosis. CMC: carboxymethyl cellulose, Hes: hesperidin, TAA: thioacetamide, CV: central vein. Six to eight rats in each group.

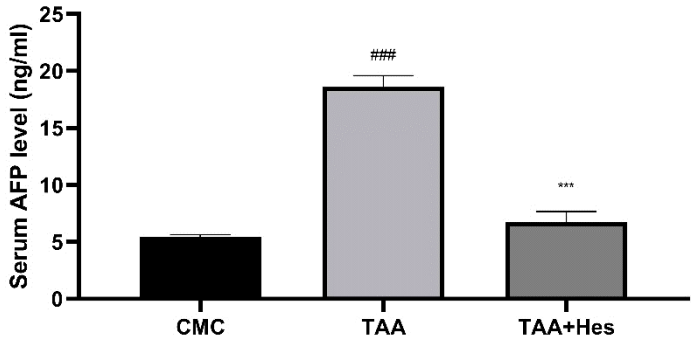

Figure 3: Effect of Hes on AFP serum level. Alpha-fetoprotein (AFP) serum level was assessed by using ELISA technique. CMC: carboxymethyl cellulose, Hes: hesperidin, TAA: thioacetamide. Six to eight rats in each group, Values: (Mean ± SEM). ###: significant difference with CMC group ($P < 0.001$). ***: significant difference with TAA group ($P < 0.001$).

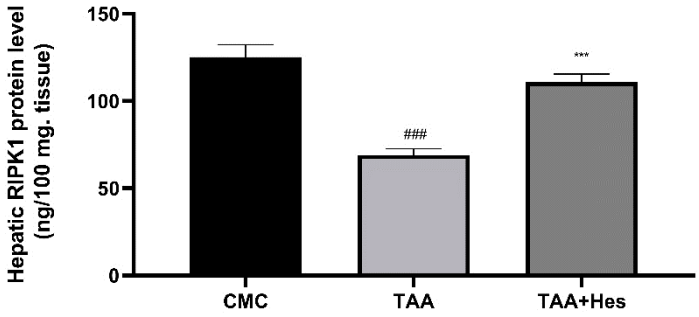

Figure 4: Effect of Hes on protein expression of hepatic RIPK1. Hepatic content of Receptor Interacting Protein Kinase-1 (RIPK1) was assessed by using ELISA techniques. CMC: carboxymethyl cellulose, Hes: hesperidin, TAA: thioacetamide. Six to eight rats in each group, Values: (Mean ± SEM). ###: significant difference with CMC group ($P < 0.001$). ***: significant difference with TAA group ($P < 0.001$)

Discussion

The present work revealed the protective effect of Hes against TAA-induced HCC via targeting RIPK1 protein as a possible mechanism. Our findings demonstrated that the TAA group showed substantial hepatocyte damage and diminished synthetic function, as seen by increased serum ALT, AST activities, and total bilirubin levels, as well as declined serum albumin and total protein levels. These results were verified through histopathological investigation. Additionally, redox imbalance was confirmed

by decreased hepatic GSH levels indicating defects in the antioxidant defence system. Moreover, hepatic MDA content increased denoting the predominance of lipid peroxidation. Decreasing GSH levels and increasing MDA content occurred due to liver cell injury induced by TAA. TAA sulfoxide and TAA disulfoxide metabolites produced after TAA metabolism resulted in hepatic necrosis in liver perivenous areas and subsequent HCC development by the production of ROS which attack hepatic macromolecules resulting in DNA damage and lipid peroxidation. Our findings were in line with other preceding studies. These findings were in line with other preceding studies [24-25]. This can be attributed to the well-known TAA genotoxic and hepato-carcinogenic effects [26-27].

The hepatoprotective effects of Hes were evidenced by minimizing TAA-induced liver damage and dysfunction and restoring the structural integrity of hepatocytes which was indicated through decreasing serum ALT and AST activities. Furthermore, Hes improved serum liver function markers by restoring the normal synthetic and excretory function of the hepatocyte which was indicated by increasing serum albumin and total protein levels as well as decreasing total bilirubin level. Moreover, Hes attenuated TAA-induced redox imbalance demonstrated by elevated hepatic GSH level and reduced hepatic MDA level due to the antioxidant activity of Hes through its free radical scavenging property. Similar effects of Hes were noted in previous studies [15-28]. This hepatoprotective effect might be attributed to their antioxidant and anti-inflammatory effects [11-12].

