

Investigation of immunomodulatory role of honey on cisplatin injected rats.

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Abstract:

The aim of the present study is to evaluate the role of honey as an immunomodulator. Rats given cisplatin injections as immune suppressor agent were used to study the effect of honey as an immunological modulator. Cisplatin treatment resulted in a reduction in WBCs and neutrophil counts, antibodies IgG and IgM, as well as Bcl2 gene expression levels an increase in IL-4 and IL-6 levels, BAX and iNOS gene expression levels, and notable histological alterations in spleen tissue sections, including the expansion of the red pulp with lymphocytic depletion and many variable-sized blood-filled voids. All study parameters are successfully improved by the co-administration of honey and cisplatin. The results obtained can be explained by the high concentration of bioactive compounds in honey, including phenolic acids and flavonoids. Therefore, honey can modulate the immune system. In conclusion, honey can mitigate the damaging effects that cisplatin has on the immune system.

Keywords: - Honey, CIS Platin, Immune system, GC-MS.

Introduction

An organism is protected from disease by a network of biological processes known as the immune system. The immune system distinguishes between cancer cells and healthy tissues of the organism to aid in the detection and elimination of a number of pathogens, including viruses, parasitic worms, and cancer cells. It comprises immune system diseases such as immune system malignancies, autoimmune disorders, allergy disorders, and immunodeficiency disorders [1].

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Research has led to the creation of new chemotherapeutic drugs, and cutting-edge therapeutic modalities like radiation, chemotherapy, stem cell and bone marrow transplantation have been successfully applied in clinical settings. Sadly, a large reduction in innate and/or acquired immunity is still a formidable foe for the bulk of these therapeutic options [2].

The chemotherapeutic medication cisplatin is used to treat a variety of human malignancies. Despite having an effective anti-cancer impact, it also has a number of undesirable side effects, such as immune system dysfunction, hepatotoxicity, ototoxicity, nephrotoxicity, and spermiotoxicity [3].

According to growing body of data [4], cisplatin-induced leukopenia is a major contributor to weakened immune function. Results of numerous experimental research confirmed that, consuming honey and other bee products in your diet has profoundly positive health effects against a variety of illnesses [5]. These products contain a significant amount of active ingredients such flavonoids, phenolic acid, phenolic compounds, terpenes, and enzymes, which have biological activities in the prevention of certain diseases and the promotion of good health [6].

Numerous studies have examined the benefits of honey in connection to modern medicine because it is thought to be a key component in traditional treatments. Honey has long used to treat patients with a variety of ailments in folk medicine [7]. Ancient Egyptians, Greeks, Romans, and Chinese all utilized it to treat intestinal wounds and illnesses. In addition, it has employed as a treatment for earaches, sore throats, and coughs [8]. The biological, physiological, and pharmacological effects of honey are widely recognized. The ability of honey to modify the immune system by creating an anti-inflammatory impact also has shown in numerous research [9, 6].

Therefore, the aim of this work was to study the role of honey as immune modulator on cisplatin injected rats.

Materials and methods

Preparation of honey: -

500 mg of honey were diluted with equal amount of distilled water and used to treat rats through rat mouth using an intragastric tube. The doses were measured on digital scales depending on the animal's weight because each gram of the experimental rat should receive 0.5 mg of honey [10].

Gas chromatography–mass spectrometry (GC–MS) analysis

The GC-MS analysis was conducted using a TRACE GC Ultra Gas Chromatograph according to [11]. Obtained mass spectra was compared with the Wiley spectral library collection, the NSIT library database, the AMDIS program (www.amdis.net), and mass spectral matching to genuine standards to identify the honey constituents.

Experimental animals

The Nile Company For Pharmaceuticals & Chemical Industries in Cairo, Egypt, provided 24 healthy male albino rats (180-200 g). Rats were kept in a well-ventilated space where they were also exposed to light (12:12 h of light and darkness). Animals were housed at a constant temperature of 25 C in metabolically tagged cages. The rats were given unlimited access to water and pellets to eat at their leisure.

Research Design

Healthy males were chosen at random and split into three groups, each with six rats.

1. The control group (CO): injected with saline only
2. The cisplatin group (PC): injected intraperitoneally with 7 mg/kg of cisplatin to induce leukopenia [12]
3. The cisplatin + Honey group (PC+H): cisplatin (7 mg/kg) was delivered intraperitoneally, and the next day, honey (500 mg/kg/day for 21 days) was given orally.

Blood and spleen tissue collection

Biological samples were collected from rats after they were slaughtered at the end of the experiment. Spleen tissue was separated and collected in preparation for weighing and dissection. The single spleen was then divided into three parts. The first section was preserved in 15% formaldehyde solution for use in histological analyses. The second fraction was preserved in triazole for use in gene expression analysis and stored in -80 °C. The third part was stored at -80°C for use in preparing tissue homogenate immediately before measuring the inflammatory markers.

Spleen tissue was isolated, dissected and weighed. Blood was collected for complete blood count (CBC) test and biochemical analyses. Spleen tissue was quickly removed and divided into three sections. The first section was stored in Trizol reagent for real-time gene expression analysis at -80 °C. The second section was immersed in 15% formaldehyde solution for pathological examination. The third section was used to prepare a tissue homogenate and was stored at -80 °C. For the biochemical analyses, the tissue homogenate was prepared immediately before starting the measurements. Blood samples were collected from each rat separately for CBC analysis.

Inflammatory markers analysis

Measurement of both IL-4 and IL-6 was conducted using tissue homogenate and following the manufacturer's instructions of (IL-4 Rat ELISA Catalog No.: MBS355442) and (IL-6 Rat ELISA Catalog No.: MBS355410) kits.

Immune parameters

Immunoglobulin G and Immunoglobulin M level was determined according to the procedure of (Rat IgG ELISA Kit Catalog No. LS-F24320) and (Rat IgM ELISA Kit Catalog No. LS-F8534).

Histological examinations

Samples of spleen tissue were prepared and fixed in 10% formalin, dried in a sequence of increasing alcohol concentrations, washed in xylol, and finally embedded with paraffin. An optical microscope (Optica light microscope (B- 350)) was used to section tissues at a thickness of 4-5 M, fix them, prepare them for H&E staining, and look for obvious cellular damage.

