JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/48383

RESEARCH ARTICLE

Hepatoprotective Effects of Capsaicin against Alcohol Induced Zebrafish Liver Damage Might be Due to its Potent Antioxidant Action

Rupa Gupta[1], Neeraj K. Sethiya[2] and Satish Sardana[1*]

[1]Amity Institute of Pharmacy, Amity University, Gurugram - 122015, Haryana, India; ssardana@ggn.amity.edu

[2]Faculty of Pharmacy, School of Pharmaceutical and Populations Health Informatics, DIT University, Mussoorie Diversion Road, Dehradun - 248009, Uttarakhand, India

Abstract

Background: Capsaicin is one of the most widely utilized global bioactive dietary ingredients exhibiting multiple health benefits to both humans and animals. However, any of earlier report lacking any evidence related to hepatoprotective activity of capsaicin on zebra fish model. **Aim:** The study demonstrates antioxidant (*in vitro*) and hepatoprotective activity (*in vivo*) of capsaicin. **Methods:** The antioxidant activity of capsaicin was performed using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging method and hepatoprotective activity were performed on alcohol induced zebrafish liver damage, respectively. **Results:** In outcome we have found that capsaicin was able to demonstrate significant antioxidant action and hepatoprotective activity as evidence from experiments. **Conclusion:** In conclusion the current study validated capsaicin role in improving liver toxicity through antioxidant mechanism and can be useful to develop many value added products in near future.

Major Findings: Capsaicin (8-methyl-N-vanillyl-6-nonenamide, from Chili peppers) was found to exhibits antioxidant and hepatoprotective activity as a scientific evidence resulted from DPPH (*in vitro*) radical scavenging method and alcohol induced zebrafish liver damage (*in vivo*). Current research is useful for designing future remedies for management of liver toxicity.

Keywords: Antioxidant, Capsaicin, Hepatoprotection, Toxicity, Zebra Fish

1. Introduction

Liver is one of the most crucial and essential organ of our body having multifunctional roles, efficiency and capabilities[1]. In brief, liver is majorly responsible for metabolism and detoxification including production of chemicals necessary for digestive transformation and clearance of destructive metabolites[2]. In addition, a number of harmful substances, such as thioacetamide, chemotherapeutic drugs, carbon tetrachloride, some antibiotics, excessive alcohol intake, and pathogenic microorganisms, can cause liver illnesses, which have now emerged as a significant global health concern[3]. Modern medical approaches to treat liver illnesses are increasingly inadequate and linked to long-term negative effects, in addition being prohibitively expensive in underdeveloped nations[4]. Presently, several natural products including traditional plants have gained worldwide attention as an alternative agent for the management of liver diseases[5-8].

Capsaicin is a pungent dietary bioactive component present mostly in *Capsicum* fruits (hot chili peppers, *mirch*) exhibits to produce multiple health benefits in both humans and animals[9-11]. As evidence, *Capsicum* fruits have been utilized as traditional recipe and found an important place of every kitchen from ancient times[12,13]. Historically, capsaicin was first time isolated in purified form in 1876 and chemical structure was

*Author for correspondence

Article Received on: 27.01.2025 Revised on: 21.03.2025 Accepted on: 02.04.2025

explained in 1919 as a non-polar phenolic compound[14]. Since, over the time several recently published studies on capsaicin shown with molecular mechanism based diversified health applications[15-23]. Beside this, capsaicin has been validated to have enormous potential to manage several liver diseases as evidence illustrated in recent published review[24,25]. Recently, zebrafish (*Danio rerio*) was proposed to offer many unique features such as small in size with extreme chemical sensitivity, external fertilization and rapid development[26]. Interestingly, the researcher currently utilizing zebrafish as an ideal animal vertebrate model for testing effects of various drugs against chemical or alcohol induced liver toxicity[27-30]. Further, no study has been conducted so far to investigate the hepatoprotective effects of capsaicin against alcohol induced liver toxicity in zebra fish models. Therefore, we proposed to investigate the antioxidant activity of capsaicin using DPPH radical scavenging assay followed by hepatoprotective effects on alcohol induced zebrafish liver damage.

