Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Protective effect of allicin and Omega-3 fatty acids against paracetamol-induced hepatic toxicity

Moamem Elsafty [1], Ahmed Abdeen [2], Mohamed Aboubakr[1*]

[1]*Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*

[2]*Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*

ARTICLE INFO

Keywords

Paracetamol
Liver
Omega-3 fatty acids
Allicin
Damage

Received 06/08/2022
Accepted 31/08/2022
Available On-Line: 09/10/2022

ABSTRACT

The most prominent over-the-counter antipyretic-analgesic drug is paracetamol (n-Acetyl-Para-Amino-Phenol, APAP). This study attempted to examine whether allicin (AC) and/or Omega-3 fatty acids (O3FA) could protect rats from the liver damage induced by APAP. Seventy rats were randomly distributed into seven groups (n=10): Control (saline), AC group received allicin (10 mg/kg, PO), O3FA group given omega-3 (100 mg/kg, PO), APAP group given paracetamol (1000 mg/kg, PO single dose on the 27th day), AC+APAP group received AC (10mg/kg) for 30 days and then given single dose of paracetamol on the 27th day (1000 mg/kg, PO), O3FA+APAP group received O3FA (100 mg/kg) for 30 days and then given single dose of paracetamol on the 27th day (1000 mg/kg, PO), and AC+O3FA+APAP group received O3FA (100 mg/kg) and AC (10 mg/kg) for 30 days and then given single dose of paracetamol on the 27th day (1000 mg/kg, PO). APAP had a significant negative impact on haematological and serum biochemical markers suggested that hepatic injury occurred in response to the APAP exposure. Histopathological examination of liver sections confirmed this hepatic damage where hepatic denegeration and necrosis were evident after APAP treatment. Allicin and/or omega 3 fatty acids treatment restore hepatic tissue architecture after treatment. Thus, pre-treatment with AC and O3FA alone or in combination was effective to reduce hepatic injury in APAP-intoxicated rats.

1. INTRODUCTION

Liver is the most vital organ in the body because it performs numerous biological functions during the metabolism of substances such as drugs, carbohydrates, proteins, and lipids (Islam et al., 2021). The primary causes of APAP-induced hepatotoxicity are hepatic cytochrome P-450, which produce N-acetyl-p-benzoquinoneimine (NAPQI) (Wang et al., 2017). NAPQI levels rise after an APAP overdose, which lowers cellular glutathione levels (GSH), alters mitochondrial proteins, causes mitochondrial oxidative stress, and ultimately leads to a high amount of reactive oxygen species (ROS) as superoxide anions (Ebada, 2018). Several studies recorded the hepatic toxicity of paracetamol (Jaeschke et al., 2020; Sinaga et al., 2021; Elshal and Abdelmageed, 2022).

More and more people are using herbal medications made from plant extracts that have natural antioxidant and pharmacological properties to counteract the potentially toxic effects of chemical agents like paracetamol. (Wu et al., 2017).

The predominant biologically active component derived from garlic is allicin, which is a diallylthiosulfinate (Wang et al., 2015). Alliinase catalyses the transformation of S-allylcysteine sulfoxide into diallylthiosulfinate or allicin once garlic has been damaged (Borlinghaus et al., 2014). A thiosulfinate compounds called allicin has a wide range of pharmacological and biological effects, including, antimicrobial (Reiter et al., 2017), hepatoprotective (Yang et al., 2017), nephroprotective (Abdel-Daim et al., 2019), and antioxidant effects (Saleh et al., 2021).

In addition to scavenging oxygen free radicals and hydroxyl radicals, the natural antioxidant allicin also inhibits the liver homogenates' lipids from oxidizing due to hydroxyl radical-induced lipid peroxidation (Chung et al., 2013). Allicin has been demonstrated to have hepatoprotective effects against paracetamol-induced hepatic damage (Samra et al., 2020) by inhibiting apoptosis, lowering the inflammasome pathway and reducing oxidative stress. Consequently, allicin may be a cutting-edge strategy in order to resist the development of APAP-caused hepatotoxicity (Samra et al., 2020).

Long-chain fatty acids called omega-3 polyunsaturated fatty acids are distinguished by the presence of a double bond at the third carbon atom of the hydrocarboxylic chain counted from the methyl end (Calder, 2018). The highest sources of docosahexanoic acid (DHA) and eicosatetranoic acid (EPA) are fish (particularly oily fish) and other seafood, despite the fact that these very long chain fatty acids can be found in a wide variety of foods (Scorletti and Byrne, 2018).