Gene Expression Analyses

Total RNA was isolated from both treated and untreated rats using Trizol reagent in accordance with the manufacturer's instructions. To obtain cDNA from 1 g of RNA, reverse transcriptase was employed. The expression level of the BAX, Bcl2, and iNOS genes were measured. Real-time PCR was conducted using an internal control, β -actin, as a standard. Table 1 illustrates sequence of the used primers, gene symbol and GenBank accession number. qPCR was conducted to measure the changes in gene expression level according to [13, 14].

Table 1: Sequence of the used primers, gene symbol and GenBank accession number

| Gene symbol | Forward | Reverse | Gene bank |
|----------------|-----------------------|-------------------------|-------------|
| Bax | CCTGAGCTGACCTTGGAGCA | GGTGGTTGCCCTTTTCTACT | U32098.1 |
| iNOS | CCAACCTGCAGGTCTTCGATG | GTCGATGCACAACCTGGGTGAAC | U26686.1 |
| Bcl2 | TGATAACCGGGAGATCGTGA | AAAGCACATCCAATAAAAAGC | NM_016993.1 |
| β -actin | TCCGTCGCCGGTCCACACCC | TCACCAACTGGGACGATATG | NM_031144.3 |

Statistical analysis

Statistical significant between means was assessed using t test via SPSS Version 20 software. The differences between the means are regarded as significant differences if the p-value is < 0.05.

Results

Gas chromatography–mass spectrometry (GC–MS) analysis

From the results of GC Mass analyzes of honey, it was possible to identify and define more than 16 compounds based on the Wiley spectral library collection and the NSIT library

database. Each compound was identified based on the retention times and % of peak area according to each compound's unique peak area in comparison to the sum of all peak areas in the GC-chromatogram (Table 2 and Figure 1).

The most prevalent compounds were HEXADECANOIC ACID, 9-OCTADECENYL ESTER, (Z)-, with a retention time of 39.92 min and a peak area of 10.54 %. and Eicosen-1-ol, cis-9- with a retention time of 40.10 min and a peak area of 60.10%.

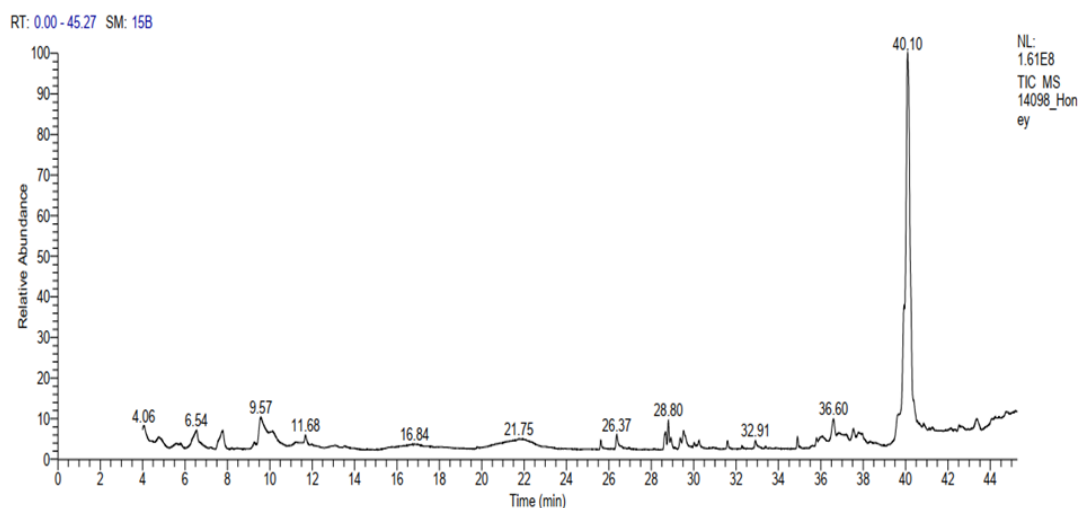
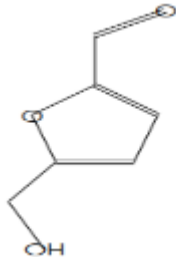







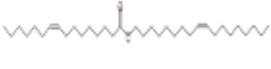




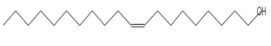


Figure 1 :- Chromatograms of honey compounds obtained from

Table 2: Chemical compounds identified by GC-MS analysis of honey

| peak | (RT) | Name of compound | Area (%) | Molecular formula | Molecular weight | Molecular structure |
|------|------|---|----------|---|------------------|---------------------|
| 1 | 6.55 | Clindamycin | 4.21 | C ₁₈ H ₃₃ ClN ₂ O ₅ S | 424 | |
| 2 | 7.78 | 2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE | 3.46 | C ₆ H ₈ O ₄ | 144 | |

| | | | | | | |
|----|-------|---|------|--|-----|---|
| 3 | 9.58 | 5-Hydroxymethylfurfural | 6.36 | C ₆ H ₆ O ₃ | 126 |  |
| 4 | 26.37 | HEXADECANOIC ACID | 1.27 | C ₁₆ H ₃₂ O ₂ | 256 |  |
| 5 | 28.68 | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- | 1.44 | C ₂₁ H ₃₆ O ₄ | 352 |  |
| 6 | 28.80 | 11-Octadecenoic acid, methyl ester | 1.63 | C ₁₉ H ₃₆ O ₂ | 296 |  |
| 7 | 28.93 | 16 OCTADECENOIC ACID, METHYL ESTER | 0.84 | C ₁₉ H ₃₆ O ₂ | 296 |  |
| 8 | 29.37 | Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester | 0.78 | C ₂₅ H ₄₂ O ₂ | 374 |  |
| 9 | 29.52 | 9-OCTADECENOIC ACID (Z)- | 2.45 | C ₁₈ H ₃₄ O ₂ | 282 |  |
| 10 | 34.90 | Z-8-Octadecen-1-ol acetate | 0.64 | C ₂₀ H ₃₈ O ₂ | 310 |  |

