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Alleviating Effects of Honey in Albino Wistar Rats with Hematological and Hepatic Variations Provoked by Methotrexate

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ABSTRACT

Background: Methotrexate is a chemotherapeutic drug with toxic consequences to the liver. Honey has been documented to have healing properties. This study aimed to explore the alleviating effects of Honey in rats with Hematological and Hepatic variations provoked by Methotrexate.

Methods: This experimental research was conducted at Baqai Medical University for one year. 45 male albino Wistar rats were randomly divided into three groups. Control Group A with no intervention, treatment Group B with a single dose of Methotrexate of 20 mg/kg intraperitoneally and protected Group C with 10g/kg honey by gastric gavage for 7 days and 20 mg/kg of Methotrexate on the 4th day. At the end of the experiment, blood and liver samples were collected for liver enzymes and microscopic investigation. SPSS v25.0 was used for statistical analysis, with values as Mean ± SD, ANOVA for group comparisons, post hoc Tukey's test, and $p<0.05$ considered significant at a 95% confidence interval.

Results: The ultimate body weight of Group B was reduced (162±2.58); whereas the relative and absolute weight of the liver increased (9.54±1.42) and (5.89±0.91) respectively. The levels of ALT (51.27±15.35), AST (103.41±2.68), ALP (353.64±2.54) and albumin (4.19±5.36) in the serum notably increased, while Group C showed significant improvement. Sections from Group A's liver appeared to have normal parenchyma whereas it was altered in Group B, however Group C showed improvement. The hepatocyte counts per reticule decreased (7.18±1.36) significantly in Group B, while hepatocyte diameter increased (17.6±1.51) and nuclear diameter decreased (5.29±1.41). In contrast, all measurements showed improvement in Group C.

Conclusion: This study indicated that Honey was able to alleviate Methotrexate induced hematological and hepatotoxic alterations.

Keywords: Methotrexate, Hepatotoxicity, Honey, Morphometry, Liver Enzymes.

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INTRODUCTION

Methotrexate (MTX) is a commonly used antifolate drug. Originally it was used as an anticancer medication; numerous cancers like prostate carcinoma, breast carcinoma, head and neck tumors, bladder cancers and leukemia were treated by it. While initially it was given in high dosage in numerous neoplastic conditions, however its use is now prevalent in lower dosage for prolonged durations for immunosuppression and anti-inflammatory treatments in diseases such as rheumatoid arthritis, systemic lupus erythematosus, and psoriasis[1]. Because of its intolerable side effects, 30% of cancer patients withdraw from the treatment with MTX[2]. In cancer cells it reversibly inhibits dihydrofolate reductase enzyme which leads to the disruption of the biosynthesis of nucleotides, in turn hindering DNA synthesis. This brings about a fall in cellular pathways essential for tumor advancement. This decline also affects other promptly splitting typical body's cells, for instance the cells present in the bone marrow and hepatocytes, producing critical hematopoietic suppression as well as hepatotoxicity, which becomes a prominent reason for the cessation of chemotherapy[1]. An inflammatory reaction commences in the hepatocytes resulting in a disarray of hepatic architecture when MTX is administered which further leads to the decline of antioxidant enzymes, precipitating oxidative injury and oxidative degradation of lipids in liver tissue[3,4].

Liver is the basic metabolic organ that metabolizes virtually all drugs that enter the body through its chemically driven biotransformation processes[5]. This biotransformation causes some drugs to become inactive whereas transforming other drugs to intensified byproducts, thus making hepatocytes more prone to injury[6]. Liver injury induced by drugs is one of the leading causes in patients with acute liver failure which accounts for approximately 5% of cases that eventually leads to hospital admission[3,4]. The biotransformation that MTX goes through creates insoluble metabolites, which results in oxidative injury in hepatocytes[1].