Hes showed improvements in the morphological and histopathological examination which is consistent with previous studies [15-28]. Besides the hepatoprotective effects of Hes, it also has anti-cancer effects. Moreover, neoplastic and dysplastic lesions were demonstrated in hepatic slices of the TAA group. Hes was able to decrease the number of neoplastic and dysplastic lesions. Importantly, Hes prevented the TAA-induced rise in serum AFP level. This result is compatible with previous research [15].

A suggested mechanism of Hes anti-cancer effects could be related to targeting the RIPK1 pathway by upregulation of its protein expression [29]. This study showed a significant decline in hepatic RIPK1 protein level in the TAA group in comparison with the control group, which matches previous results [29-30]. However, some studies reported contradictory results which alleged that RIPK1 might be upregulated in HCC [31-32]. This conflict may be explained as RIPK1 having both pro-and anti-carcinogenic functions. That depends on the cellular context and its post-translational modifications which control the equivalence between RIPK1 pro-survival scaffolding activity and its kinase-activity-mediated pro-apoptotic function. RIPK1 kinase inhibitors might be useful in restricting hepatic injury and inflammation if utilized before the development of neoplastic lesions. In opposition, intervening with the pro-survival action of RIPK1 by enhancing RIPK1's cytotoxic potential could improve HCC susceptibility to apoptosis.

Conclusion

Hes efficiently protected rats from TAA-induced HCC through their antioxidant activities and upregulation of the expression of RIPK1 protein.

Author contributions

Fatima A. Abdelhameid: Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Software; Writing - original draft; Writing. **Yara A. Samra:** Conceptualization; Methodology; Writing - review & editing. **Mamdouh M El-Shishtawy:** Conceptualization; Visualization; Data Curation; Writing - review & editing. All authors approved the final version of the manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability

All the data generated and analyzed during this study are included in our manuscript. Any additional data is available from the corresponding author upon reasonable request.

Statements and Declarations

Conflict of Interests

Fatima Abdelhameid, Yara Samra, and Mamdouh El-Shishtawy declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

References

1. McGlynn, K. A., Petrick, J. L., and El-Serag, H. B. (2021). Epidemiology of hepatocellular carcinoma. Hepatology, 73, 4-13.
2. Rashed, W. M., Kandeil, M. A. M., Mahmoud, M. O. (2020). Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview. Journal of the Egyptian National Cancer Institute, 32(1), 1-11.
3. Petrick, J. L., Florio, A. A., Znaor, A. (2020). International trends in hepatocellular carcinoma incidence, 1978–2012. International journal of cancer, 147(2), 317-330.
4. Rekha, R. D., Amali, A. A., Her, G. M. (2008). Thioacetamide accelerates steatohepatitis, cirrhosis and HCC by expressing HCV core protein in transgenic zebrafish Danio rerio. Toxicology, 243(1-2), 11-22.
5. Al-Attar, A. M., Alrobai, A. A., and Almalki, D. A. (2016). Effect of Olea oleaster and Juniperus procera leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice. Saudi journal of biological sciences, 23(3), 363-371.
6. Zargar, S., Wani, T. A., Alamro, A. A.. (2017). Amelioration of thioacetamide-induced liver toxicity in Wistar rats by rutin. International journal of immunopathology and pharmacology, 30(3), 207-214.
7. Hou, J., Ju, J., Zhang, Z. (2019). Discovery of potent necroptosis inhibitors targeting RIPK1 kinase activity for the treatment of inflammatory disorder and cancer metastasis. Cell death & disease, 10(7), 1-13.
8. Kondylis, V., and Pasparakis, M. (2019). RIP kinases