| | | | | | | |
|----|-------|--|-------|--|-----|---|
| 11 | 36.59 | 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- | 2.62 | C ₃₄ H ₆₄ O ₂ | 504 |  |
| 12 | 37.54 | Oleyl oleate | 1.23 | C ₃₆ H ₆₈ O ₂ | 532 |  |
| 13 | 39.64 | Ethanol, 2-(9-octadecenyl)-, (Z)- | 1.58 | C ₂₀ H ₄₀ O ₂ | 312 |  |
| 14 | 39.69 | cis-10-Nonadecenoic acid | 0.87 | C ₁₉ H ₃₆ O ₂ | 296 |  |
| 15 | 39.92 | HEXADECANOIC ACID, 9-OCTADECENYL ESTER, (Z)- | 10.54 | C ₃₄ H ₆₆ O ₂ | 506 |  |
| 16 | 40.10 | Eicosen-1-ol, cis-9- | 60.10 | C ₂₀ H ₄₀ O | 296 |  |

Effect of honey treatment on spleen weight

Spleen weight was measured, and the data obtained showed that treatment with cisplatin significantly reduces spleen weight in the (PC) group when compared to other groups. While using honey significantly increases spleen weight (Figure 2).

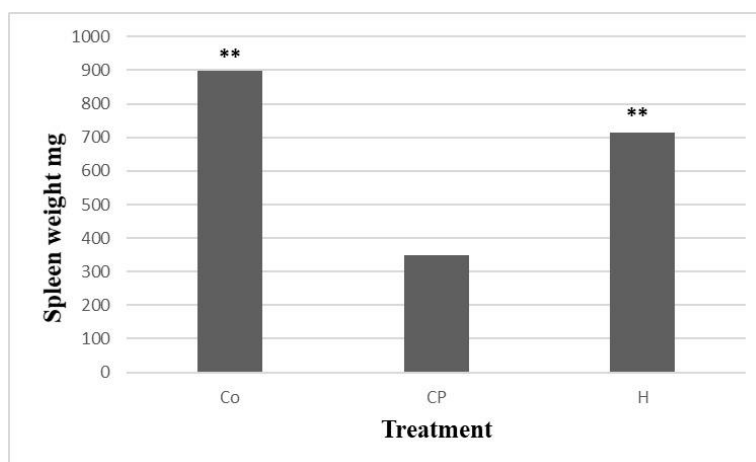


Figure 2: Effects of honey and cisplatin on weight of spleen

Effect of honey of WBCs and Neutrophils counts

CBC test was carried out to detect the effect of honey on WBCs and neutrophils counts. Obtained data showed that treatment with cisplatin significantly reduces WBCs and neutrophils counts (PC) group when compared to other groups. While using honey significantly increases both WBCs and neutrophils counts (Figures 3&4).

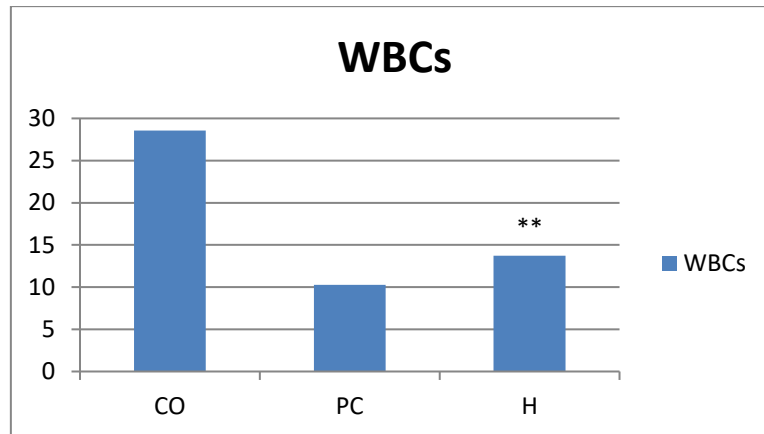


Figure 3:- Effect of honey and cisplatin on

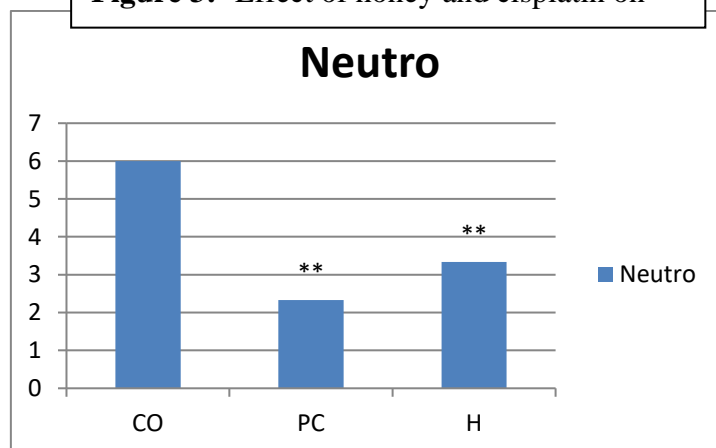


Figure 4:- Effect of honey and cisplatin on

Effect of honey treatment on inflammatory markers

Results in Figure 5 demonstrate the treatment with honey on IL-4 and IL-6. The cisplatin group (PC) group showed a significant increase ($p \leq 0.05$) in IL-4 and IL-6 levels compared to the control group (CO). In contrast, when the (PC) group was treated with honey the levels of IL-4 and IL-6 were markedly decreased ($p \leq 0.05$) compared to the PC group (figure 5)

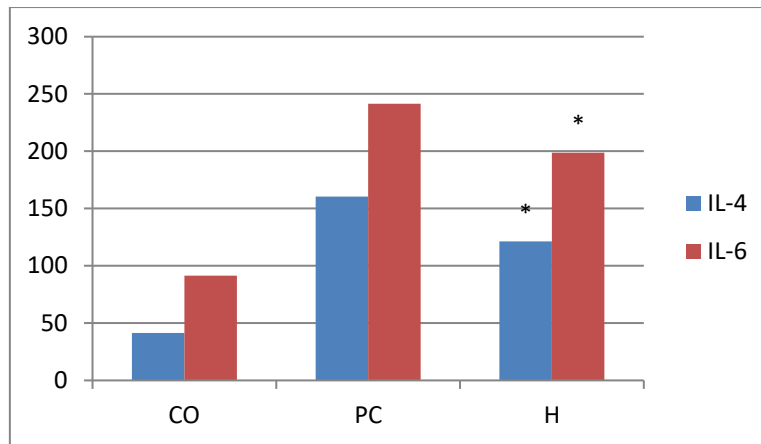


Figure 5:- Effect of honey and cisplatin on Interleukin 4, Interleukin 6 inflammatory markers