Research has reported that the antioxidative influence of several chemicals and herbs can protect the liver tissue from injury induced by drugs[7]. Honey is a natural, ready-to-eat and an over enriched product, produced by honeybees. It is reported to have therapeutic properties[8]. It possesses substantial potential to mitigate oxidative stress and exhibits anti-metastatic, immune-modulatory, anti-inflammatory, anti-proliferative and pro-apoptotic effects[9,10]. In research, there has been documented liver-protective effects of honey[11,12].

It was hypothesized that honey could help lessen the toxic effects produced on the liver by MTX. The rationale for the study was the need to explore natural antioxidants that can mitigate the liver damage induced by this commonly used drug through oxidative stress. The aim of the study was to examine the hematologic and hepatic cytotoxicity caused by MTX and assess the potential protective effects of Honey in rat models through hematological and microscopic histological interpretation.

METHODS

This experimental study was carried out in the animal house of Baqai Medical University and the Anatomy Department of Jinnah Medical and Dental College. Ethical approval was granted by the Baqai Board of Advance Research and Studies (BMU:EC-2017/04). For the study, a 50 mg of locally manufactured MTX injection (Unitraxate®) and pure honey (authorized by Pakistan Council of Scientific and Industrial Research Laboratories, Karachi) was acquired.

Forty-five, 10-12 weeks old male Wistar Albino rats were selected in this study, through random sampling. They weighed between 180 and 200 grams. The rats which were diseased and underweight were eliminated. They were accommodated in environmentally controlled clear plastic cages. They received laboratory pellet diet and limitless access to water. Prior to experimentation, they were habituated for 15 days. Keeping in terms with the ethical standards of Pakistan, all the animals were well taken care of accordingly.

Rats were randomly grouped as A, B and C, 15 rats in each. Group A was the control group that received no intervention. Group B, was the treatment group, received MTX on the 4th day of study at the dose of 20 mg/kg intraperitoneally. Group C was the protected group which was given 10 g/kg honey via gastric gavage for 7 days alongside 20 mg/kg dose of MTX through intraperitoneal route on day 4.

Animals received oral dose of honey after overnight starvation. For concurrent dosing of honey and MTX, honey was administered orally first and after an hour, MTX was given through an insulin needle. Weights were recorded daily. On the study's eighth day, animals were anesthetized, dissected with a midline incision and their organs were uncovered.

In a 5cc syringe, blood sample was obtained through cardiac puncture and was later stored in Serum Separator Tube to analyze serum albumin and the liver enzymes biochemically; Alkaline Phosphatase (ALP), Alanine Transaminase or Serum Glutamate Pyruvate Transaminase (ALT/SGPT), Aspartate aminotransferase or Serum Glutamic-oxaloacetic Transaminase (AST/SGOT).

The liver was reaped, examined grossly and the

weight was measured. Afterwards it was washed using normal saline and preserved in 10% formalin. Later, it was run through a tissue processor and made into paraffin blocks. Using a microtome, sections of 5 micrometers in thickness were made. Histological slides of these sections were made with H&E staining. Light microscope was used to observe the tissue.

Micrometric observations of the diameter of a hepatocyte and its nucleus were manually recorded at 100X and 400X magnification, maneuvering a stage micrometer and an ocular micrometer scale. Hepatocyte count was determined with the help of an ocular reticule that consisted of 100 squares with an area of 625 µm2. Five random microscopic fields from each liver were selected and their average was documented.

SPSS version 25.0 was used to statistically analyze the data. The values were stated as mean and standard deviation (Mean ± SD). ANOVA was used to compare means among different groups, with post hoc Tukey's test to follow. A p-value of <0.05 was considered statistically significant at a 95% confidence interval.

