

Research Article

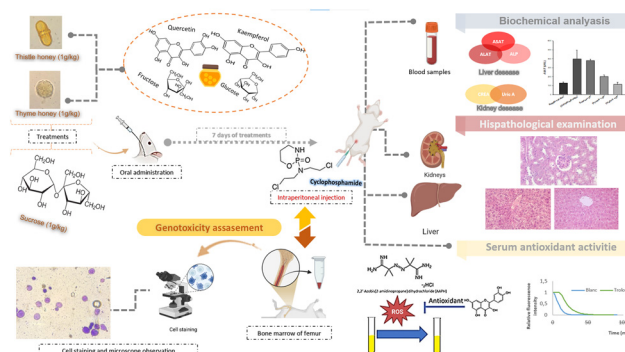
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Ameliorative effects of thistle and thyme honeys on cyclophosphamide-induced toxicity in mice

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Abstract: Cyclophosphamide (CP) is a potent anticancer agent widely used to treat various malignancies and autoimmune diseases after organ transplantation. However, its therapeutic benefits are often accompanied by severe toxicity, primarily attributable to oxidative stress. In contrast, Moroccan honey, including varieties, such as thyme and thistle, is known for its multifaceted medicinal properties, including potent antioxidant activity. This study sought to investigate the protective potential of Moroccan honey against CP-induced genotoxic and cytotoxic effects in mouse bone marrow cells. The results revealed a significant increase in genotoxicity and cytotoxicity following CP administration (20 mg/kg), as evidenced by elevated micronuclei frequency and a reduced ratio of polychromatic to normochromatic erythrocytes. However, pretreatment with Moroccan honey (1 g/kg) for a duration of 6 days effectively attenuated these adverse



Graphical abstract

effects. Furthermore, biochemical analysis demonstrated that mice receiving honey exhibited notable improvements in liver function, as indicated by decreased levels of alanine aminotransferase, aspartate aminotransferase, and uric acid. Histological examination revealed reduced hepatic damage characterized by diminished steatosis, apoptosis, necrosis, and inflammatory cell infiltration. These findings underscore the potential of thyme and thistle honeys, as a promising adjunctive therapy to mitigate the deleterious effects associated with CP treatment, offering potential applications in complementary chemotherapeutic strategies.

Keywords: cyclophosphamide, Moroccan honey, bioactive compounds, genotoxicity, micronucleus formation

Abbreviations

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the decay curve
CP	cyclophosphamide
CREA	creatinine
MNPCE	micronucleated polychromatic erythrocyte
NCE	normochromatic erythrocyte
ORAC	oxygen radical absorbance capacity
PCE	polychromatic erythrocyte
TE	Trolox equivalent

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UA uric acid;
UI International unit of enzyme's catalytic activity

1 Introduction

Cancer is a global threat and is responsible for 9.74 million deaths [1]. The situation is expected to become even worse by 2040 when the number of cancer deaths could exceed 5.3 million [1]. In response to the dramatic increase in the incidence and prevalence of cancer worldwide, various protocols using chemotherapeutic and radiotherapeutic approaches have been created as anticancer treatments. Cyclophosphamide (CP) is widely used as an anticancer drug [2]. It serves as an alkylating agent commonly employed in antineoplastic treatments and as an immune-suppressive drug at high doses for bone marrow transplantation or to treat autoimmune diseases [3]. It is commonly prescribed for the management of myeloblastoma, leukemias, ovarian carcinoma, and similar conditions, often as part of combination therapy with other chemotherapeutic drugs [4]. Cancer treatment is currently based on administering a low dose of CP, typically ranging from 1 to 5 mg/kg daily [5].

The primary pharmacological effect of alkylating agents such as CP involves disruption of cell development, differentiation, and function through the induction of cross-links as well as single and double breaks in DNA [6]. The ability of CP to disrupt all rapidly multiplying tissues underlies its therapeutic potential but also contributes to its various toxic effects. Indeed, DNA-damaging compounds, including alkylating agents such as CP and cisplatin, are potent anticancer drugs in clinical practice. However, the inability to repair DNA damage induced by these anticancer agents could have negative consequences for the cells present and cause several side effects, including adverse genetic, histological, or even reproductive effects [7].

The precise processes underlying CP toxicity are not well known; however, several studies have shown biochemical and genomic changes due to exposure to this drug [8]. It has been demonstrated that metabolic activation of CP occurs via the cytochrome P450 mixed functional system, resulting in the production of phosphoramidate mustard and acrolein; these metabolites are the leading causes of oxidative stress, which explains its potential toxicity [9].