2. Materials and Methods

2.1 Chemicals and Reagents

DPPH was purchased from CDH Pvt. Ltd., New Delhi (India) and Capsaicin (99.13%) was obtained as a gift sample from Herbo Nutra Extract Pvt Ltd, Gautam Buddha Nagar, Uttar Pradesh, India. All other chemicals and solvents utilized in this current work are analytical grade.

2.2 Animals

Total 40 adult wild-type male zebrafish (6-8 month old with average weight of 0.45±0.05 g) were acclimatized for a period of 1 week (14:10 light/dark cycle) prior to the start of the experiment with normal food 2 times a day (10 mg/fish/meal/10 minutes). Animal experiment was conducted in accordance with accepted ethical standards and authorized by the Institutional Animal Ethical Committee (Project proposal number: MB/IAEC/2024/03/15, dated 19th September 2024), Mass Biotech, Chengalpet, Tamil Nadu, India.

2.3 DPPH Radical Scavenging Antioxidant Activity

Methanol solution of DPPH (100 M) was mixed with equal volumes of capsaicin and positive standard vitamin E (3 replicates) at varying doses (0, 50, 100, 150, 200, 250, and 300 µg/ml) in a test tubes. After 15 min of incubation (room temperature), the absorbance at 517 nm was recorded. The concentration of each sample needed to scavenge (50%) DPPH free radicals scavenging effects were shown by the IC_{50} values[31].

2.4 Hepatoprotective Activity of Capsaicin against Alcohol Induced Zebrafish Liver Damage

The hepatoprotective activity of capsaicin was performed as per the reported standard method with slight modification on alcohol induced zebrafish liver damage with reference to Silymarin as positive standard drug[27,28]. The experimental animals (n= 10) showing no rejection to feed intake with normal swim pattern and without stress will be allocated randomly to the four different groups as follows.

2.4.1 Grouping of Animals

Four distinct groups were created from the animals (n = 10).

1. Group I (Normal control): Vehicle treated.
2. Group II (Negative control): Treated with 0.5% alcohol for 30 days.
3. Group III (Positive control): Received 0.5% alcohol for 30 days followed by Silymarin (420 mg/day/orally) for 14 days[32].
4. Group IV (Treatment): Received 0.5% alcohol for 30 days followed by Capsaicin (75 mg/day/orally) for 14 days[33].

2.4.2 Ethanol Induced Liver Toxicity

Hepatotoxicity was induced by letting the zebra fishes with 0.5% alcohol (change of water for every 2 days) for 30 days (Groups II, III and IV). The animals of Group III and IV were treated by blending the calculated amount of Silymarin and Capsaicin, respectively with commercial feed for a period of 14 days. After complete treatments, each group in the individual study tanks were anesthetized with -4° C water followed by open excision procedures to sacrifice the animals. Thereafter, toxicity analysis by histopathology was performed for each animal by collecting tissue samples from liver under general anesthesia condition. Zebrafish larvae were homogenized in cold saline solution in order to analyze the hepatic transaminase activity. The homogenates were centrifuged for 10 minutes at 4° C at 2500 rpm[34]. The

auto biochemistry analyzer BS-120 (Mindray, Gurugram, India) was then used to evaluate the activities of Lactate Dehydrogenase (LDH), Aspartate Transaminase (AST), and Alanine Transaminase (ALT) in the resultant supernatants as per manufacturer's protocol and all the results were expressed as IU/L.

3. Statistical Analysis

The mean ± SD was used to report all results. Dunnett's multiple comparison post-test has been used to evaluate one-way ANOVA using non-parametric techniques in Graph Pad Prism 10 in order to quantify the variation in a set of data. At $P<0.05$, statistical differences were deemed significant.

4. Results

4.1 DPPH Radical Scavenging Antioxidant Activity

The radical scavenging effects of capsaicin and positive standard vitamin E are represented in Figure 1. The stable, purple DPPH radical was found to be 50% reduced by both capsaicin and vitamin E. The IC_{50} value were found to be 85.0668 ± 2.5780 µg/ml for capsaicin and 96.2823 ± 11.0112µg/ml for vitamin E, respectively.