RESULTS

Table 1: Statistics of Weight

Groups	Weight-Initial (gm) Mean ± SD	Weight-Final (gm) Mean ± SD	Absolute weight of Liver (gm) Mean ± SD	Relative weight of Liver (gm) Mean ± SD
A-Control	187.02 ± 2.59	201 ± 2.58	6.2 ± 1.50	3.03 ± 0.76
B-MTX Treated	203.83 ± 2.31	162 ± 2.58 [a]	9.54 ± 1.42 [a]	5.89 ± 0.91 [a]
C-MTX + Honey	198.98 ± 2.57	181 ± 1.49 [ab]	7.11 ± 0.67 [b]	3.95 ± 0.38 [ab]

a statistically significant (P<0.05) in comparison to group A
b statistically significant (P<0.05) in comparison to group B

Table 1 shows the final and initial body and organ of the different groups as Mean with SD. Group B treated with MTX, their final body weight declined significantly when compared with the controls. However, Group C treated with MTX + Honey, their final body weight was significantly more than the final weight of Group B. In both Group B and C, the liver weights (relative and absolute both) increased significantly in comparison to the control group whereas in the protected Group C a significant decrease was noticed when compared to the treatment Group B.

Table 2 Hematological Parameters of the Different Groups

Groups	Levels of ALT in Serum (µ/L) Mean ± SD	Levels of AST in Serum (µ/L) Mean ± SD	Levels of ALP in Serum (µ/L) Mean ± SD	Levels of Albumin in Serum (G/Dl) Mean ± SD
A-Control	33.7 ± 1.51	59.96 ± 2.47	190.71 ± 2.33	3.22 ± 0.49
B-MTX Treated	51.27 ± 15.35 [a]	103.41 ± 2.68 [a]	353.64 ± 2.54 [a]	4.19 ± 5.36
C-MTX + Honey	41.1 ± 1.46 [ab]	63.72 ± 1.18 [b]	205.6 ± 1.51 [ab]	3.65 ± 0.66

[a] statistically significant (P<0.05) in comparison to group A
[b] statistically significant (P<0.05) in comparison to group B

The mean values with standard deviation (SD) of biochemical parameters, including levels of ALP, ALT, AST, and albumin in the serum are given in Table 2. ALP, ALT, and AST in group B and C appeared to have a significant surge in their levels in contrast to the control whereas Group C's levels showed a decline when compared with group B. Serum albumin level exhibited a similar pattern but the difference between the groups was statistically insignificant **(Table 2)**.

Regular parenchyma was observed on the liver sections of the control group A. Central vein is seen in the middle indicating a typical hepatic lobule. Hepatic cords are radiating out from the central vein which have hepatocytes arranged alongside it. A portal triad can be seen at the periphery containing a hepatic artery, portal vein and a bile duct. Narrow blood sinusoids lined with epithelium are present between the hepatic chords. Round vesicular nuclei with acidophilic cytoplasm around it are present in the polyhedral hepatocytes **(Figure 1)**.

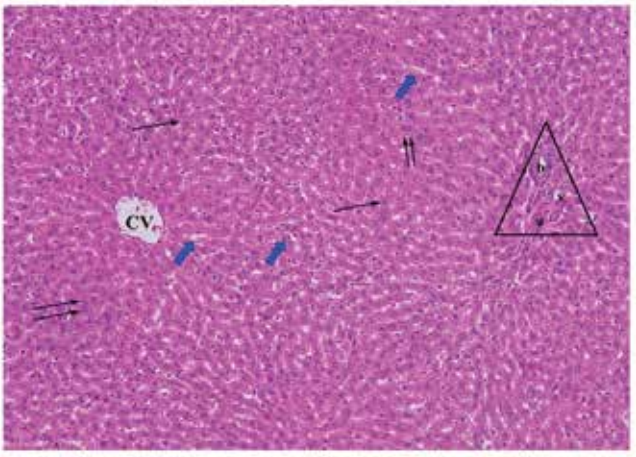

Figure 1: Shows the control liver at 100X; Typical hepatic lobule with a portal triad (Δ) at the periphery which contains a hepatic artery (a), bile duct (b) and a portal vein (v). It also contains a central vein (CV) in the middle; Endothelium lined Sinusoids (Blue arrow); Polyhedral Hepatocytes with vesicular nuclei and acidophilic cytoplasm (single arrow), some are binucleated (double arrow).