Effect of honey treatment on Immune parameters

Obtained data showed that treatment with cisplatin significantly reduces antibodies IgG and IgM in (PC) group when compared to control group. While using honey alone or a mixture significantly increases antibodies IgG and IgM (figures 6 & 7)

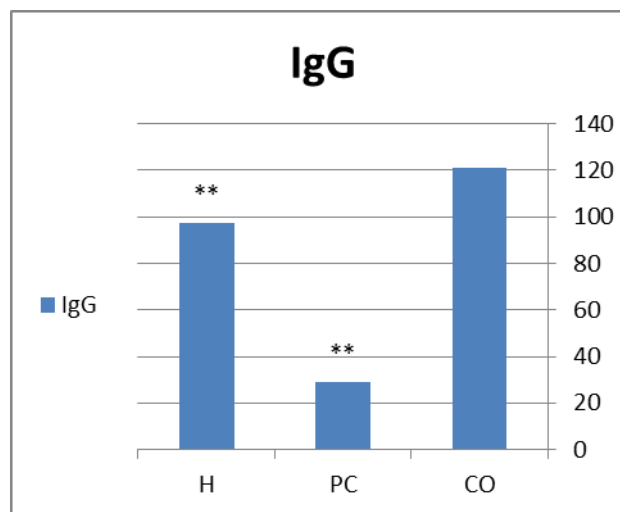


Figure 6 : Effect of honey and cisplatin on Immunoglobulin G

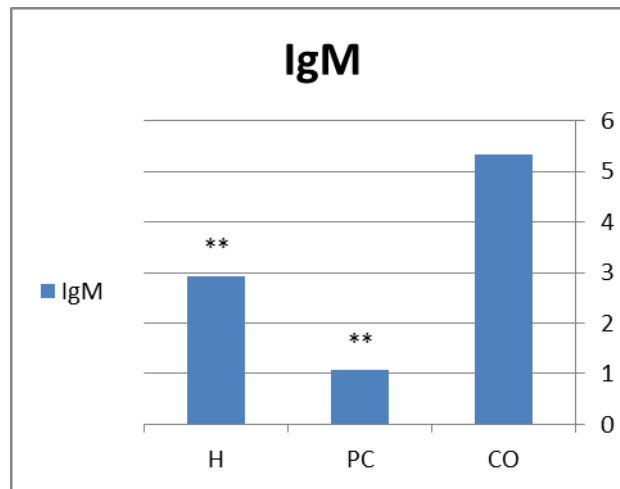


Figure 7: Effect of honey and cisplatin on

Histological analysis

Microscopic examination of splenic tissue from the CO group revealed normal histology of splenic parenchyma that appeared formed of white and red pulps; red pulp was represented by splenic cords and sinusoids while the splenic white pulp was consisted of three compartments; Periarterial lymphatic sheath (PALS), lymphatic follicles and the marginal zone (Figure 8). Concerning PC group (Figure 8), serious pathological alterations were detected in the affected splenic tissue. Expansion of splenic red pulps associated with atrophy of some white pulps were commonly observed that also displayed lymphoid depletion. Apoptosis was seen in affected lymphoid follicles and characterized by shrinkage of lymphocytes, nuclear chromatin condensation, and fragmentation of apoptotic cells into membrane-bound bodies (apoptotic bodies) that were engulfed by macrophages giving the characteristic tingible body macrophages. Variable number of megakaryocytes were detected among the splenic parenchyma. Angiectasis was noticed in several examined section that characterized by existence of variable-size blood-filled spaces occupied the splenic parenchyma and lined by delicate to inapparent endothelium. Partial protection was observed in H group (Figure 8) that showed numerous variable sized white pulps with apparently normal lymphoid follicles. Atrophied lymphoid follicles were limited in few examined sections that were accompanied by expanded red pulps. Focal hemorrhagic area and angiectasis were observed in splenic parenchyma in some examined sections. apparently normal lymphoid follicles. Atrophied lymphoid follicles were limited in few examined sections that were accompanied by expanded red pulps. Focal hemorrhagic area and angiectasis were observed in splenic parenchyma in some examined sections.