4.2 Hepatoprotective Activity of Capsaicin Against Alcohol Induced Zebrafish Liver Damage

The groups treated with a combination of ethanol and silymarin (Group III) and ethanol and capsaicin, exhibited significant hepatoprotective activity as evidence from reduction of AST, ALT and LDH activity compared to the negative and normal control groups (Figure 2). This suggests that silymarin and capsaicin both have preventive effects against liver

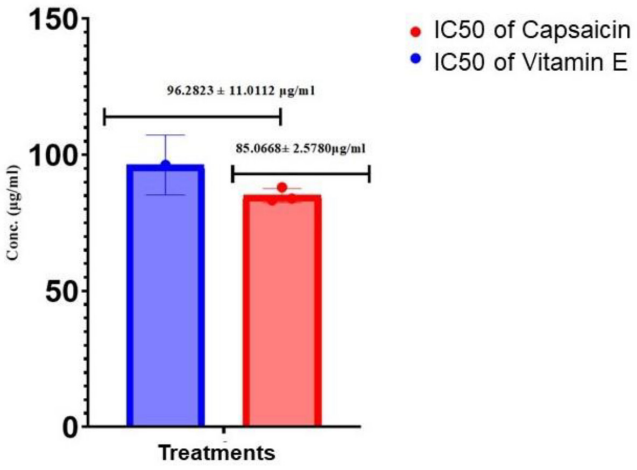


Figure 1. The radical scavenging effects of capsaicin and vitamin E.

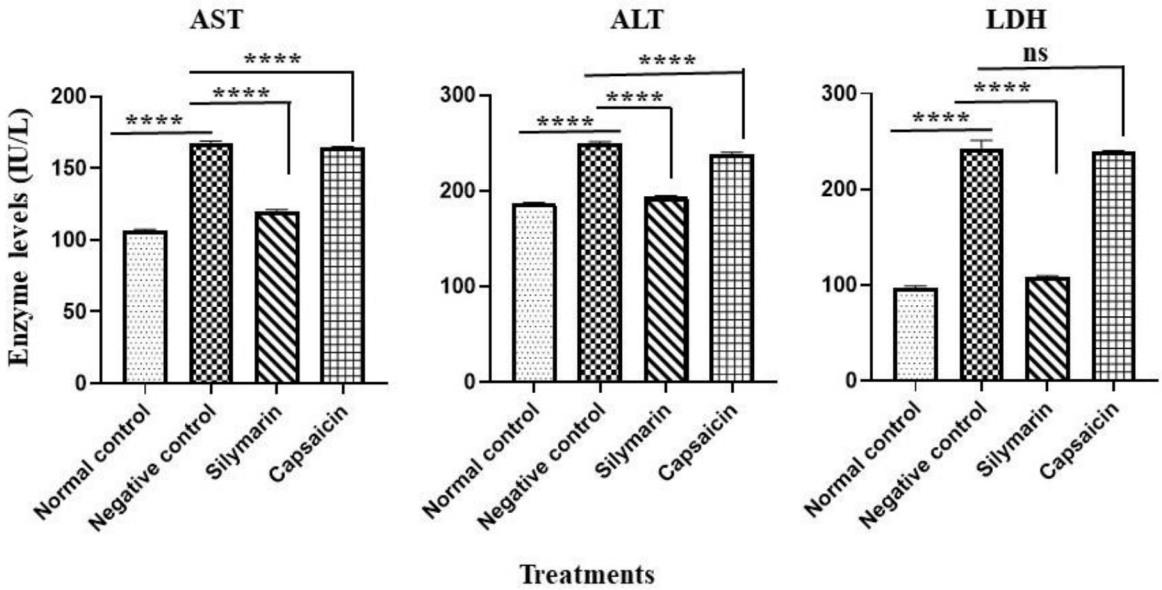


Figure 2. Hepatoprotective effects of capsaicin on alcohol induced liver toxicity of zebra fish.
(Where $p < 0.0001$ is **** by Oneway Annova followed by Dunnett's multiple comparisons test).
*ns denotes non-significant.

damage brought by alcohol. Additionally, capsaicin was observed to reduce AST, ALT, and LDH activity levels in a manner equivalent to silymarin at lower doses, indicating that capsaicin effectively mitigates alcohol-induced liver damage.

The results of histopathology of zebra fish liver sections were shown in Figure 3. The results revealed that the symptom of hepatotoxicity with moderate ballooning and fatty degeneration of hepatocytes were observed in negative control group, when compare with normal control. Additionally, both silymarin and capsaicin treated group were able to reverse the changes associated with hepatotoxicity.