The Group B, MTX treated group's liver section revealed altered liver morphology with disarrangement of typical hepatic lobules. Disorganization of the hepatocytes' radial cords was noticed. Zones were showing degeneration, hemorrhage, and necrosis. The sinusoids and the central vein were enlarged but showing congestion, with lymphocytic infiltration seen around the central vein. Hydropic ballooning degeneration was exhibited by the hepatocytes while nuclear changes included fragmentation and pyknosis. The portal triad showed evidence of infiltration of mononuclear cells present within and around it (Figure 2 & 3)

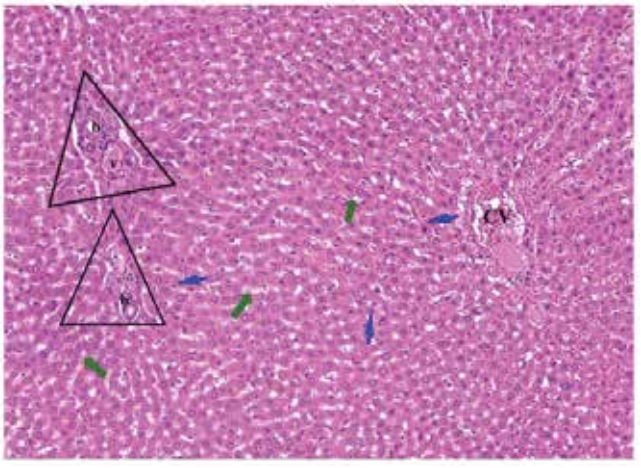

Figure 2. Liver treated with MTX shown at 100X; Dilated sinusoids with hemorrhage (green arrows) and inflammatory cellular infiltration seen in the dilated central vein (CV); altered portal triad (Δ); Hepatocytes with hydropic ballooning in the cytoplasm and nucleus with pyknosis (blue arrow).

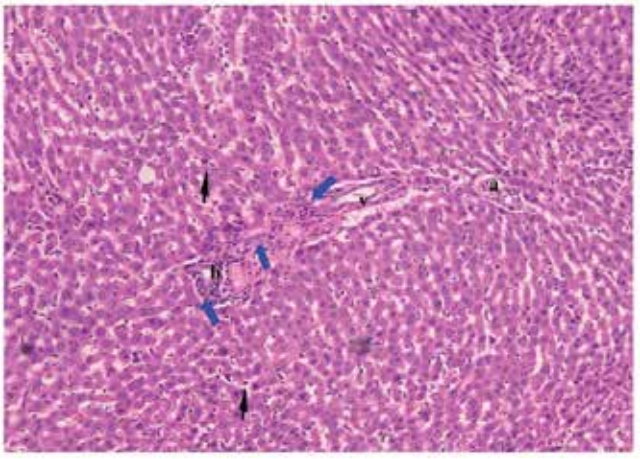

Figure 3. showing liver treated with MTX at 200X; Parenchyma seen with an influx of inflammatory cell inside the portal and periportal zones (blue arrows); Hepatocytes with a nucleus showing fragmentation and pyknosis with cytoplasmic hydropic (black arrow).

In the group treated with MTX and honey which was the protected Group C, the sections of the liver with morphological alterations showed improvement. The hepatocyte cords projecting from the central vein displayed a usual pattern. The sinusoids showed no dilation whereas the portal veins and central veins were only scarcely dilated. The periportal region displayed mild lymphocytic infiltration (Figure 4).

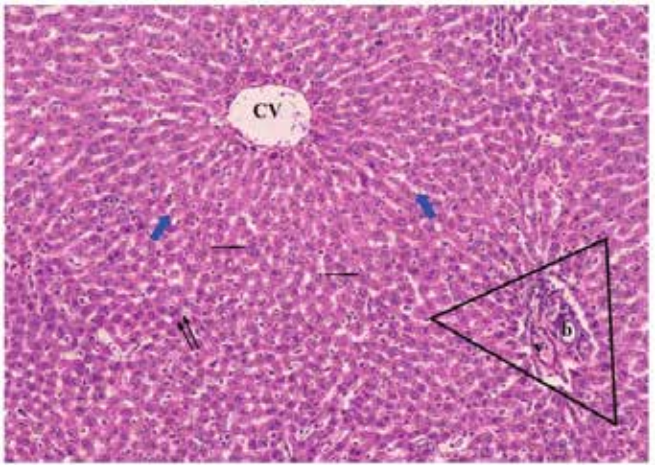

Figure 4. showing liver protected with honey at 200X; liver sinusoids (blue arrows) and moderately enlarged central vein (CV); Binucleated hepatocytes with vesicular nucleus (one arrow) and normal cytoplasm (two arrows).