Antioxidants play an essential role in protecting cells from the toxic effects of free radicals. The consumption of these chemopreventive compounds in the diet has been proposed as a practical approach to mitigate the adverse effects of carcinogens [10]. In this context, bee products such as propolis, royal jelly, bee venom, and honey have

demonstrated promising anticancer effects in preclinical studies. Royal jelly and honey possess immunomodulatory properties and may help enhance the body's natural defense mechanisms against cancer [11,12]. Compared to chemical drugs, natural products from bees often exert their effects through multiple pathways, which could reduce the likelihood of drug resistance and enhance the treatment efficacy. Additionally, honey, as a naturally sweet product, is considered a food or sweetener and a source of bioactive compounds. Its consumption benefits human health [13]. These favorable findings have focused on different biological activities, such as antibacterial, antioxidant, and antihepatotoxic effects, as well as beneficial physiological and metabolic properties extensively reported in the scientific literature, highlighting that honey has potential biological activities with promising health-promoting properties [14]. Honey is considered a rich source of bioactive compounds, and the preventive potential of each type of honey varies significantly according to the concentration of several bioactive molecules, such as phenolic compounds [15]. Thistle and thyme honey are among the most known varieties of honey and are esteemed not only for their distinct and delightful flavors but also for their substantial health benefits [15]. These honeys are rich in antioxidants, antimicrobial properties, and essential nutrients and have been used traditionally in various cultures to promote wellness and treat a range of diseases. The unique botanical origins of thistle and thyme impart specific bioactive compounds that contribute to their therapeutic potential. Indeed, the chemical profile and biological activities of thyme and thistle honeys have distinct characteristics that contribute to their health benefits. Thyme honey is rich in phenolic compounds such as ellagic acid, vanillin, and quercetin, which are known for their antioxidant and anti-glycation properties. These compounds, present in relatively high concentrations in thyme honey, play an important role in reducing the production of advanced glycation end products. On the other hand, thistle honey also exhibits high bioactivity attributed to its phenolic content, particularly ferulic acid and ellagic acid. These compounds, along with quercetin, contribute to the antioxidant and anti-glycation activities of thistle honey [15].

Several authors have documented the diverse biological and pharmacological properties of propolis, from antibiotic to antigenotoxic and antimutagenic activities [16,17]. Additionally, bee venom has been confirmed to have therapeutic effects on cancer, multiple sclerosis, dementia, osteoarthritis, and rheumatoid arthritis [18]. However, studies focusing on the protective role of honey against the genotoxicity of CP are lacking.

This study aimed to assess the protective effects of thistle and thyme honeys on DNA damage caused by the

Table 1: Geographical origin of the two types of Moroccan honey samples

Sample	Common name	Geographical origin	Harvest year	Predominant pollen (%)	Other important minor pollen (%)
H 1	Thyme	Rich (East-Morocco)	2018	Thymus sp. (76)	Eucalyptus sp. (21), Cardus spp. (4)
H 2	Thistle	Rural community Bourrous (Marrakesh-Morocco)	2018	Eryngium ilicifolium (66)	Bupleurum sp. (8), Carduus spp. (3)

anticancer drug CP. This study also established the connection between these protective activities and the chemical composition of these natural products.

2 Materials and methods

2.1 Materials

2.1.1 Chemicals and reagents

2.1.1.1 Honey samples

The present study used two reputed Moroccan honeys (i.e., thyme and thistle honeys). The samples were kept in sealed glass vessels until analysis in the dark at ambient temperature (25 ± 5°C). The regions from which the honey samples were collected and the verification of botanical origin based on pollen grain percentages are indicated in Table 1 and Figure 1. Analyses were carried out at least in duplicate.

2.1.1.2 Animals

Male Swiss albino mice aged 8 weeks (20–28 g) were obtained from the Central Animal Facility of the Department of Biology, Faculty of Science Semlalia, Cadi Ayyad University of Marrakech (Morocco). The animals were acclimatized for 1 week before the study and had access to standard laboratory food and water *ad libitum*. All animals received treatment in compliance with the recommendations of the Moroccan Ethics Committee for Animal Research, which approved our protocol (Ref. UCA-FMP-06/2022). All efforts were made to reduce the number of animals used and to minimize any animal suffering.

2.2 Methods

2.2.1 Experimental animals

Male Swiss albino mice were divided into five groups, each comprising five mice. The first group received saline water. The second group received a single intraperitoneal injection of freshly dissolved distilled water (CP; 20 mg/kg). Sucrose (1 g/kg) was given orally to the third group for seven consecutive days. In addition, groups VI and V were orally treated with thyme and thistle honey, respectively, at 1 g/kg. On the last day (day 7) of pretreatment, CP (20 mg/kg) was given to all groups except for the first group (Figure 2). The animals were sacrificed after being treated for 24 h with CP.