The DHA and EPA that are biochemically active downstream fatty acids from omega-3 fatty acids, are metabolised to anti-inflammatory and proresolving mediators (Calder, 2012). They have a number of different hypothesised modes of action, but the most important ones would include regulating cell proliferation, controlling fatty acid metabolism, preventing lipogenesis, and lowering inflammation and oxidative stress. (Wang et al., 2017). Therefore, the current study attempts to elucidate the

* Corresponding author: Mohamed Aboubakr, Department of Pharmacology, Faculty of Veterinary Medicine, Benha University – Moshtohor, Kalyobiya 13736, Egypt. E mail: mohamed.aboubakr@fvtm.bu.edu.eg

preventive role of AC and/or O3FA in preventing liver damage caused by APAP.

2. MATERIAL AND METHODS

2.1. Chemicals:
Paracetamol (APAP, 1 g) was bought as Panadol® from GlaxoSmithKline Pharmaceuticals Company (Brentford, United Kingdom). Allicin, was bought as pure powder (35% Conc.) from Delta Vet Center (Cairo, Egypt). Omega-3 fatty acids, was bought as pure fish oil (Conc.100%) from Sigma Pharmaceutical Industries (Cairo, Egypt). The used kits were bought from Bio-diagnostic Company (Giza, Egypt).

2.2. Experimental animals:
Seventy male albino Wister rats, 2 months age weighing 160-200 gm were obtained from the Center of Laboratory Animals, Faculty of Veterinary Medicine, Benha University, Egypt. Prior to the experiment, the rats were left for acclimatization for 14 days (temperature 25°C) and were fed ideal laboratory commercial diet and water ad libitum. Ethical approval from Animals Care and Use Committee Research Ethics Board was obtained from Faculty of Veterinary Medicine, Benha University (BUFVTM 07–03-22).

2.3. Experimental design:
Rats were divided into 7 equal groups (10 rats in each group). Group 1 (Control); had been given distilled water. Group (2); AC (10 mg/kg b.wt, orally). Group (3); O3FA (100 mg/kg b.wt, orally). These doses of AC according to Samra et al. (2021) and for O3FA (El-Gendy et al. 2021). Group (4); APAP toxic control group that received saline, orally once daily and a single dose of APAP 1 g/kg b.wt orally on the 27th day of the experiment. Group (5); (AC+APAP). Group (6); (O3FA+APAP). Group (7); (AC+O3FA+APAP). rats in these groups have been received allicin, omega-3 and APAP as described before. Saline, allicin, and omega-3 were administered for 30 days.

2.4. Sampling:
Rats were euthanized at 31st day of the experiment, blood samples were collected from Retro-bulbar venous plexus. The liver tissues were taken out for histopathological investigation.

2.5. Hematological analysis:
The whole blood samples were used directly after collection on EDTA for estimation of hematological parameters including the red blood cells (RBCs) count, hemoglobin (Hb) concentration, white blood cells (WBCs) count, hematocrit value (PCV%) and platelets (Plt) count. These parameters were estimated using automated hematology analyzer (Mindray BC-2800, China).

2.6. Serum biochemical analysis:
The biochemical markers were AST and ALT (Reitman and Frankel, 1957), ALP (Tietz et al. 1983), triglycerides (TG) and cholesterol (Shah et al. 2011), albumin (Doumas et al. 1971) and total protein (Doumas and Biggs, 1975). The previous biochemical tests were evaluated in accordance with data protocol provided by using commercial kits (Bio-Diagnostic Company, Giza, Egypt).

2.7. Histopathological alteration:
The liver tissue from each rat was fixed quickly in 10% neutral-buffered formalin for histopathology. The liver were progressively dehydrated, embedded in paraffin, cut into 5-µm sections, and stained with the hematoxylin and eosin (H&E) for histological inspection according to the method described by Bancroft and Gamble (2008). Finally, light microscopy was used to examine liver tissue sections (Leica, Germany).

2.8. Statistical analysis:
Statistical analysis was carried out using SPSS (Version 20; SPSS Inc., Chicago, USA). The significant divergence through multiple groups comparisons were analyzed by one-way ANOVA and Duncan test as a post hoc test was used. All values are expressed as mean ± SE, with significance considered at $P \leq 0.05$.