Table 3. The Hepatic Morphometry

Groups	Hepatocyte Count Per reticule Mean ± SD	Hepatocyte Diameter (µM) Mean ± SD	Nuclear Diameters (µM) Mean ± SD
A-Control	16.12 ± 1.51	13.34 ± 0.66	7.28 ± 1.51
B-MTX Treated	7.18 ± 1.36 [a]	17.6 ± 1.51 [a]	5.29 ± 1.41 [a]
C-MTX + Honey	13.81± 0.64 [ab]	15.15 ± 0.7 [ab]	6.7 ± 0.61 [b]

[a] statistically significant (P<0.05) in comparison to group A

[b] statistically significant (P<0.05) in comparison to group B

Table 3 shows the Mean with SD of the Morphological indices which are the diameter of the nucleus, diameter of the hepatocyte and the hepatocyte count per reticule. Group B and Group C in comparison to the control showed a significantly reduced hepatocyte per reticule count whereas it significantly grew in Group C when compared to Group B. Diameter of the hepatocyte enlarged significantly in Group B and C than that of Group A. However, the diameters of the hepatocytes of Group C, in contrast to Group B, showed significant reduction. The diameter of the nucleus of both Group B and Group C in comparison to Group A showed significant reduction but enlargement was noticed when Group C was compared to Group B.

DISCUSSION

This study illustrated substantial evidence of honey's protective effects against the hematological and hepatotoxic alterations induced by MTX in rat model. MTX is used as a treatment for neoplasms and long-term inflammatory conditions; often showed grave side effects including impaired hematopoiesis, toxic lung injury and hepatic impairment[13,14]. Prevalence of liver damage in patients can be as high as 50% with MTX treatment[15].

The present study determined that treatment with MTX led to a reduction in overall body weight, while causing an increase in liver weight[16]. Previous studies have stated the same decreased body weights when compared with control group[16,17]. Secondary to MTX administration, decreased food intake, gastrointestinal toxicity and diarrhea explains the results best[14]. Another study saw a similarl increase in absolute and relative weights of the liver[18]. In our study, the addition of honey to MTX led to a notable increase in the weights of rats' body and liver weight as compared to the ones given MTX alone. These findings were well supported by a study that documented honey's protective role on body weight[12]. A study reinforced these results as pure honey has nutrient rich elements, like bioactive phenols and carbohydrates[10].

The MTX treatment of rats, in this study, resulted in a significant elevation of the liver enzyme levels i.e. serum ALT, AST, ALP whereas the albumin levels in the serum between Group B and C remained negligible. Conclusions from another study were found to be aligned with these findings, with a reported increase in oxidative stress markers[18]. A disruption in the cell membrane is noticed with the elevated activity of these liver enzymes, resulting in hepatocellular injury[19]. Hoque et al. supported these findings who observed that long term use or high dosage of MTX led to hepatocellular toxicity with significant increase in serum liver enzyme levels[20]. In the current study, improvement was observed across all enzymatic parameters when honey was co-administered with MTX. A study, previously conducted, also found that honey holds effective hepatoprotective properties against liver injury and oxidative stress exemplified by a significant decline in liver enzyme levels, predominantly of AST and ALP[21]. Improvement in the liver enzymes were also observed by Omar[12]. A deduction can be made that vital reason for the healing properties of honey is being enriched with both enzymatic and non-enzymatic antioxidants, can be because of its antioxidant function[8,10].