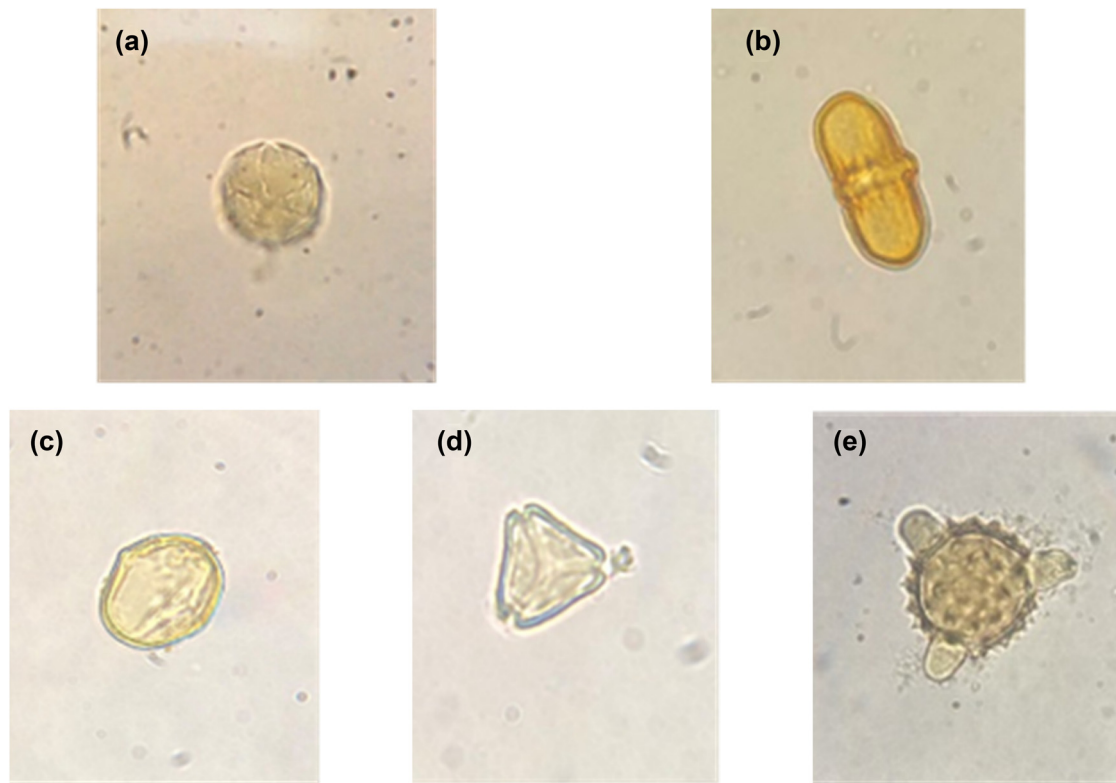


Figure 1: Light microscopy photographs of pollen grains observed in the two honey samples: (a) *Thymus* sp., (b) *Eryngium ilicifolium*, (c) *Bupleurum* sp., (d) *Eucalyptus* sp., and (e) *Carduus* sp.