3. RESULTS

3.1. Hematological examination:
Figure 1 depicts the haematological analysis results. When compared to the control group, exposure to APAP significantly reduced the values of RBC counts, Hb concentrations, Plateletes counts (PL), and packed cell volumes (PCV), while increasing the values of WBC counts. Allicin and/or omega3 administration reduced the harmful effects of APAP by reversing these changes in haematological parameters to the values observed in control rats.

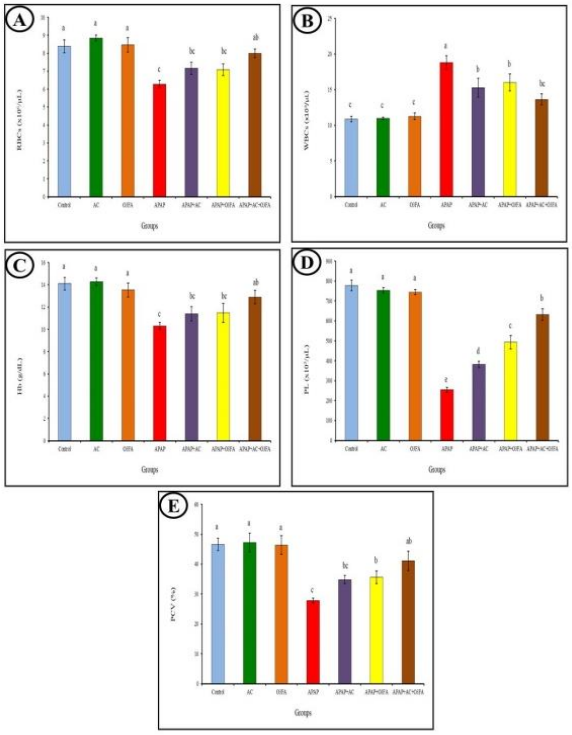

Figure (1) Effect of allicin and/or omega3 and paracetamol on hematological parameters

3.2. Biochemical analysis:
The increased serum levels of the liver biomarkers showed the induction of hepatotoxicity (Figure 2). When compared to the control rats, the effects of APAP toxicity significantly elevated the AST, ALT, and ALP activities as well as the cholesterol and triglycerides levels. Also, APAP decreases

the concentrations of total protein and albumin levels in serum.Contrarily, the case is different where these parameters were significantly decreased in the APAP treated-rats with AC, O3FA, or combination treatment (AC and O3FA) in comparison with the APAP group. When APAP-intoxicated rats were treated with both AC and O3FA, these parameters were restored almost to normal levels when compared to treatment with either AC or O3FA alone. Thus, combination of AC and O3FA showed better protection from liver damage caused by APAP than either alone.

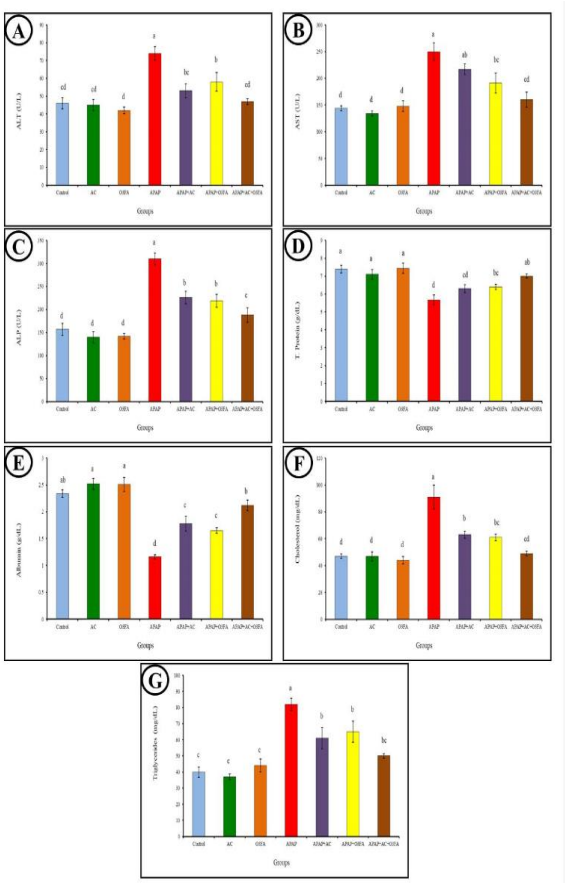

Figure (2) Effect of allicin and/or omega3 on hepatic damage induced by paracetamol indicated by liver biomarkers including, ALT, AST, ALP, total protein, albumin, cholesterol, and triglycerides.