As evidence from Figure B the moderate ballooning and fatty degeneration of hepatocytes were observed. However, normal morphology of hepatocytes were observed in peri-portal and centri-lobular liver regions by positive control group *i.e.* silymarin and treatment group *i.e.* capsaicin and comparable with normal control.

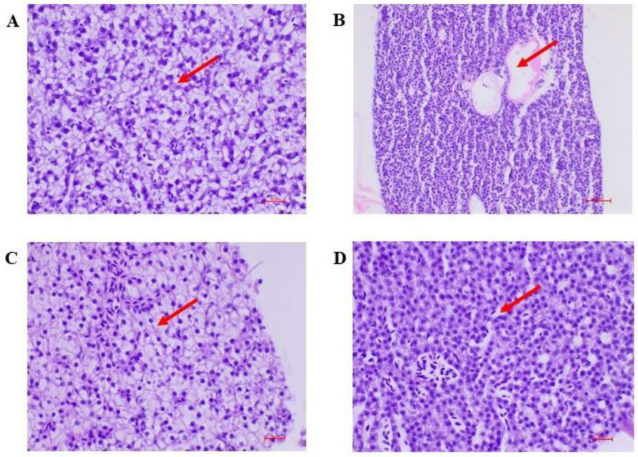


Figure 3. Histopathology of zebra fish liver, where **(A)** Group I (Normal control), **(B)** Group II (Negative control), **(C)** Group III (Positive control) and **(D)** Group IV (Treatment).

5. Discussion

It is commonly known that the pathophysiology of several liver illnesses, especially alcoholic liver disease, is significantly influenced by oxidative stress[35]. Therefore, any test drugs can be assessed for liver protection *via* screening of antioxidant activity[2,6]. Capsaicin is one of the known pungent principle have been found to exhibits *in vitro* free radical scavenging effects by DPPH method as per earlier report[36]. Similarly, a previous study reported higher antioxidant activity of capsaicin, when compared with vitamin E[37]. Additionally, by stopping ROS from oxidizing glutathione, capsaicin has been demonstrated to reduce oxidative stress and boost cellular antioxidant capability as per earlier report[38,39]. Our study also shown higher antioxidant activity of capsaicin in comparison with vitamin E and supported the outcome of earlier finding.

As per earlier report, chronic exposure of ethanol to zebrafish was established as model of alcoholic liver disease lead to increase in both oxidative and ER (Endoplasmic Reticulum) stress in the liver[27]. Further, a recent study reviewed capsaicin as promising hepatoprotective agents against several diseases including NAFLD, liver injury, liver cancer, and liver fibrosis *via* antioxidant, antisteatosis, antiinflammatory, and antifibrosis effects[25]. Similarly, a previous study has shown that capsaicin was able to ameliorate alcohol-induced acute liver injury in mice partly *via* improvement of hepatic antioxidant status[40]. Surprisingly, effects of capsaicin against alcohol induced zebrafish liver damage were still not investigated and may be useful as it leads to establish capsaicin as more effective liver protective agents. Therefore, present study was designed to demonstrate the effects of capsaicin on alcohol induced zebrafish liver damage. Moreover, ethanol induced liver toxicity causes leakage of ALT, AST, and LDH enzymes to the medium due to the loss of cell membrane functional integrity[41]. Similarly, in our study chronic long term exposure of ethanol causes the liver toxicity and elevate the levels of ALT, AST, and LDH as evidence from negative control group. As shown by results silymarin and capsaicin both have preventive effects against liver damage brought by alcohol as evidence from reduction of AST, ALT, and LDH activity *via* improvement of hepatic antioxidant status as evidence from DPPH free radical scavenging activity. Additionally, as per previous histological finding aqueous extract of *C. annum* L. (major source of capsaicin) can ameliorate the hepato-cellular damage in liver of rats caused by ethanol[42]. Similarly, in our study capsaicin are able to reverse the changes of hepato-cellular damage in zebra fish liver as evidence. Therefore, our results strongly support the earlier findings of capsaicin associated with liver protection.