In this study, the biochemical changes in MTX treated rats were reinforced by obvious histopathological changes. As previously observed, this group displayed cellular and vascular deterioration with nuclear variations in the hepatocytes and inflammatory cell permeation18. It is suggested that MTX toxicity can be contributed by multiple processes, with inflammation prompted apoptosis caused by oxidative stress, being implied as a key causative factor[22]. The results of the morphometric analysis also showed injury to the liver tissue in the MTX-treated rats.

The decline in the hepatocyte counts per reticule, and hepatocyte and nuclear diameter changes can be explained by a climb in the number of toxic metabolites and the resultant damage to the cell membrane. Prior research has found comparable results particularly when MTX is administered chronically and in high doses in vivo[14]. Literature accredited that apoptosis transpired due to hepatotoxic inflammation and oxidative injury[23]. An increase in the oxidative degradation of lipids level and decrease in the level of key biomarkers; glutathione, glutathione-S-transferase and superoxide dismutase activities was confirmed [24]. In Group C, the signs of hepatotoxicity were significantly diminished with the incorporation of honey. Laaroussi et al. outlined similar findings with honey solely and concurrently with olive oil[25].

El Kutry demonstrated visibly improved MTX-induced intestinal toxicity with honey alone or in combination with other natural substances[26]. Evidently, honey exhibited enzymatic and microscopic hepatoprotective effects with carbon tetrachloride also[12]. Literature confirmed that in patients with breast cancer who were given Fluorouracil, Doxorubicin, and Cyclophosphamide treatment regimen showed correction in oxidative stress markers with the use of honey[27].

CONCLUSION

The present study investigated the protective effects of honey against the toxicity induced by MTX. It provides evidence that when administered with

honey the weights of the liver, functions of the liver enzymes and the morphological features of the liver at a microscopic level improved successfully. These consequences can be attributed to the antioxidative phenolic components of the honey as well as the other additional antioxidants present in it. Clinically, the incorporation of honey as an adjunct therapy may help alleviate chemotherapy induced side effects suffered by patients undergoing these anticancer treatments who often endure these complications with more duress more often than the symptoms caused by their primary disease itself. More research is still needed to explore and understand the mechanisms of honey supplementation at a molecular level and its beneficial effects in mitigating liver and other organ damage.

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CONFLICT OF INTEREST
None

FUNDING
None

ETHICAL APPROVAL
The study received ethical approval from the Ethical Review Committee of Baqai medical University, under reference number (BMU:EC-2017/04). Animals were taken care of according to Pakistan standards. https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf

AUTHORS' CONTRIBUTIONS
NY and **TK** conceptualized the study, designed the methodology, analyzed and interpreted data, and contributed to the manuscript. **TK** also collected data and performed histological examinations, while **NY** conducted histomorphological assessments. **MS** wrote the manuscript, analyzed, and tabulated data. **AA and ABA** contributed to data analysis, interpretation, and manuscript writing, with **ABA** also performing histological examinations. SM analyzed data, created tables, and contributed to writing, while **RN** critically proofread the manuscript.