in liver cell death, inflammation and cancer. Trends in molecular medicine, 25(1), 47-63.
9. Soerjomataram, I., Oomen, D., Lemmens, V. (2010). Increased consumption of fruit and vegetables and future cancer incidence in selected European countries. European Journal of Cancer, 46(14), 2563-2580.
10. Man, M. Q., Yang, B., and Elias, P. M. (2019). Benefits of hesperidin for cutaneous functions. Evidence-Based Complementary and Alternative Medicine, 2019.
11. Pandey, P., and Khan, F. (2021). A mechanistic review of the anticancer potential of hesperidin, a natural flavonoid from citrus fruits. Nutrition Research, 92, 21-31.
12. Xiong, Y. J., Chu, H. W., Lin, Y. (2016). Hesperidin alleviates rat postoperative ileus through anti-inflammation and stimulation of Ca2+-dependent myosin phosphorylation. Acta Pharmacologica Sinica, 37(8), 1091-1100.
13. Aboismaiel, M. G., El-Mesery, M., El-Karef, A. (2020). Hesperetin upregulates Fas/FasL expression and potentiates the antitumor effect of 5-fluorouracil in rat model of hepatocellular carcinoma. Egyptian Journal of Basic and Applied Sciences, 7(1), 20-34.
14. Helmy, S. A., El-Mesery, M., El-Karef, A. (2018). Chloroquine upregulates TRAIL/TRAILR2 expression and potentiates doxorubicin anti-tumor activity in thioacetamide-induced hepatocellular carcinoma model. Chemico-biological interactions, 279, 84-94.
15. Zaghloul, R. A., Elsherbiny, N. M., Kenawy, H. I., El. (2017). Hepatoprotective effect of hesperidin in hepatocellular carcinoma: Involvement of Wnt signaling pathways. Life sciences, 185, 114-125.
16. Gallagher, C. H., Gupta, D. N., Judah, J. D.. (1956). Biochemical changes in liver in acute thioacetamide intoxication. The Journal of Pathology and Bacteriology, 72(1), 193-201.
17. Balakrishnan, A., and Menon, V. P. (2007). Protective effect of hesperidin on nicotine induced toxicity in rats.
18. Elrazik, N. A. A., El-Mesery, M., and El-Shishtawy, M. M. (2022). Sesamol protects against liver fibrosis induced in rats by modulating lysophosphatidic acid receptor expression and TGF-β/Smad3 signaling pathway. Naunyn-Schmiedeberg's Archives of Pharmacology, 1-14.
19. Chung, B. H., Wilkinson, T., Geer, J. C., and Segrest, J. P. (1980). Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. Journal of lipid research, 21(3), 284-291.
20. Funk, G. M., Hunt, C. E., Epps, D. E. (1988). Use of a rapid and highly sensitive fluorescamine-based procedure for the assay of plasma lipoproteins. Journal of lipid research, 27(7), 792-795.
21. Jendrassik, L. (1938). Vereinfachte photometrische methoden zur bestimmung des blutbilirubins. Biochem z, 297, 81-89.
22. Beutler, E. (1963). Improved method for the determination of blood glutathione. J. lab. clin. Med., 61, 882-888.
23. Hiroshi, O., Nobuko, O., and Kunio, Y. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351-358.
24. Chen, B., Ning, M., and Yang, G. (2012). Effect of paeonol on antioxidant and immune regulatory activity in hepatocellular carcinoma rats. Molecules, 17(4), 4672-4683.
25. Abass, S. A., Abdel-Hamid, N. M., Abouzed, T. K., and El-Shishtawy, M. M. (2018). Chemosensitizing effect of Alpinia officinarum rhizome extract in cisplatin-treated rats with hepatocellular carcinoma. Biomedicine & Pharmacotherapy, 101, 710-718.
26. Al-Attar, A. M., and Shawush, N. A. (2015). Influence of olive and rosemary leaves extracts on chemically induced liver cirrhosis in male rats. Saudi journal of biological sciences, 22(2), 157-163.
27. Meng, Z., Meng, L., Wang, K. (2015). Enhanced hepatic targeting, biodistribution and antifibrotic efficacy of tanshinone IIA loaded globin nanoparticles. European Journal of Pharmaceutical Sciences, 73, 35-43.
28. Mo'men, Y. S., Hussein, R. M., and Kandeil, M. A. (2019). Involvement of PI3K/Akt pathway in the protective effect of hesperidin against a chemically induced liver cancer in rats. Journal of Biochemical and Molecular Toxicology, 33(6), e22305.
29. Schneider, A. T., Gautheron, J., Feoktistova, M., R. (2017). RIPK1 suppresses a TRAF2-dependent pathway to liver cancer. Cancer cell, 31(1), 94-109.
30. Koppe, C., Verheugd, P., Gautheron, J. (2016). IκB kinaseα/β control biliary homeostasis and hepatocarcinogenesis in mice by phosphorylating the cell-death mediator receptor-interacting protein kinase 1. Hepatology, 64(4), 1217-1231.
31. Huang, H., Chen, T., Zhou, Y. (2018). RIPK1 inhibition enhances pirarubicin cytotoxic efficacy through AKT-P21-dependent pathway in hepatocellular carcinoma. International journal of medical sciences, 15(14), 1648.
32. Wang, C., Yao, B., Xu, M.. (2016). RIP1 upregulation promoted tumor progression by activating AKT/Bcl-2/BAX signaling and predicted poor postsurgical prognosis in HCC. Tumor Biology, 37(11), 15305-15313.