6. Conclusion

Capsaicin is a pungent dietary bioactive component reported to exhibits multiple health benefits including liver protective effects. Similarly, our study provides evidence that the capsaicin was found to be beneficial in mitigating alcohol-induced liver hepatotoxicity in a zebrafish model. These effects include improvement in liver enzymes and hepatic cell pathophysiology. Further future research is warranted to demonstrate the underlying mechanisms and validate these findings in humans.

7. Acknowledgements

We here with acknowledge institutional administrative supports enabled us to complete the current work.

8. Author Contribution

Rupa Gupta conducted research, analyzed the results and wrote the research paper draft. Neeraj K. Sethiya and Satish Sardana designed, supervised, and revised the paper draft. All authors have read and agreed to publish current version of manuscript.

9. References

1. Michalopoulos GK. Liver regeneration. J Cell Physiol. 2007; 213(2):286-300. https://doi.org/10.1002/jcp.21172
2. Bairwa NK, Sethiya NK, Mishra SH. Protective effect of stem bark of *Ceiba pentandra* Linn. against Paracetamol-induced hepatotoxicity in Rats. Pharmacogn Res. 2010; 2(1): 26-30. https://doi.org/10.4103/0974-8490.60584
3. Seto WK, Mandell MS. Chronic liver disease: Global perspectives and future challenges to delivering quality health care. Plos One. 2021; 16(1):e0243607. https://doi.org/10.1371/journal.pone.0243607
4. Okaiyeto K, Nwodo UU, Mabinya L, Okoh AI. A review on some medicinal plants with hepatoprotective effects. Phcog Rev. 2018; 12(24):186-199. https://doi.org/10.4103/phrev.phrev_52_17
5. Hong M, Li S, Tan HY, Wang N, Tsao SW, Feng Y. Current status of herbal medicines in chronic liver disease therapy: The biological effects, molecular targets and future prospects. Int J Mol Sci. 2015; 16:28705-28745. https://doi.org/10.3390/ijms161226126
6. Sethiya NK, Shah P, Rajpara A, Nagar PA, Mishra SH. Antioxidant and hepatoprotective effects of mixed micellar lipid formulation of phyllanthin and piperine in carbon tetrachloride-induced liver injury in rodents. Food Funct. 2015; 6(11):3593-3603. https://doi.org/10.1039/c5fo00947b
7. Sethiya NK, Ahmed NM, Shekh RM, Kumar V, Singh PK, Kumar V. Ethnomedicinal, phytochemical and pharmacological updates on *Hygrophila auriculata* (Schum.) Hiene: An overview. J Integr Med. 2018; 16(5):299-311. https://doi.org/10.1016/j.joim.2018.07.002
8. Sardana S, Gupta R, Madan K, Bisht D, Rana VS, Bhargava S, Sethiya NK. Advance drug delivery and combinational drug approaches for hepatoprotective action of berberine: A progressive overview with underlying mechanism. RPS Pharm Pharmacol Rep. 2023; 2(1). https://doi.org/10.1093/rpsppr/rqad002
9. Hayman M, Kam PCA. Capsaicin: A review of its pharmacology and clinical applications. Curr Anaesth Crit Care. 2008; 19(5-6):338-343. https://doi.org/10.1016/j.cacc.2008.07.003
10. Fattori V, Hohmann MSN, Rossaneis AV, Pinho-Ribeiro FA, Verri WA. Capsaicin: Current understanding of its mechanisms and therapy of pain and other pre-clinical and clinical uses. Molecules. 2016; 21(7):844. https://doi.org/10.3390/molecules21070844
11. Batiha GE, Alqahtani A, Ojo OA, Shaheen HM, *et al*. Biological properties, bioactive constituents, and pharmacokinetics of some *Capsicum* spp. and capsaicinoids. Int J Mol Sci. 2020; 21(15):5179. https://doi.org/10.3390/ijms21155179
12. Nelson EK, Dawson LE. The constitution of capsaicin, the pungent principle of Capsicum III. J Am Chem Soc. 1923; 45(9):2179-2181. https://doi.org/10.1021/ja01662a023
13. Kaga H, Miura M, Orito K. A facile procedure for synthesis of capsaicin. J Org Chem. 1989; 54(14):3477-3478. https://doi.org/10.1021/jo00275a040
14. Nelson EK. The constitution of capsaicin, the pungent principle of Capsicum. J Am Chem Soc. 1919; 41(7):1115-1121. https://doi.org/10.1021/ja02228a011
15. Dludla PV, Cirilli I, Marcheggiani F, Silvestri S, Orlando P, Muvhulawa N, *et al*. Bioactive properties, bioavailability profiles, and clinical evidence of the potential benefits of Black pepper (*Piper nigrum*) and Red pepper (*Capsicum annum*) against diverse metabolic complications. Molecules. 2023; 28(18):6569. https://doi.org/10.3390/molecules28186569
16. Mandal SK, Rath SK, Logesh R, Mishra SK, Devkota HP, Das N. *Capsicum annuum* L. and its bioactive constituents: A critical review of a traditional culinary spice in terms of its modern pharmacological potentials with toxicological issues. Phytother Res. 2023; 37(3):965-1002. https://doi.org/10.1002/ptr.7660
17. Periferakis AT, Periferakis A, Periferakis K, Caruntu A, Badarau IA, Savulescu-Fiedler I, Scheau C, Caruntu C.