REFERENCES
1. Ezhilarasan D. Hepatotoxic potentials of methotrexate: Understanding the possible toxicological molecular mechanisms. Toxicol. 2021 Jun;458:152840. doi:10.1016/j.tox.2021.152840
2. Hasan Khudhair D, Al-Gareeb AI, Al-Kuraishy HM, El-Kadem AH, Elekhnawy E, Negm WA, Saber S, Cavalu S, Tirla A, Alotaibi SS, Batiha GE. Combination of vitamin C and curcumin safeguards against methotrexate-induced acute liver injury in mice by synergistic antioxidant effects. Front Med. 2022 Apr;9:866343. doi:10.3389/fmed.2022.866343
3. Goudarzi M, Kalantar M, Sadeghi E, Karamallah MH, Kalantar H. Protective effects of apigenin on altered lipid peroxidation, inflammation, and antioxidant factors in methotrexate-induced hepatotoxicity. Naunyn Schmiedebergs Arch Pharmacol. 2021 Mar;394:523-531. doi:10.1007/s00210-020-01991-2
4. Ezhilarasan DJT. Hepatotoxic potentials of methotrexate: Understanding the possible toxicological molecular mechanisms. Toxicol. 2021 Jun;458:152840. doi:10.1016/j.tox.2021.152840
5. Orhan H, Karakuş F, Ergüç A. Mitochondrial biotransformation of drugs and other xenobiotics. Curr Drug Metab. 2021 Jul;22(8):657-669. doi:10.2174/1389200222666210628125020
6. Rai M, Paudel N, Sakhrie M, Gemmati D, Khan IA, Tisato V, Kanase A, Schulz A, Singh AV. Perspective on quantitative structure–toxicity relationship (QSTR) models to predict hepatic biotransformation of xenobiotics. Livers. 2023 Aug;3(3):448-462. doi:10.3390/livers3030032
7. Zhou Y, Wang J, Zhang D, Liu J, Wu Q, Chen J, Tan P, Xing B, Han Y, Zhang P, Xiao X. Mechanism of drug-induced liver injury and hepatoprotective effects of natural drugs. Chin Med. 2021 Dec;16(1):135. doi:10.1186/s13020-021-00543-x
8. Silva B, Biluca FC, Gonzaga LV, Fett R, Dalmarco EM, Caon T, Costa AC. In vitro anti-inflammatory properties of honey flavonoids: A review. Food Res Int. 2021 Mar;141:110086. doi:10.1016/j.foodres.2020.110086
9. Anastasiou IA, Eleftheriadou I, Tentolouris A, Samakidou G, Papanas N, Tentolouris N. Therapeutic properties of honey for the management of wounds; is there a role in the armamentarium of diabetic foot ulcer treatment? Results from in vitro and in vivo studies. Int J Low Extrem Wounds. 2021 Dec;20(4):291-299. doi:10.1177/15347346211026819
10. Afrin S, Haneefa SM, Fernandez-Cabezudo MJ, Giampieri F, Al-Ramadi BK, Battino M. Therapeutic and preventive properties of honey and its bioactive compounds in cancer: An evidence-based review. Nutr Res Rev. 2020 Jun;33(1):50-76. doi:10.1017/S0954422419000192
11. Akinpelu AT, Akinloye OM, Olubanjo FO, Busuyi KD, Fatoki JO, Stephen OA, Adewale OO. Ameliorative and Synergetic Effects of Co-Administration of Honey and Vernonia amygdalina (Bitter leaf) Extract on Lead-Induced Wistar Rats. Trop J Nat Prod Res. 2024 Jun;8(6). doi:10.26538/tjnpr/v8i6.14
12. Omar S. The Activity of Some Herbs with or Without Honey in Protecting the Liver against