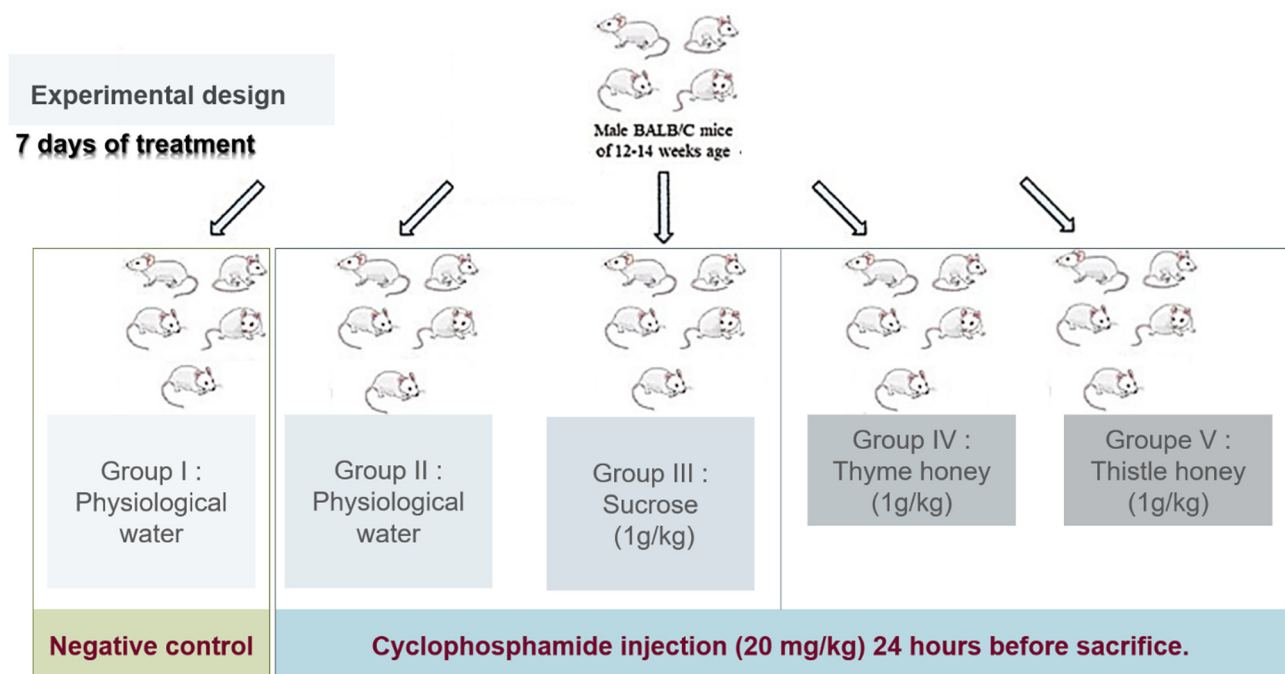


Figure 2: Experimental design of the assessment of genotoxicity and cytotoxicity of different treatments in mice.

2.2.2 Micronucleus assay

The micronucleus test on mouse bone marrow was conducted as described by Jain and Pandey [19]. In summary, the extremities of the femurs were cut off, and a syringe filled with Hanks' balanced salt solution (HBSS) (pH 7.4) was inserted into the bone cavity. The bone marrow was then evacuated by gently pushing the plunger, allowing the cell suspension to be collected in a centrifuge tube for processing. The slides were stained with Giemsa and May-Grünwald following Schmid's methods [19]. A total of 1,500–2,000 normochromatic erythrocytes (NCEs) and polychromatic erythrocytes (PCEs) were counted per animal using an Olympus CX33 trinocular microscope to calculate the micronucleated PCE (MNPCE) frequencies (Figure 3). To identify potential cytotoxic effects, the PCE/NCE ratio was computed for 200 erythrocytes per animal following the methods of Bhaskar Gollapudi and McFadden [20]. Slides were examined at 1,000× magnification under a light microscope.

2.2.3 Serum biochemical parameters

2.2.3.1 Liver function test

Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and alkaline phosphatase (ALP) levels were measured by using an ARCHITECT Abbott ci4100 automated system.

2.2.3.2 Kidney function test

Uric acid (UA) and creatinine (CREA) levels were measured using an ARCHITECT Abbott ci4100 automated system.

2.2.4 Histopathological examination of liver and kidney tissues

After sacrifice, organs from all animals were dissected and then immersed in a 10% formaldehyde solution for 48–72 h.

All organs were washed with tap water for half an hour, dehydrated, cleaned with xylene, impregnated with soft kerosene, and immersed in hard kerosene. Thin sections of tissues were stained with hematoxylin and eosin for histopathological examination.

2.2.5 Oxygen radical absorbance capacity (ORAC) assay of serum

The antioxidant capacity of mouse serum in each group was determined using the ORAC assay following the method described by Zulueta et al. [21]. The standard curve was generated using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (0.02 mM Trolox). The loss of fluorescein signal (excitation and emission wavelengths of 485 and 535 nm, respectively) was continuously monitored for 1 h at 30°C on a spectrofluorometer. The ORAC values, expressed as mM Trolox equivalents (mM TE), were calculated as follows:

$$\text{ORAC(mM TE)} = \frac{C_{\text{Trolox}} \times (\text{AUC}_{\text{Sample}} - \text{AUC}_{\text{Blank}})}{(\text{AUC}_{\text{Trolox}} - \text{AUC}_{\text{Blank}})} \times k,$$

where C_{Trolox} is the concentration of Trolox, $\text{AUC}_{\text{Sample/Blank/Trolox}}$ is the area under the decay curve of the sample/blank/Trolox, respectively, and k is the dilution factor of the sample.

2.2.6 Statistical analysis

All the results are expressed as the mean ± standard deviation (SD). SPSS 18.0.0 was used for one-way analysis of variance (ANOVA). The differences between the mean values were statistically significant at the 95% confidence level, with a corresponding p -value less than 0.05.

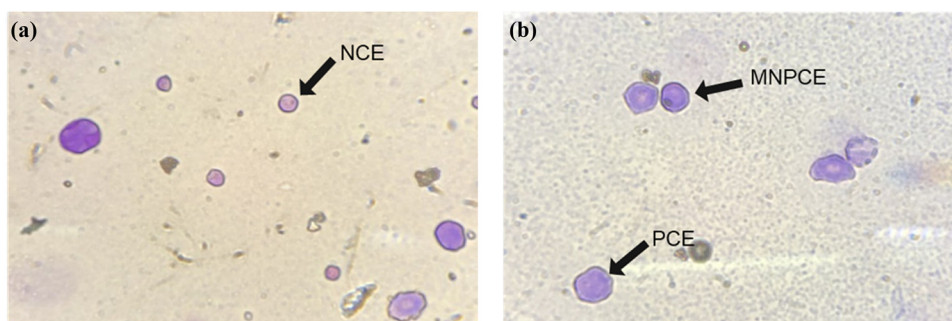


Figure 3: Mouse bone marrow smear for micronucleus analysis stained with May-Grünwald and Giemsa (1,000× magnification). NCE: normochromatic erythrocyte (a); PCE: polychromatic erythrocyte and MNPCE: micronucleated polychromatic erythrocyte (b).