Antimicrobial properties of capsaicin: Available data and future research perspectives. Nutrients. 2023; 15(19):4097. https://doi.org/10.3390/nu15194097
18. Rezazadeh A, Hamishehkar H, Ehsani A, Ghasempour Z, Kia EM. Applications of capsaicin in food industry: Functionality, utilization and stabilization. Crit Rev Food Sci Nutr. 2023; 63(19):4009-4025. https://doi.org/10.1080/10408398.2021.1997904
19. Sales EK, Mohaddes G, Alipour MR. Hepatoprotection of capsaicin in alcoholic and non-alcoholic fatty liver diseases. Arch Physiol Biochem. 2024; 130(1):38-48. https://doi.org/10.1080/13813455.2021.1962913
20. Petran EM, Periferakis A, Troumpata L, Periferakis AT, Scheau AE, Badarau IA, *et al*. Capsaicin: Emerging pharmacological and therapeutic insights. Curr Issues Mol Biol. 2024; 46(8):7895-7943. https://doi.org/10.3390/cimb46080468
21. Zhang Q, Huang S, Soladoye OP, Zhang Y, Fu Y. Preparation, pungency, and bioactivity of capsaicin: A review. Int J Food Sci Technol. 2024; 59(9):6659-6687. https://doi.org/10.1111/ijfs.17291
22. Ferreira LGB, Faria JV, Santos JPSD, Faria RX. Capsaicin: TRPV1-independent mechanisms and novel therapeutic possibilities. Eur J Pharmacol. 2020; 887:173356. https://doi.org/10.1016/j.ejphar.2020.173356
23. Zhang W, Zhang Y, Fan J, Feng Z, Song X. Pharmacological activity of capsaicin: Mechanisms and controversies (review). Mol Med Rep. 2024; 29(3). https://doi.org/10.3892/mmr.2024.13162
24. Scheau C, Badarau IA, Caruntu C, Mihai GL, Didilescu AC, Constantin C, Neagu M. Capsaicin: Effects on the pathogenesis of hepatocellular carcinoma. Molecules. 2019; 24(13):2350. https://doi.org/10.3390/molecules24132350
25. Li S, Hao L, Yu F, Li N, Deng J, Zhang J, Xiong S, Hu X. Capsaicin: A spicy way in liver disease. Front Pharmacol. 2024; 15. https://doi.org/10.3389/fphar.2024.1451084
26. Shimizu N, Shiraishi H, Hanada T. Zebrafish as a useful model system for human liver disease. Cells. 2023; 12(18):2246. https://doi.org/10.3390/cells12182246
27. Park KH, Kim SH. Low dose of chronic ethanol exposure in adult zebrafish induces hepatic steatosis and injury. Biomed Pharmacother. 2019; 117:109179. https://doi.org/10.1016/j.biopha.2019.109179
28. Park KH, Kim SH. Adult zebrafish as an *in vivo* drug testing model for ethanol induced acute hepatic injury. Biomed Pharmacother. 2020; 132:110836. https://doi.org/10.1016/j.biopha.2020.110836
29. Wang X, Zhao J, Zhang R, Liu X, Ma C, Cao G, Wei Y, Yang P. Protective effect of *Hedyotis diffusa* Wild. Ethanol extract on isoniazid-induced liver injury in the zebrafish model. Drug Des Devel Ther. 2022; 16:1995-2015. https://doi.org/10.2147/DDDT.S358498
30. Rana S, Sharma N, Dutt S, Patil RD. Hepatoprotective effects of *Carissa spinarum* extract on carbon tetra chloride induced liver damage in zebrafish (*Danio rerio*). Med Pharm J. 2023; 2(4):206-220. https://doi.org/10.55940/medphar202362
31. Sethiya NK, Trivedi A, Mishra S. The total antioxidant content and radical scavenging investigation on 17 phytochemicals from dietary plant sources used globally as functional food. Biomed Prev Nutr. 2014; 4(3):439-444. https://doi.org/10.1016/j.bionut.2014.03.007