3.3. Histopathological changes of liver:

Liver sections from control, AC and O3FA treated rats exhibited normal hepatic histo-architecture. Hepatocytes organized in cords radiating from central veins and separated by regular sinusoids (Figs. 3A, B, C). Otherwise, APAP intoxicated rats revealed several histological changes represented by severe congestion of the central veins and hydropic degeneration of the hepatocytes (Fig.3D), necrosis of some hepatocytes as well as inflammatory cells aggregation (Fig. 3E). Liver sections from paracetamol+allicin treated rats represented mild congestion in the central veins with few inflammatory cells infiltration (Fig. 4A). The examined liver of rats in paracetamol+O3FA group revealed few inflammatory cellular infiltrations in addition to congestion of blood sinusoids (Fig. 4B). While the liver in paracetamol +AC+O3FA group showed congestion of central veins and sinusoids with no inflammatory cellular infiltration (Fig. 4C).

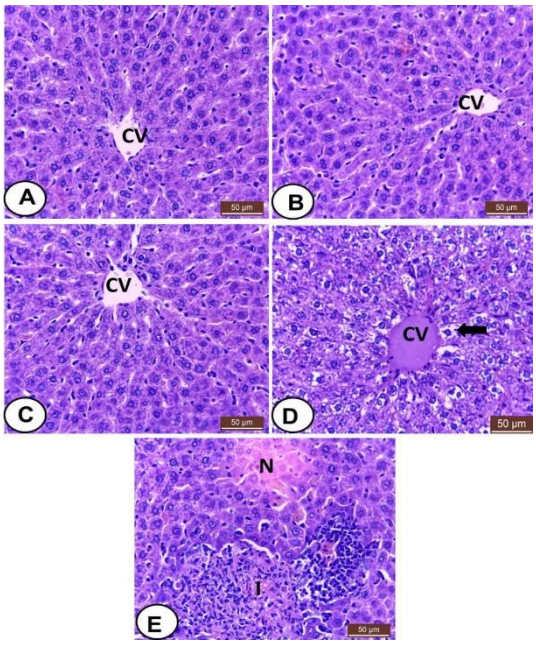

Figure (3) Histopathological sections of livers from control, allicin, omega 3 and paracetamol. A, B and C; Control, allicin and omega 3 groups showed normal hepatic histo-architecture. Hepatocytes organized in cords radiating from central vein (CV). D-E; Paracetamol intoxicated rats showed several histological changes. D; severe congestion of the central vein (CV) and hydropic degeneration of the hepatocytes (Thick arrow). E; inflammatory cells aggregation (I). H&E stain, scale bars=50µm.

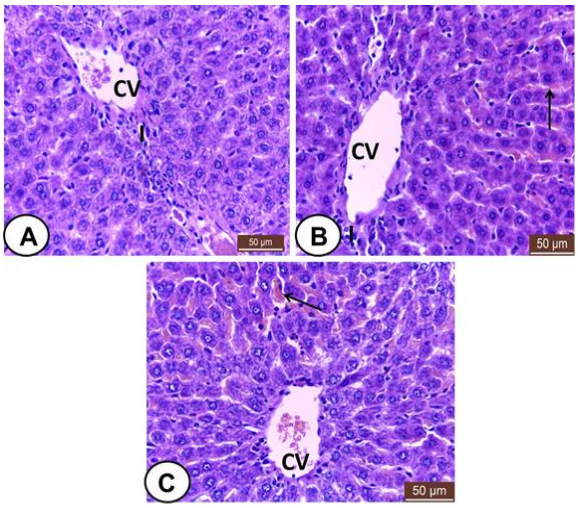

Figure (4) Histopathological sections of livers from the rest of paracetamol+allicin, paracetamol+omega 3, and paracetamol+allicin+omega 3 groups groups. A, B and C; respectively. A; showed mild congested central vein (CV) with some inflammatory cells infiltration (I). B; showed few inflammatory cells infiltration (I) in addition to congestion of blood sinusoids (thin arrow). C; showed congestion of central vein (CV) without inflammatory cells infiltration. H&E stain, scale bars=50µm.