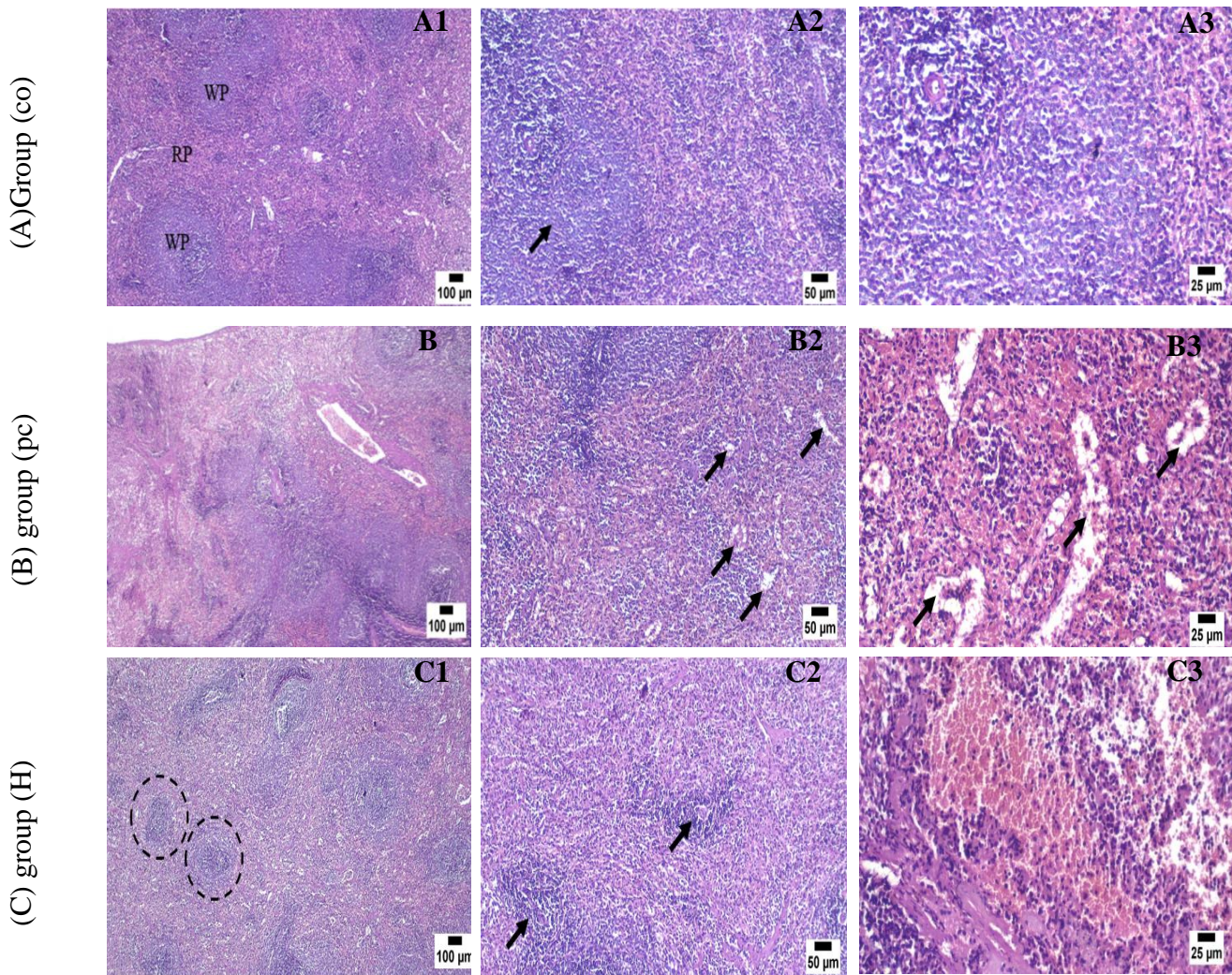


Figure 8:- (A1) Spleen of rat, CO group showing normal histological structure of splenic white pulp (WP) and red pulp (RP) (H&E). (A2) Spleen of rat, CO group higher magnification showing normal lymphoid tissue (white pulp) with germinal center (arrow) (H&E). (A3) Spleen of rat, CO group higher magnification showing normal lymphocytes of white pulp (H&E). (B1) Spleen of rat, PC group expansion of the red pulp with lymphocytic depletion (H&E). (B2) Spleen of rat, PC group showing multiple variable sized blood-filled spaces (arrows) (H&E). (B3) Spleen of rat, PC group showing higher magnification of multiple variable sized blood-filled spaces (arrows) (H&E). (C1) Spleen of rat, H group showing atrophy of some lymphoid

Gene expression analysis

Cisplatin treatment causes significant differences in gene expression level of BAX, BCL2 and iNOS genes in comparison with other two groups. Whereas, honey treatment modulate the effect of cisplatin in all studied groups. Cisplatin induce upregulation of both BAX and iNOS gene and downregulate the Bcl2 gene. However, obtained results indicated that honey modulate such effect of cisplatin treatment throughout downregulation of both BAX and iNOS genes as well as upregulation of Bcl2 gene (Figure 9).

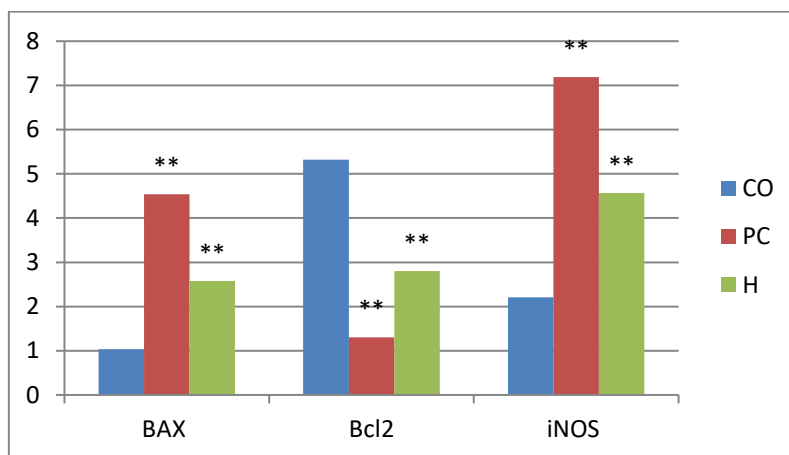


Figure 9:- Effects of honey and cisplatin on gene expression of BAX, BCL2 and iNOS genes

Over many ages, honey has had a significant influence on alternative medicine. In addition to its abilities to heal wounds, operate as an anti-microbial, and have antioxidant properties, numerous lines of evidence have highlighted the usefulness of honey and related bioactive chemicals as anti-tumor agents against a range of cancer types. A few in vitro and in vivo investigations have demonstrated that honey can influence the immune system. The present work aimed to delve into studying and investigate the mechanism of the role of honey as an immune modulator on several biochemical, histological and molecular levels. Treatment with cisplatin led to decrease in spleen weight, reduction of WBCs count, neutrophils count, elevate of the IL-4 and IL-6, histological changes in spleen tissue and changes in mRNA level of BAX, Bcl2 and iNOS genes. Moreover, honey treatment of cisplatin-injected rats led to improving the immune system, as demonstrated by analyses of the effect of honey on spleen weight, WBCs, neutrophils, IL-4, IL-6 and gene expression of BAX, BCL2 and iNOS genes. Additionally, honey stopped the histological alterations caused by CISP that were seen in the rat spleen tissue. These findings showed that honey alters the immunological response in cisplatin-induced immune-suppressed rats.