32. Karimi G, Vahabzadeh M, Lari P, Rashedinia M, Moshiri M. "Silymarin", a promising pharmacological agent for treatment of diseases. Iran J Basic Med Sci. 2011; 14(4):308-317. PMCID: PMC3586829. PMID: 23492971.
33. Glinsukon T, Stitmunnaithum V, Toskulkao C, Buranawuti T, Tangkrisanavinont V. Acute toxicity of capsaicin in several animal species. Toxicon. 1980; 18(2):215-220. https://doi.org/10.1016/0041-0101(80)90076-8
34. Park K, Mohamedelfatih H, Makki M, Kim SH, Chung HJ, Jung J. Narirutin ameliorates alcohol-induced liver injury by targeting MAPK14 in zebrafish larvae. Biomed Pharmacother. 2023; 166:115350. https://doi.org/10.1016/j.biopha.2023.115350
35. Arauz J, Tovar ER, Muriel P. Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. Ann Hepatol. 2016; 15(2):160-173. PMID: 26845593. https://doi.org/10.5604/16652681.1193701
36. Thongin S, Den-udom T, Uppakara K, Sriwantana T, Sibmooh N, Laolob T, *et al*. Beneficial effects of capsaicin and dihydrocapsaicin on endothelial inflammation, nitric oxide production and antioxidant activity. Biomed Pharmacother. 2022; 154:113521. https://doi.org/10.1016/j.biopha.2022.113521
37. Kursunluoglu G, Taskiran D, Kayali HA. The investigation of the antitumor agent toxicity and capsaicin effect on the electron transport chain enzymes, catalase activities and lipid peroxidation levels in lung, heart and brain tissues of rats. Molecules. 2018; 23(12):3267. https://doi.org/10.3390/molecules23123267
38. Kogure K, Goto S, Nishimura M, Yasumoto M, Abe K, Ohiwa C, Sassa H, Kusumi T, Terada H. Mechanism of potent antiperoxidative effect of capsaicin. Biochim Biophys Acta - Gen Subj. 2002; 1573(1):84-92. https://doi.org/10.1016/s0304-4165(02)00335-5
39. Li Q, Li L, Wang F, Chen J, Zhao Y, Wang P, Nilius B, Liu D, Zhu Z. Dietary capsaicin prevents nonalcoholic fatty liver disease through transient receptor potential vanilloid 1-mediated peroxisome proliferator-activated receptor δ activation. Pflugers Arch. 2013; 465:1303-1316. doi: https://doi.org/10.1007/s00424-013-1274-4
40. Koneru M, Sahu BD, Mir SM, Ravuri HG, Kuncha M, Kumar JM, Kilari EK, Sistla R. Capsaicin, the pungent principle of

peppers, ameliorates alcohol-induced acute liver injury in mice *via* modulation of matrix metalloproteinases. Can J Physiol Pharmacol. 2018; 96(4):419-427. https://doi.org/10.1139/cjpp-2017-0473

41. Fernando CD, Soysa P. Evaluation of hepatoprotective activity of *Eriocaulon quinquangulare in vitro* using porcine liver slices against ethanol induced liver toxicity and free radical scavenging capacity. BMC Complement Altern Med. 2016; 16(74). https://doi.org/10.1186/s12906-016-1044-x
42. Das M, Basu S, Banerjee B, Sen A, Jana K, Datta G. Hepatoprotective effects of green *Capsicum annum* against ethanol induced oxidative stress, inflammation and apoptosis in rats. J Ethnopharmacol. 2018; 227:69-81. https://doi.org/10.1016/j.jep.2018.08.019