3 Results and discussion

3.1 CP-induced genotoxic and cytotoxic effects

Significant progress has been made by the pharmaceutical industry in developing new and highly effective drugs for a wide range of diseases [22]; one such treatment is CP, which is recognized as one of the most effective and commonly used antineoplastic medications [5]. However, it has been reported that CP has several toxic effects, including bone marrow suppression, cardiac and gonadal toxicity, hemorrhagic cystitis, and carcinogenesis, with the cumulative dose being the principal risk factor as does genotoxicity [8,23]. Cancer and non-cancer drugs were evaluated by several *in vivo* test systems, and the micronucleus test is widely used because it is a simple and rapid assay for detecting potential carcinogenic agents [24].

The PCE/NCE ratio was used to determine the cytotoxicity, and the MNPCE percentage was used to assess genotoxicity in bone marrow cells from mice pretreated for 6 days (Figure 4a). The results of CP-exposed groups were compared with those of the negative control group. Exposure to 20 mg/kg CP alone without any pretreatment significantly decreased the ratio of PCE to NCE (PCE/NCE) compared to that of the negative control ($p < 0.05$); this ratio reached 0.48 ± 0.15 compared with 0.94 ± 0.11 for the negative control, demonstrating that CP exhibited a highly selective destruction potential for cells that are actively dividing in the erythropoietic system. CP is considered toxic to cells through a free-radical-mediated mechanism involving the toxic byproduct acrolein.

The results of PCE/NCE ratio are complementary to those of the MNPCE frequency since the decrease in the PCE/NCE ratio obtained for the CP group was accompanied by significantly greater frequencies of MNPCE in comparison with the negative control ($p < 0.001$), i.e., an average frequency of 28.40 ± 1.78 for the CP group and 6.40 ± 1.25 for the negative control group (Figure 4b). In previous studies, CP was shown to be responsible for genotoxicity in an *in vitro* model using lymphocyte cultures [25] and *in vivo* tests using bone marrow cells [26]. The relationship between genotoxicity and oxidative stress has been well established in many experimental animal models [27].

The PCE/NCE ratio was significantly greater in mice pretreated with sucrose than in those in the negative control group or in the groups pretreated with honey samples, with cytotoxicity levels close to those obtained for the group treated with CP (Figure 4a). Similarly, pretreatment with 1 g/kg body weight sucrose resulted in an MNPCE

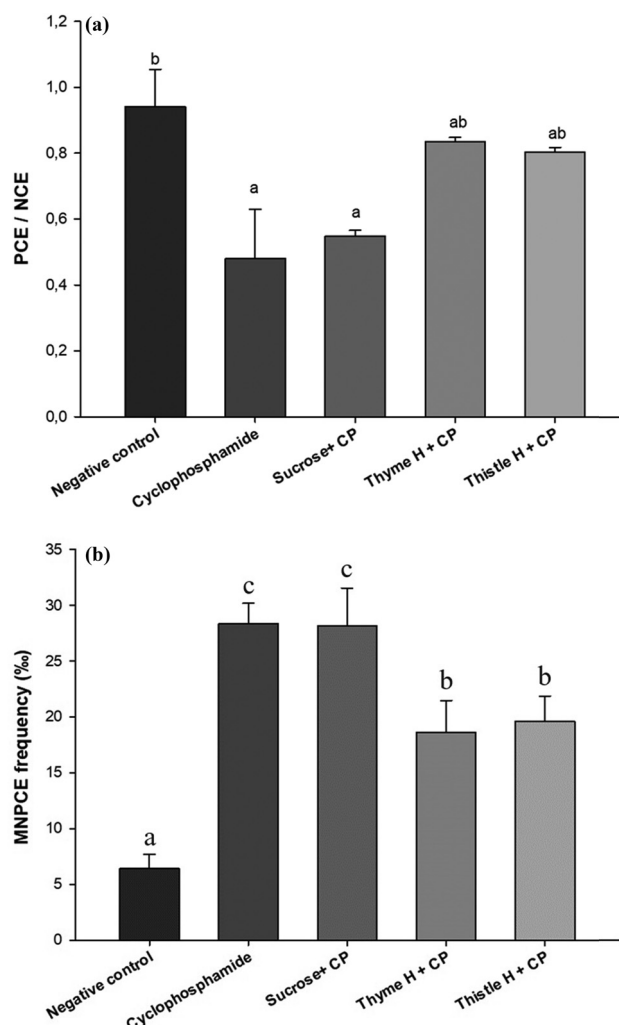


Figure 4: Counting of PCE–NCE ratio (a) and the micronucleated polychromatic erythrocyte (MNPCE) frequency (b) of different types of treatments. All values are expressed as mean $\pm$ SD; $n = 5$ for each treatment group. Values with different letters are significantly different ($p < 0.05$).

frequency of 28.20 ± 3.36 out of 2,000 PCE, which was significantly ($p < 0.001$) greater than that in the negative control group but similar to that recorded in the CP group (Figure 4b).