4. DISCUSSION

The goal of the current study was to establish a scientific basis for the use of allicin and omega-3 combination therapy in conventional medicine by examining its protective effects against APAP-induced hepatotoxicity in Wistar rats. One of the most commonly used experimental models for assessing a drug's hepatoprotective capabilities is APAP-induced liver

damage. Indeed, APAP-treated rats showed higher serum levels of each of these indicators than did controls, which supported the existence of hepatic injury and demonstrated the model's viability.

Drug-induced hepatotoxicity is one of the leading causes of mortality in the world (Bhawna & Kumar, 2009). Because of its analgesic and antipyretic effects, APAP is used all over the world; nevertheless, if taken in excess, it can induce acute liver damage (Akhilraj et al., 2021).

Both APAP and its NAPQI, often referred to as N-acetyllimidoquimone, have harmful impact on the liver. NAPQI destroys liver cells directly and depletes the natural antioxidant glutathione in the liver, which results in liver failure (Akhilraj et al., 2021). Additionally, APAP plays a direct role in oxidative stress development, which results in lipid peroxidation, antioxidants depletion, and the reduction of ATP generation, all of which contribute to liver damage (Rabiul et al., 2011).

The current investigation showed that a single acute overdose of APAP led to a significant changes in some hematological and biochemical parameters. Oyedeji et al., (2013) stated that erythrocyte deformability is decreased and membrane permeability is increased in toxic APAP doses, which lowers erythrocyte survival. Our results recorded decreased values of RBCs, Hb, PCV, and PL counts in APAP-intoxicated rats. Therefore it was presumed that APAP increases the degradation rate of erythrocytes. The rise in WBCs seen in the APAP-treated rats is consistent with the result of Matić et al. (2021) and that may be an indicator to acute inflammation. The harmful effects were reduced by allicin and/or omega3 treatment, which returned changes in the haematological parameters to the recorded values in the control group.

Furthermore, APAP intoxication revealed significant elevations of serum ALT, AST, ALP, triglycerides (TG) and total cholesterol (TC). Both serum ALT and AST concentrations as a diagnostic for hepatic necrosis. Also, APAP caused reduction in serum levels of total protein and albumin. The reductive transfer of amino acids from alanine or aspartate, respectively, to alpha ketoglutarate to produce pyruvate or oxaloacetate, is carried out by both the ALT and AST enzymes. Hepatocytes that have been damaged discharge their contents, including ALT and AST, into the extracellular space (Islam et al., 2021).

The hepatic cells are harmed by the NAPQI which created by an excess intake of paracetamol through lipid peroxidation, which damages cellular permeability and raises blood levels of ALT and AST (Islam et al., 2021).

Furthermore, a rise of ALP serum level seen may be due to impaired hepatic excretion or enhanced ALP production by hepatic parenchymal or duct cells in the presence of rising biliary pressure. (Iyanda and Adeniyi, 2011). The lipoprotein metabolism appears to be compromised by APAP overdose (Kobashigawa and Kasiske, 1997) resulting in a change in the metabolism and level of triglycerides and cholesterol in intoxicated group in comparison with control groups. The reduction in total protein and albumin levels seen in APAP-treated rats suggests the destruction of many hepatic cells, which may result in a decrease in hepatic capacity to synthesis protein as most plasma proteins are synthesized by hepatocytes (Chaphalkar et al., 2017).

Freshly crushed garlic's main active compound is allicin. Anti-inflammatory and antioxidant activities have been documented for it (Shang et al., 2019). In addition to scavenging oxygen free radicals and hydroxyl radicals, the natural antioxidant allicin also stops the liver homogenates' lipids from oxidising due to hydroxyl radical-induced lipid peroxidation (Zhang et al., 2012). According to the results of the current investigation, dietary allicin can reduce the toxicity of APAP to some extent.