Carbon Tetrachloride Hepatotoxicity in Female Albino Rats. Medicine. 2022 Sep;8(42):173-200. doi:10.21608/jedu.2022.108442.1534
13. Ghoneum M, El-Gerbed MS. Human placental extract ameliorates methotrexate-induced hepatotoxicity in rats via regulating antioxidative and anti-inflammatory responses. Cancer Chemother Pharmacol. 2021 Dec;88:961-971. doi:10.1007/s00280-021-04349-4
14. Hamed KM, Dighriri IM, Baomar AF, Alharthy BT, Alenazi FE, Alali GH, Alenazy RH, Alhumaidi NT, Alhulayfi DH, Alotaibi YB, Alhumaidan SS. Overview of methotrexate toxicity: A comprehensive literature review. Cureus. 2022 Sep;14(9). doi: 10.7759/cureus.29518
15. El-Sheikh AA, Morsy MA, Abdalla AM, Hamouda AH, Alhaider IA. Mechanisms of thymoquinone hepatorenal protection in methotrexate-induced toxicity in rats. Mediat Inflamm. 2015 May;2015(1):859383. doi:10.1155/2015/859383
16. Ilamkar S, Rathod P, Ingole R, Hajare S, Khodke M. Ameliorative effect of hydro-ethanolic extract of Tinospora cordifolia against methotrexate induced toxicity in mice. J Entom Zool Studies. 2020 Jan;8:505-510. https://www.entomoljournal.com/archives/2020/vol8issue1/PartI/7-4-317-206.pdf
17. Hassanein EH, Mohamed WR, Khalaf MM, Shalkami AG, Sayed AM, Hemeida RA. Diallyl disulfide ameliorates methotrexate-induced nephropathy in rats: Molecular studies and network pharmacology analysis. J Food Biochem. 2021 Jun;45(6):e13765. doi:10.1111/jfbc.13765
18. Abdul-Hamid M, Abdel-Reheim ES, Hegazy W, Allam A, Othman SI, Alwaele MA, Abdel-Kawi SH. Effect of gervital in attenuating hepatotoxicity caused by methotrexate or azathioprine in adult albino rats. Environ Sci Pollut Res Int. 2022 Feb;29(31):46788-46801. doi:10.1007/s11356-022-18903
19. Di Martino V, Verhoeven DW, Verhoeven F, Aubin F, Avouac J, Vuitton L, Lioté F, Thévenot T, Wendling D. Busting the myth of methotrexate chronic hepatotoxicity. Nat Rev Rheumatol. 2023 Feb;19(2):96-110. doi:10.1038/s41584-022-00883-4
20. Hoque MR, Razzaque MA, Zawad AN, Rahman SW. Status of Renal and Liver Function in Rheumatoid Arthritis (RA) Patients of Chattogram, Bangladesh Treated with Methotrexate (MTX). J Biosci Med. 2023 Aug;11(9):114-126. doi:10.4236/jbm.2023.119010.
21. Omidipour R, Zarei L, Boroujeni MB, Rajabzadeh A. Protective effect of thyme honey against valproic acid hepatotoxicity in Wistar rats. BioMed Res Int. 2021 Feb;2021(1):8839898. doi:10.1155/2021/8839898
22. Solomon DH, Glynn RJ, Karlson EW, Lu F, Corrigan C, Colls J, et al. Adverse effects of low-dose methotrexate: a randomized trial. Ann Intern Med. 2020 Mar;172(6):369-380. doi.org/10.7326/M19-3369
23. Dogra A, Gupta D, Bag S, Ahmed I, Bhatt S, Nehra E, Dhiman S, Kumar A, Singh G, Abdullah ST, Sangwan PL. Glabridin ameliorates methotrexate-induced liver injury via attenuation of oxidative stress, inflammation, and apoptosis. Life Sci. 2021 Aug;278:119583. doi:10.1016/j.lfs.2021.119583
24. Tufan E, Sivas GG, Gürel-Gökmen B, Yılmaz-Karaoğlu S, Dursun E, Çalışkan-Ak E, Muhan A, Özbeyli D, Şener G, Tunali-Akbay T. Whey protein concentrate ameliorates the methotrexate-induced liver and kidney damage. Br J Nutr. 2023 Nov;130(10):1704-1711. doi:10.1016/j.lfs.2021.119583
25. Laaroussi H, Bakour M, Ousaaid D, Ferreira-Santos P, Genisheva Z, El Ghouizi A, Aboulghazi A, Teixeira JA, Lyoussi B. Protective Effect of Honey and Propolis against Gentamicin-Induced Oxidative Stress and Hepatorenal Damages. Oxidative Med Cell Longev. 2021 Sep;2021(1):9719906. doi:10.1155/2021/9719906
26. El Kutry MS. Potential protection effect of using honey, ginger, and turmeric as a natural treatment against chemotherapy of intestinal toxicity. J Biol Active Prod Nature. 2020 Mar;10(2):86-99. doi:10.1080/22311866.2020.1745686
27. Sidabutar DFM, Budijitno S, Prasetyo A . The effectiveness of multiflora honey to prevent hepatotoxicity in invasive ductal breast cancer patients with FAC chemotherapy. Bali Med J. 2021 Dec;10(3):982-987. 7. doi:10.15562/bmj.v10i3.2660