In this study, sucrose served as a neutral control in the experimental design, allowing the differentiation between the protective effects exerted by the honeys and any potential nonspecific responses induced by additional dietary components. By including sucrose-treated groups alongside honey-treated groups, we aimed to elucidate the unique contributions of honey's bioactive compounds to mitigating the adverse effects induced by CP.

Therefore, pretreatment with sucrose did not alter the cytotoxicity or genotoxicity induced by CP injection. This

effect of sucrose can be explained by its impact on the level of oxidative stress. Previous research has shown that sucrose does not cause oxidative stress in the colon [28]. Additionally, Hansen et al. [29] reported that simple carbohydrates had the same level of genotoxicity, and the genotoxicity of these compounds may be related to the modification of the chemical environment, which may be modified in the case of our study by the injection of CP.

The combined effect of thistle and thyme honey samples, as shown in Figure 4a, indicated that the PCE/NCE ratio reached 0.80 ± 0.01 for the group pretreated with thistle honey and 0.83 ± 0.02 for the group pretreated with thyme honey; these values were significantly greater ($p < 0.05$) than those of the CP and CP + sucrose groups. Moreover, this attenuation of the cytotoxic effect was accompanied by an attenuation of the genotoxic effect (Figure 4b) since the frequencies recorded (19.60 ± 2.28 and 18.60 ± 2.88 for the thyme and thistle honey groups, respectively) were lower ($p < 0.001$) than those recorded for the CP and CP + sucrose groups.

The protective effect of the two types of honey (thyme and thistle honey samples) against the toxicity induced by CP may be explained by its potent antioxidant activity. Additionally, honey's ability to modulate immune responses and enhance DNA repair mechanisms might also contribute to the observed protective effects [30]. In addition, to the free-radical-scavenging activity mechanism, we cannot exclude the possibility that thistle and thyme honeys exert their protective effects by enhancing the defense mechanisms and promoting the activity of detoxifying enzymes in the liver, such as glutathione S-transferase (GST) and other phase II detoxification enzymes. These enzymes may help in the conjugation and elimination of toxic metabolites of CP, reducing their harmful effects on liver and kidney tissues. These mechanisms collectively contribute to the ameliorative

effects of honey against CP-induced genotoxicity in liver and kidney tissues. However, the exact mechanisms can vary depending on the type and composition of honey, as well as the specific biological context. Further research is needed to elucidate these mechanisms in detail and to identify the most effective types and doses of honey for protective purposes.

3.2 Effects on serum biochemical parameters

The serum biochemistry data are summarized in Figures 5 and 6. In the normal animal group, ASAT, ALAT, and ALP levels were significantly lower than those in the other studied groups (Figure 4). Moreover, the levels of these enzymes increased, with mean values of 398.50 ± 96.50 , 70.86 ± 6.28 , and 47.69 ± 23.68 UI/L for ASAT, ALP, and ALAT, respectively, in the group that received an intraperitoneal injection of CP alone (Figure 5). Moreover, significant changes in the listed biochemical parameters were observed in groups of animals subjected to different pretreatments. ASAT, ALAT, and PAL were not significantly greater than those in the negative control and thistle groups. However, significant differences were detected between the negative control and thyme groups for ALAT and ALP. Moreover, compared with those in the CP and sucrose groups, a significant decrease in the aminotransferase activity was detected in animals pretreated with the two honey samples, demonstrating the protective effect of these two types of honey (Figure 5).

Serum UA and CREA have been used as markers of renal function. The present study showed that CREA levels varied from 24.71 ± 1.51 to 30.09 ± 2.73 $\mu\text{mol/L}$ (Figure 6a), with non-significant differences ($p > 0.05$) between the