Since cytosolic aminotransferases and ALP seep into the blood after exposure to chemicals, including medicines and hazardous compounds, they are utilised as a marker for hepatocellular membrane damage (Al-Brakati et al., 2019). The APAP-treated group showed a considerable increase in these indicators. Serum liver functions, including ALT, AST, ALP, TG, and cholesterol, were significantly reduced. Additionally, allicin elevated total protein and albumin level when compared to toxic group. Remarkably, the elevated serum liver function markers that occurred after APAP administration were decreased by allicin supplementation. These findings suggest that allicin protects the liver from damage caused by APAP exposure by maintaining the hepatocyte membrane's shape and integrity.

Nowadays, daily intakes of omega-3 long-chain polyunsaturated fatty acids (Omega-3 PUFAs) are advised due to their anti-inflammatory and antioxidant properties (El-Gendy et al., 2021). The most significant of their various hypothesised modes of action could be be attributed to regulating cell proliferation, controlling fatty acid metabolism, preventing lipogenesis, as well as reducing oxidative stress and inflammation (Huang et al., 2015). Numerous studies have demonstrated the hepatoprotective effects of omega-3 fatty acids (Adeyemi and Olayaki, 2017; Eraky and Abo El-Magd, 2020).

A considerable increase in blood ALT, AST, ALP activities, TG and cholesterol concentrations and significant reduction in total protein and albumin levels compared with normal group, which indicates cell damage with cell membrane breakdown, leading to cellular leakage and hepatic dysfunction, was conducted to prove the APAP-induced hepatocellular injury while administration of O3FA fatty acids altered these liver markers almostly to normal and prevented hepatic impairment.

Additionally, the results of histopathological examination of the liver confirmed the serum biochemical findings which have been reported in other studies and showed that APAP hepatic damages tissue by causing oxidative damage (Saritas et al., 2014; Uysal et al., 2016). The liver tissue was arranged in lobules according to the histological report of the normal control group. Significant and widespread necrosis with degenerative changes, central veins dilatation, were seen in the paracetamol group and these result compatible with previous study (Akhilraj et al., 2021). While the treated rats with allicin or omega 3 represented mild congested central veins with some inflammatory cells infiltration in addition to congestion of blood sinusoids in the combined treated rats, the examined liver showed congestion of central vein and sinusoids without inflammatory cells infiltration.

5. CONCLUSION

Pretreatment with allicin and/or omega-3 fatty acids had better protective effects on APAP-induced liver injury than either one alone. Therefore, combining allicin with omega-3 therapy could be a unique way to slow the progression of APAP-induced hepatotoxicity.