This can be explained by the observation of [15] that Cisplatin works as a representative redox cyclor, resulting in direct injury to multiple organs and considerable weight loss. Similar to the conclusions of [16, 4, 17]. Several *in vivo*, *in silico*, *in vitro*, and clinical research have suggested that honey plays a part in weight management. [17, 18, 19, 20, 21]. Male honey-fed rats had larger pancreatic and caecum weights. The intestinal bacteria in the caecum and the pancreas' enzymatic activity may work more efficiently during digestion as a result of the weight gain of the organs. In addition, rats fed honey displayed improved intestinal villi growth and no disease in their abdominal viscera, suggesting that honey may have nutritional benefits [22]. Aflatoxins treatment decreased body weight in mice, while treatment with honey increased body weight in mice injected with aflatoxins and honey for 90 days [23]. Honey also prevents the CISP-induced histological alterations seen in the rat spleen. [24] showed a protective effect of royal jelly and bee honey against CISP-induced nephrotoxicity in cancer patients treated with CISP, which is consistent with our findings. Royal jelly and honey also provided protection for rats against CISP-induced nephrotoxicity [25, 26].

In other studies, the modulatory properties of honey have been examined using pre-clinical cancer models. The histopathological grade was improved when Malaysian Tualang honey (TH) or MH were given orally to Sprague-Dawley rats after tumors were palpable [27]. Manuka honey also lessens the histological alterations in the kidneys and liver that are induced by CISP. The histological alterations in the liver caused by CISP were lessened by acacia honey [28]. The results obtained indicated that honey has an anti-inflammatory role and this was shown by reducing the levels of interleukin 4 and 6 after treatment with honey in rat PC group. Previous studies indicate that such an anti-inflammatory role can be attributed to bioactive compounds found in honey, such as phenolic and flavonoids contents honey [6]. The potential health advantages of honey against inflammation were supported by both *in vivo* and *in vitro* research on the effect of honey various types [29, 30, 31, 32, 33].

The basic components of the immune response are immunoglobulins (Igs) or antibodies (Abs) [34]. In another study, CY injection decreased IgG and IgM levels in serum [35], whereas honey therapy increased antibody production (IgG, IgM) [36]. This is consistent with what we found. Another study found that giving coriander honey to Ehrlich ascites carcinoma mice increased IgG and IgM levels [37].

The regulation of CISP-induced apoptosis involves both the pro-apoptotic and anti-apoptotic molecules BAX and the Bcl-2 [38]. In the present study, CISP upregulate the expression level of BAX and iNOS and downregulate Bcl2 expression level in spleen. [28] reported that consuming of MAN and TALH honey reduced BAX and caspase-3 expression while

increasing Bcl-2 expression in the both liver and kidney tissue . They explained these results as, the attachment of BAX to the mitochondrial membrane resulted in the release of chemicals such as cytochrome C, a caspase Smac and Omi activator, and other compounds into the cytosol. Both activate caspase-9 and caspase-3, which both have the ability to cleave a wide range of protein substrates and trigger apoptosis. Both also activate the procaspase-9 initiator. The Bcl-2 proto-oncogene encodes the protein Bcl-2, which is one of the molecular members of the Bcl-2 family of cell survival factors and one of the components regulating apoptotic pathways [39].

According to [40], Bcl-2 prevents apoptosis by blocking the release of cytochrome C from the mitochondria. Nitric oxide (NO), which is produced by the enzyme inducible nitric oxide synthase (iNOS) from l-arginine, is a crucial mediator of immunological activation and inflammation, which is present in a significant number of human disorders. Numerous illnesses have been linked to overexpressed or dysregulated iNOS [41]. The level of iNOS gene expression, which had increased as a result of cisplatin injection, was decreased as a result of honey treatment. According to the findings of [42], Gelam honey has a dose-dependent effect on reducing edema in inflamed rat paws. which in turn suppresses the production of iNOS in the paw tissue. Such positive effects of honey can be attributed or explained through the results obtained from the chemical analysis of honey by GC-MAS analysis. It was found that there are chemical compounds that are most prevalent in honey (HEXADECANOIC ACID, 9-OCTADECENYL ESTER, (Z)- and Eicosen-1-ol, cis-9-). According to research [43], n-Hexadecanoic acid has anti-inflammatory, antioxidant, hypocholesterolemic, and cancer-prevention properties. According to [44], 9-octadecenoic acid methyl ester, (E)- has the highest score (0.401 kcal/mol) against urate oxidase receptor. A study conducted by [45] demonstrated the antioxidant efficacy of 9-octadecenoic acid methyl ester and 9-hexadecenoic acid methyl ester. The immune system is stimulated by (Z)-11-eicosenol, methyl cis-11-eicosenoate, and cis-11-eicosenoic acid [46].

Conclusion & Recommendations

The results obtained from this study support the role of honey as an immune modulator. This is through the bioactive compounds honey contains that have an anti-inflammatory role and reduce oxidative stress. This is explained by honey's ability to restore improvement in spleen histology and counts of white blood cells and neutrophils. In addition to the inflammatory markers interleukins 4 and 6, as well as the gene expression of Bax, BCL2, and iNOS genes. Despite these encouraging findings, more investigation is required to expand upon the present findings and determine the precise pathways by which honey triggers its immunomodulatory action.