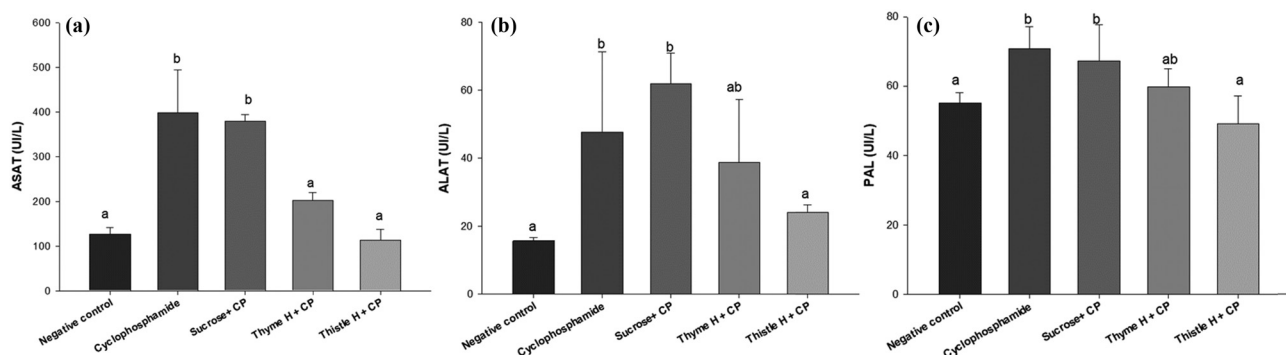


Figure 5: Effects of different types of treatments on serum aspartate aminotransferase (ASAT) (a), alanine aminotransferase (ALAT) (b), and alkaline phosphatase (PAL) (c). All values are expressed as mean $\pm$ SD; $n = 5$ for each treatment group. Values with different letters are significantly different ($p < 0.05$).

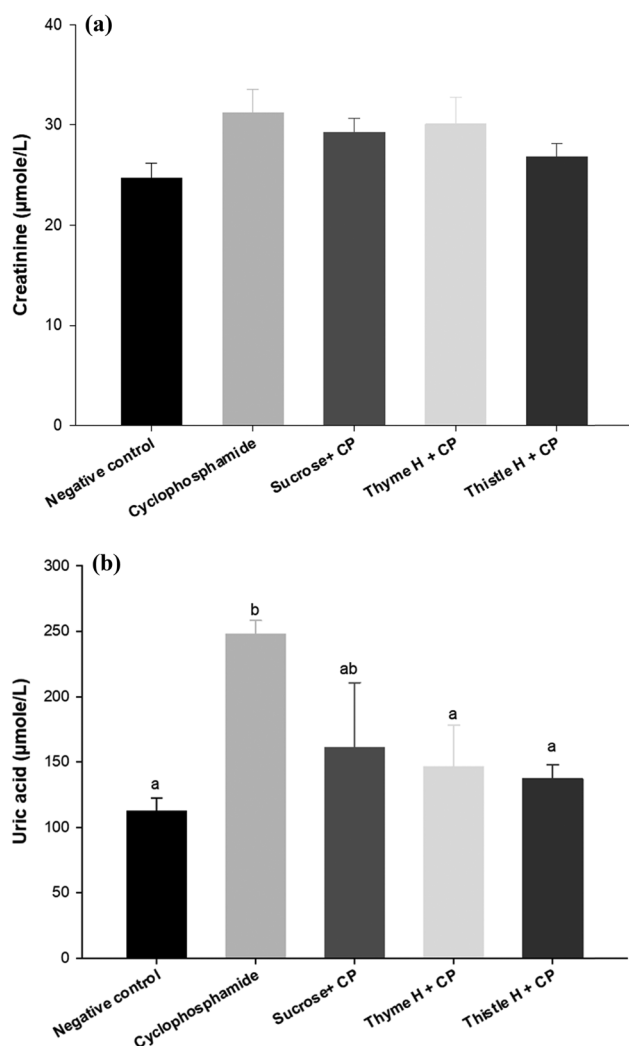


Figure 6: Effects of different types of treatments on serum CREA (a) and UA (b). All values are expressed as mean $\pm$ SD; $n = 5$ for each treatment group. Values with different letters are significantly different ($p < 0.05$).

negative control group, the CP group, and the groups that received different types of pretreatments. On the other hand, the CREA level slightly increased in mice injected with CP but did not reach the significance threshold. Concerning the level of UA, the results (Figure 6b) showed that, compared with mice in the CP group, mice that were injected with CP and pretreated with thyme and thistle honey exhibited a significant reduction ($p < 0.05$) in these values. On the other hand, they were not significantly elevated compared with those in the negative control group. For the mice injected with CP pretreated with sucrose, the level of UA increased considerably compared to that in the mice in groups pretreated with honey samples ($p < 0.05$).

These findings on the serum biochemical parameters suggested that thyme and thistle honey had protective effects on the high toxicity of CP on liver and renal

functions. Although pretreatment of the honey samples did not restore the values to normal levels, these effects were not altered by treatment with sucrose.

The positive effects of pretreatment with the two honey samples on most of the biochemical parameters may be associated with the antioxidant capacities of these substances. It has been documented that certain antioxidant compounds are valuable for alleviating the adverse effects of anticancer drugs. This approach could regulate oxidative stress and improve the outcomes of chemotherapy [31].