6. REFERENCES

1. Abdel-Daim MM, Abushouk AI, Donia T, Alarifi S, Alkahtani S, Aleya L, Bungau SG (2019). The

nephroprotective effects of allicin and ascorbic acid against cisplatin-induced toxicity in rats. Environ Sci Pollut Res Int; 26(13):13502-13509.
2. Adeyemi WJ, Olayaki LA (2017). Diclofenac - induced hepatotoxicity: Low dose of omega-3 fatty acids have more protective effects. Toxicol Rep.16;5:90-95.
3. Akhilraj AR, Bhat S, Priyalatha B, Vimala KS (2021). Comparative hepatoprotective activity of detoxified roots of Plumbago zeylanica L. and Plumbago rosea L. in Wistar rats. J Ayurveda Integr Med;12(3):452-457.
4. Al-Brakati AY, Fouda MS, Tharwat AM, Elmahallawy EK, Kassab RB, Abdel Moneim AE(2019). The protective efficacy of soursop fruit extract against hepatic injury associated with acetaminophen exposure is mediated through antioxidant, anti-inflammatory, and anti-apoptotic activities. Environ Sci Pollut Res Int;26(13):13539-13550.
5. Attah AI, Eneji EG, Hogan IE (2020). Effects of Carica Papaya Seeds on Acetaminophen-Induced Hepatotoxicity in Male Wistar Rats. Trop J Nat Prod Res.; 4(8):463-470.
6. Bancroft JD, Gamble M (2008) Theory and Practice of Histological Techniques. 6th Edition, Churchill Livingstone, Elsevier, China.
7. Beutler E, Duron, O, Kelly, M.B. (1963). Improved method for the determination of blood GSH . I.Lab Clin Med., 61: 883-887.
8. Bhawna S, Kumar SU (2009). Hepatoprotective activity of some indigenous plants. Int J Pharm Tech Res, 4, 1330-1334.
9. Borlinghaus J, Albrecht F, Gruhlke MC, Nwachukwu ID, Slusarenko AJ (2014). Allicin: chemistry and biological properties. Molecules. 19;19(8):12591-12618.
10. Calder PC (2012). Mechanisms of action of (n-3) fatty acids. J Nutr;142(3):592S-599S.
11. Calder PC (2018). Very long-chain n-3 fatty acids and human health: fact, fiction and the future. Proc Nutr Soc;77(1):52-72.
12. Chaphalkar R, Apte KG, Talekar Y, Ojha SK, Nandave M (2017). Antioxidants of Phyllanthus emblica L. Bark Extract Provide Hepatoprotection against Ethanol-Induced Hepatic Damage: A Comparison with Silymarin. Oxid Med Cell Longev;2017:3876040. doi: 10.1155/2017/3876040.
13. Chung ML, Lee KY, Lee CY (2013). Profiling of oxidized lipid products of marine fish under acute oxidative stress. Food Chem Toxicol; 53:205-213.
14. Doumas B, Biggs H (1975) Colourimetric determination of total protein in serum or plasma. Clin. Chem 21(8): 1159-1166.
15. Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31(1): 87-96.
16. Ebada ME (2018). Essential oils of green cumin and chamomile partially protect against acute acetaminophen hepatotoxicity in rats. An Acad Bras Cienc;90(2 suppl 1):2347-2358.
17. El-Gendy ZA, El-Batran SA, Youssef S, Ramadan A, Hotaby WE, Bakeer RM, Ahmed RF (2021). Hepatoprotective effect of Omega-3 PUFAs against acute paracetamol-induced hepatic injury confirmed by FTIR. Hum Exp Toxicol;40(3):526-537.
18. Elshal M, Abdelmageed ME (2022). Diacerein counteracts acetaminophen-induced hepatotoxicity in mice via targeting NLRP3/caspase-1/IL-1β and IL-4/MCP-1 signaling pathways. Arch Pharm Res;45(3):142-158.
19. Eraky SM, Abo El-Magd NF (2020). Omega-3 fatty acids protect against acetaminophen-induced hepatic and renal toxicity in rats through HO-1-Nrf2-BACH1 pathway. Arch Biochem Biophys.15;687:108387. doi: 10.1016/j.abb.2020.108387.
20. Huang W, Wang B, Li X, Kang JX (2015). Endogenously elevated n-3 polyunsaturated fatty acids alleviate acute ethanol-induced liver steatosis. Biofactors;41(6):453-462.
21. Islam MT, Quispe C, Islam MA, Ali ES, Saha S, Asha UH, Mondal M, Razis AFA, Sunusi U, Kamal RM, Kumar M, Sharifi-Rad J (2021). Effects of nerol on paracetamol-induced liver damage in Wistar albino rats. Biomed Pharmacother; 140:111732. doi: 10.1016/j.biopha.2021.111732.
22. Iyanda AA, Adeniyi FA (2011). Biochemical and histologic presentations of female Wistar rats administered with different doses of paracetamol/ methionine. Niger J Physiol Sci. 20;26 (2):151-160.
23. Jaeschke H, Akakpo JY, Umbaugh DS, Ramachandran A (2020). Novel Therapeutic Approaches Against Acetaminophen-induced Liver Injury and Acute Liver Failure. Toxicol Sci. 1;174(2):159-167.
24. Kobashigawa JA, Kasiske BL (1997). Hyperlipidemia in solid organ transplantation. Transplantation. Feb 15;63(3):331-338.
25. Matić MM, Paunović MG, Milošević MD, Ognjanović BI, Saičić ZS(2021). Hematoprotective effects and antioxidant properties of β-glucan and vitamin C against acetaminophen-induced toxicity: an experimental study in rats. Drug Chem Toxicol;44(3):302-309.
26. Mihara, M. and Uchiyama, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituic acid test. Anal. Biochem., 86:271-278.
27. Oyedeji, K. O., Bolarinwa, A. F., & Ojeniran, S. S. (2013). Effect of paracetamol (acetaminophen) on haematological and reproductive parameters in male albino rats. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), 4(6), 1-6.
28. Rabiul, Haque, Mondal Subhasish, Sarmita Sinha, Modhurupa Ghose Roy, Devlina Sinha, and Sunita Gupta (2011). "Hepatoprotective activity of Clerodendron inerme against paracetamol induced hepatic injury in rats for pharmaceutical product." International Journal of Drug Development and Research 3(1): 118-126.
29. Reiter J, Levina N, van der Linden M, Gruhlke M, Martin C, Slusarenko AJ (2017). Diallylthiosulfinate (Allicin), a Volatile Antimicrobial from Garlic (Allium sativum), Kills Human Lung Pathogenic Bacteria, Including MDR Strains, as a Vapor. Molecules. 12;22(10):1711.
30. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28(1): 56-63.
31. Saleh DO, Mansour DF, Fayez AM (2021). Thioacetamide-induced acute hepatic encephalopathy: central vs peripheral effect of Allicin. Metab Brain Dis.36(6):1331-1340.
32. Samra YA, Hamed MF, El-Sheakh AR (2020). Hepato-protective effect of allicin against acetaminophen-