References

- [1] **Sompayra L.** "How the Immune System Works" 7th Edition by Wiley-Blackwell , 2022, 176 Pages.
- [2] **Nurgali K., Jagoe R. T. and Abalo R.** "Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae?" Front. in Pharm., 2018, 9, 1-3
- [3] **Dasari S., Njiki S., Mbemi A., Yedjou C. G. and Tchounwou P. B.** "Pharmacological Effects of Cisplatin Combination with Natural Products in Cancer Chemotherapy" International Journal of Molecular Sciences, 2022, 23(3), 1532.
- [4] **Shruthi S., Vijayalaxmi K. K., and Shenoy K. B.** "Immunomodulatory effects of gallic acid against cyclophosphamide-and cisplatin-induced immunosuppression in swiss albino mice" Ind. J. of Pharm. Sci., 2018, 80(1), 150– 160.
- [5] **Terzoa S., Mulè F., Amato A.** "Honey and obesity-related dysfunctions: a summary on health benefits" J. of Nut. Biochem., 2020, 82 , 108401
- [6] **Masad R. J., Haneefa S. M., Mohamed Y. A., Al-Sbiei A., Bashir G., Fernandez-Cabezudo M. J. and al-Ramadi B. K.** "The Immunomodulatory Effects of Honey and Associated Flavonoids in Cancer" Nut., 2021, 13(4), 1269.
- [7] **Samarghandian S., Farkhondeh T., Samini F.** "Honey and Health: A Review of Recent Clinical Research" Pharma. Res. 2017, 9(2), 121–127.
- [8] **Rao P. V., Krishnan K. T., Salleh N., and Gan S. H.** "Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review" Rev. Bra. de Farma., 2016, (26)5, 657–664.
- [9] **Liu, J.R., Ye, Y.L., Lin, T.Y., Wang, Y.W. and Peng, C.C.** "Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan" Food Chem., 2013, 139, 938–943.
- [10] **Waykar, and Alqadhi,** "protective role of honey and royal jelly on cisplatin induced oxidative stress in liver of rat" I. J.P. S. R., 2019, 10(8), 3898-3904
- [11] **Salam, H.S., Tawfik, M.M., Elnagar, M.R., Mohammed, H.A., Zarka, M.A., Awad, N.S.** "Potential Apoptotic Activities of *Hylocereus undatus* Peel and Pulp Extracts in MCF-7 and Caco-2 Cancer Cell Lines" Plants, 2022, 11(17), 2192. <https://doi.org/10.3390/plants11172192>.
- [12] **Waykar, B. and Alqadhi, Y. A.** "Administration of Honey and Royal Jelly Ameliorate Cisplatin Induced Changes in Liver and Kidney Function in Rat" Biomed. & Pharma. J., 2018, 11(4), 2191-2199.

- [13] **Wang, L., Liu, X., Chen, H., Chen, Z., Weng, X., Qiu, T. and Liu, L.** "Effect of picroside II on apoptosis induced by renal ischemia/reperfusion injury in rats" *Exp. Ther. Med.*, **2015**, 9, 817–822.
- [14] **Fujii, A., Okuyama, T., Wakame, K., Okumura, T., Ikeya, Y. and Nishizawa, M.** "Identification of anti-inflammatory constituents in Phellodendri Cortex and Coptidis Rhizoma by monitoring the suppression of nitric oxide production" *J. of Nat. Med.* **2017**, 71, 745–756.
- [15] **Chirino, Y. and Pedraza-Chaverri, J.** Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. *Exp. and Tox. Path.*, **2009**, 61(3), 223–242.
- [16] **Park, H., Kim, S. H., Yee, S. T., Byun, M. W. and Jo, S. K.** "Effect of a herb mixture (HIM-I) on the protection of the hematopoietic-immune system and self-renewal tissues against radiation damage" *J. of the Kor. Soc. of Food Sci. and Nut.*, **2005**, 34(5), 605–612.
- [17] **Kim, S., Kwon, D., Lee, H., Kim, H. and Kim, W.** "Preventive Effect of the Herbal Preparation, HemoHIM, on Cisplatin-Induced Immune Suppression" *Evi.-Bas. Com. and Alt. Med.*, **2019**, 3494806, <https://doi.org/10.1155/2019/3494806>.
- [18] **Samat, S., Nor, N. A. M., Nor Hussein, F. and Ismail W. I. W.** "Effects of gelam and Acacia honey acute administration on some biochemical parameters of Sprague Dawley rats" *BMC Com.and Alter. Med.*, **2014**, 14(146), 1-8.
- [19] **Nurmasitoh, T. and Pramaningtyas, M. D.** "Honey improves lipid profile of diet-induced hypercholesterolemic rats" *Uni. Med.*, **2016**, 34(3),177-186.
- [20] **Samat, S., Salleh, M. A. M., Adam, Z. and Wan Ismail, W. I.** "Pineapple honey inhibits adipocytes proliferation and reduces lipid droplet accumulation in 3T3-L1 adipocytes" *Malaysian Applied Biology*, **2019**, 48(1), 21-26.
- [21] **Lori, G., Cecchi, L., Mulinacci, N., Melani, F., Caselli, A., Cirri, P., Pazzagli, L., Luti, S., Mazzoli, L. and Paoli, P.** "Honey extracts inhibit PTP1B, upregulate insulin receptor expression, and enhance glucose uptake in human HepG2 cells" *Bio. & Pharma.*, **2019**, 113, 108752.
- [22] **Ajibola, A., Chamunorwa, J. P. and Erlwanger, K.H.** "Comparative effect of cane syrup and natural honey on abdominal viscera of growing male and female rats" *Ind. J. Exp. Bio.*, **2013**, 51(4), 303-312.
- [23] **Yaman, T., Yener, Z. and Celik, I.** "Histopathological and biochemical investigations of protective role of honey in rats with experimental aflatoxicosis" *BMC Com. and Alter.Med.*, **2016**, 16, 232.
-

- [24] **Osama, H., Abdullah, A., Gamal, B., Emad, D., Sayed, D., Hussein, E., Mahfouz, E., Tharwat, J., Sayed, S., Medhat, S., Bahaa, T. & Abdelrahim, M. E. A.** "Effect of Honey and Royal Jelly against Cisplatin-Induced Nephrotoxicity in Patients with Cancer" *J. of the Am. Coll. of Nut.*, **2017**, 36(5), 342-346.
- [25] **Silici, S., Ekmekcioglu, O., Kanbur, M. and Deniz, K.** "The protective effect of royal jelly against cisplatin-induced renal oxidative stress in rats. *World Journal of Urology*, **2011**, 29(1), 127–132.
- [26] **Mohamed, H. K., Mobasher, M. A., Ebiya, R. A., Hassen, M. T., Hagag, H. M., El-Sayed, R., Abdel-Ghany, S., Said, M. M. and Awad, N. S.** "Anti-Inflammatory, Anti-Apoptotic, and Antioxidant Roles of Honey, Royal Jelly, and Propolis in Suppressing Nephrotoxicity Induced by Doxorubicin in Male Albino Rats" *Antioxidants*, **2022**, 11(5), 1029.
- [27] **Khalid, A., Tan, J. & Yong, Y.** "Malaysian Tualang Honey and Its Potential Anti-Cancer Properties: A Review" *Sains Malaysiana*, **2018**, 47(11), 2705–2711.
<http://dx.doi.org/10.17576/jsm-2018-4711-14> .
- [28] **Neamatallah, T., El-Shitany, N. A., Abbas, A. T., Ali, S. S. and Eid, B. G.** "Honey protects against cisplatin-induced hepatic and renal toxicity through inhibition of NF-κB-mediated COX-2 expression and the oxidative stress dependent BAX/Bcl-2/caspase-3 apoptotic pathway" *journal The Royal Society of Chemistry (Food & Function)*, **2018**, 9(7), 3743–3754.
- [29] **Tonks, A. J., Cooper, R. A., Jones, K. P., Blair, S., Parton, J. and Tonks, A.** "Honey stimulates inflammatory cytokine production from monocytes" *Cytokine*, **2003**, 21(5), 242-247.
- [30] **O’Sullivan, A. M., O’Callaghan, Y. C., O’Connor, T. P. and O’Brien, N. M.** "Comparison of the antioxidant activity of commercial honeys, before and after in-vitro digestion" *Polish Journal of Food and Nutrition Sciences*, **2013**, 63(3), 167–171.
- [31] **Mesaik, M. A., Dastagir, N., Uddin, N., Rehman, K. and Azim, M. K.** "Characterization of immunomodulatory activities of honey glycoproteins and glycopeptides" *Journal of Agricultural and Food Chemistry*, **2015**, 63(1), 177–184.
- [32] **Minden-Birkenmaier, B. A., Meadows, M. B., Cherukuri, K., Smeltzer, M. P., Smith, R. A., Radic, M. Z. and Bowlin, G. L.** "Manuka honey modulates the release profile of a dHL-60 neutrophil model under anti-inflammatory stimulation" *Journal of Tissue Viability*, **2020**, 29(2), 91–99.

- [33] **Silva, B., Biluca, F. C., Mohr, E. T. B., Caon, T., Gonzaga, L. V., Fett, R., Dalmarco, E. M. and Costa, A. C. O.** Effect of Mimosca scabrella Bentham honeydew honey on inflammatory mediators. *Journal of Functional Foods*, **2020**, 72, 104034.
- [34] **Mix, E., Goertsches, R. & Zett, U. K.** "Immunoglobulins—Basic considerations" *Journal of Neurology* **2006**, 253, 9–17.
- [35] **Yan H., Lu J., Wang J., Chen L., Wang Y., Li L., Miao L. and Zhang H.** "Prevention of Cyclophosphamide-Induced Immunosuppression in Mice With Traditional Chinese Medicine Xuanfei Baidu Decoction" *Frontiers in Pharmacology*, **2021**, 12, 730567.
- [36] **Umogbai, O. O., Ogli, S. A., Agaba, E. I. and Yongo, M. A.** "Natural honey pre-treatment protect against immune suppression in cyclophosphamide exposed wistar rats" *Highland Medical Research Journal*, **2022**, 22(1), 20-26.
- [37] **Hegazi, A. G., Abdel-Rahman, E. H., Abd-Allah, F. and Abdou, A. M.** "Influence of Honey on Immune Status in Mice-Bearing Ehrlich Carcinoma" *Journal of Clinical & Cellular Immunology*, **2015**, 6(1), 1000295
- [38] **Jiang, M., Wang, C. Y., Huang, S., Yang, T., Dong, Z.** "Cisplatin-induced apoptosis in p53-deficient renal cells via the intrinsic mitochondrial pathway" *American Journal of Physiology-Renal Physiology*, **2009**, 296(5), 983-993.
- [39] **Warren, C.F.A., Wong-Brown, M.W. & Bowden, N.A.** "BCL-2 family isoforms in apoptosis and cancer" *Cell Death & Disease*, **2019**, 10, 177.
- [40] **Hassan, H. A., Edrees, G. M., El-Gamel, E. M. and El-Sayed, E. A.** "Amelioration of cisplatin-induced nephrotoxicity by grape seed extract and fish oil is mediated by lowering oxidative stress and DNA damage" *Cytotechnology*, **2014**, 66(3), 419-429.
- [41] **Cinelli, M. A., Do, H. T., Miley, G. P. and Silverman, R. B.** "Inducible nitric oxide synthase: Regulation, structure, and inhibition" *Medicinal Research Reviews*, **2019**, 40(1), 158-189.
- [42] **Hussein, S.Z., Mohd Yusoff, K., Makpol, S. and Mohd Yusof, Y.A.** "Gelam Honey Inhibits the Production of Proinflammatory Mediators NO, PGE(2), TNF-alpha, and IL-6 in Carrageenan-Induced Acute Paw Edema in Rats" *Evidence-Based Complementary and Alternative Medicine*, **2012**, 109636.
- [43] **Kalpna, D., Shanmugasundaram, R. and Mohan, V.** "GC–MS analysis of ethanol extract of Entada pursaetha DC seed. *Bioscience Discovery*, **2012**, 3(1), 30-33.
- [44] **Reza, A. S. M. A., Haque, A., Sarker, J., Nasrin, S., Rahman, M., Tareq, A., Khan, Z., Rashid, M., Sadik, G., Tsukahara, T. and Alam, K.** "Antiproliferative and antioxidant

potentials of bioactive edible vegetable fraction of *Achyranthes ferruginea* Roxb. in cancer cell line" *Food Science & Nutrition*, **2021**, 9(7), 3777-3805.

[45] **Shaheed, K., AlGaraawi, N., Alsultany, A., Abbas, Z., Khshayyish, I., & Al Khazali, M.** "Analysis of bioactive phytochemical compound of (*Cyperus iria* L.) By using gas chromatography–mass spectrometry. *Earth and Environmental Science*, **2019**, 388, 012064.

[46] **Alqarni, A., Dissanayake, T., Nelson, D., Parkinson, J., Dufton, M., Ferro, V. and Watson, D.** "Metabolomic Profiling of the Immune Stimulatory Effect of Eicosenoids on PMA-Differentiated THP-1 Cells. *Vaccines*, **2019**, 7(4), 142