induced liver injury: Role of inflammasome pathway, apoptosis, and liver regeneration. J Biochem Mol Toxicol.34(5): e22470. doi: 10.1002/jbt.22470.
33. Saritas A, Kandis H, Baltaci D, Yildirim U, Kaya H, Karakus A, Colakoglu S, Memisogullari R, Kara IH (2014). N-Acetyl cysteine and erdosteine treatment in acetaminophen-induced liver damage. Toxicol Ind Health.30(7):670-678.
34. Scorletti E, Byrne CD (2018). Omega-3 fatty acids and non-alcoholic fatty liver disease: Evidence of efficacy and mechanism of action. Mol Aspects Med. 64:135-146.
35. Shah SS, Shah GB, Singh SD, Gohil PV, Chauhan K (2011) Effect of piperine in the regulation of obesity-induced dyslipidemia in high-fat diet rats. Indian J Pharmacol 43(3): 296-299.
36. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, Mavumengwana V, Li HB (2019). Bioactive Compounds and Biological Functions of Garlic (Allium sativum L.). Foods. 8(7):246. doi: 10.3390/ foods8070246.
37. Sinaga E, Fitrayadi A, Asrori A, Rahayu SE, Suprihatin S, Prasasty VD (2021). Hepatoprotective effect of Pandanus odoratissimus seed extracts on paracetamol-induced rats. Pharm Biol.59(1):31-39.
38. Tietz NW, Burtis CA, Duncan P, Ervin K., Petitclerc CJ, Rinker AD, Shuey D, Zygowicz ER (1983) A reference method for measurement of alkaline phosphatase activity in human serum. Clin. Chem. 29: 751–761.
39. Wang ET, Chen DY, Liu HY, Yan HY, Yuan Y (2015). Protective effect of allicin against glycidamide-induced toxicity in male and female mice. Gen Physiol Biophys. 34(2):177-187.
40. Wang M, Zhang X, Ma LJ, Feng RB, Yan C, Su H, He C, Kang JX, Liu B, Wan JB (2017). Omega-3 polyunsaturated fatty acids ameliorate ethanol-induced adipose hyperlipolysis: A mechanism for hepato-protective effect against alcoholic liver disease. Biochim Biophys Acta Mol Basis Dis.1863(12):3190-3201.
41. Wang X, Wu Q, Liu A, Anadón A, Rodríguez JL, Martínez-Larrañaga MR, Yuan Z, Martínez MA (2017). Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and in vitro. Drug Metab Rev. 49(4):395-437.
42. Wu H, Zhang G, Huang L, Pang H, Zhang N, Chen Y, Wang G (2017). Hepatoprotective Effect of Polyphenol-Enriched Fraction from Folium Microcos on Oxidative Stress and Apoptosis in Acetaminophen-Induced Liver Injury in Mice. Oxid Med Cell Longev. 2017:3631565. doi: 10.1155/2017/3631565.
43. Yang D, Lv Z, Zhang H, Liu B, Jiang H, Tan X, Lu J, Baiyun R, Zhang Z (2017). Activation of the Nrf2 Signaling Pathway Involving KLF9 Plays a Critical Role in Allicin Resisting Against Arsenic Trioxide-Induced Hepatotoxicity in Rats. Biol Trace Elem Res. 176(1):192-200.
44. Zhang L, Zhang H, Miao Y, Wu S, Ye H, Yuan Y (2012). Protective effect of allicin against acrylamide-induced hepatocyte damage in vitro and in vivo. Food Chem Toxicol. 50(9):3306-3312.