3.3 Histopathological investigation

Histopathological examinations revealed that the hepatic lobules of animals in the first group (negative control) had a uniform cellular architecture with distinctive liver cells, a perisinusoidal space, and a central vein (Figure 7e). However, histopathological examination of liver sections from the CP group revealed hepatic degeneration and pale staining, necrosis of many hepatocytes with pyknosis, hydropic degeneration, hepatocyte ballooning, and apoptosis (Figure 7a). In the CP + sucrose group, evident hepatic lesions, principally involving steatosis, hydropic degeneration, necrosis, and hepatocyte ballooning, were observed after histopathological examination (Figure 7b). However, microscopic observation of liver sections from the CP and thistle honey groups revealed a notable improvement in distorted hepatic lobules, characterized by a restoration toward a more normal hepatic structure and increased stainability (Figure 7c). The same findings were also observed for the thyme honey-pretreated group. A markedly decreased number of hepatocellular lesions and inflammatory cell infiltration were observed, with less hepatocellular necrosis around the hepatic portal and central veins (Figure 7d).

As reported by Lata *et al.* [32], the primary mechanism of hepatotoxicity in CP is the excessive production of ROS, which leads to alterations in the transport function and membrane permeability of hepatocytes. Under normal conditions, high concentrations of serum aminotransferases are present in the liver.

In contrast, lower serum enzyme levels indicate increased plasma membrane strength and liver tissue repair. Therefore, it appears that pretreatment with honey prevents the leakage of intracellular enzymes by enhancing the stability of the hepatocyte membrane. This effect is essentially related to the high content of antioxidant constituents, such as phenolics and flavonoids, in the two types of honey, which can delay or inhibit the generation of oxidative stress [33].

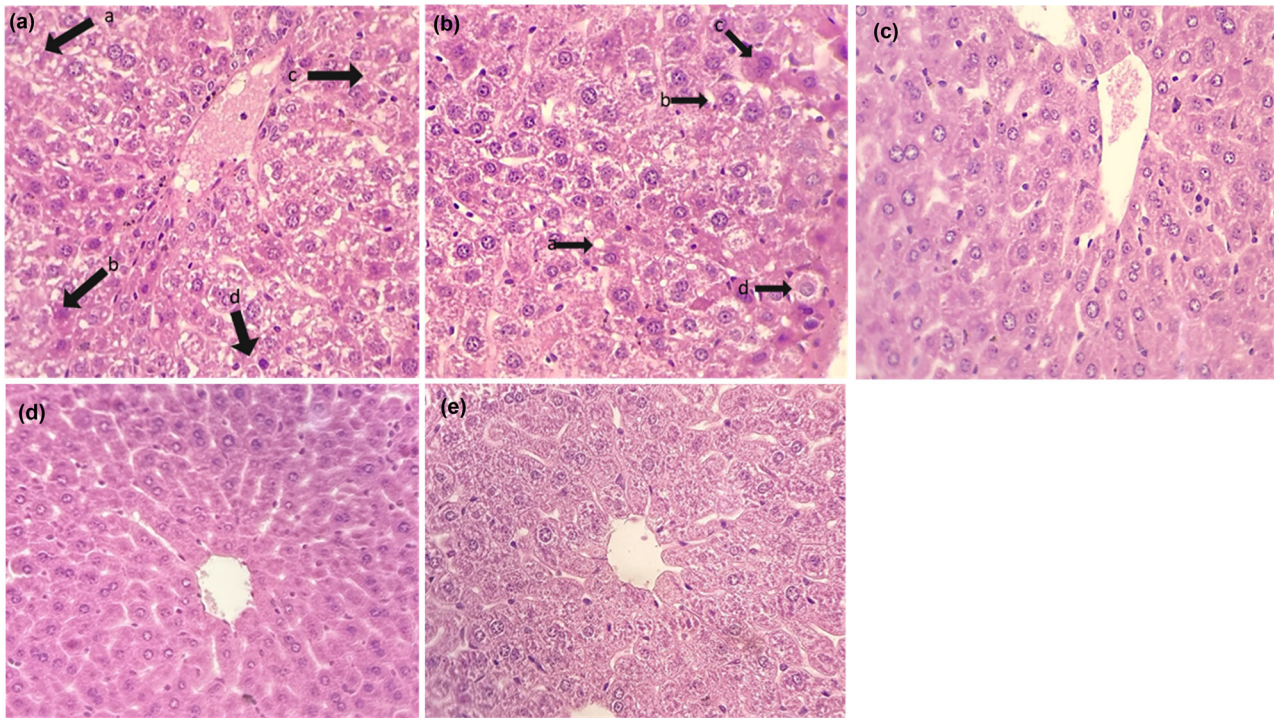


Figure 7: Representative photomicrographs of liver sections (400× magnification). The liver sections from the CP mice (a) showed a: hydropic degeneration, b: necrosis, c: hepatocyte ballooning, and d: apoptosis. The section of liver tissue from a mouse received CP + sucrose (b) showed a: steatosis, b: hydropic degeneration, c: necrosis, and d: hepatocyte ballooning. The pretreatment of animals with thistle honey (c) and thyme honey (d) revealed a better preservation of the normal liver. The liver section from control animals (e) showed a regular cellular architecture with distinct hepatic cells.

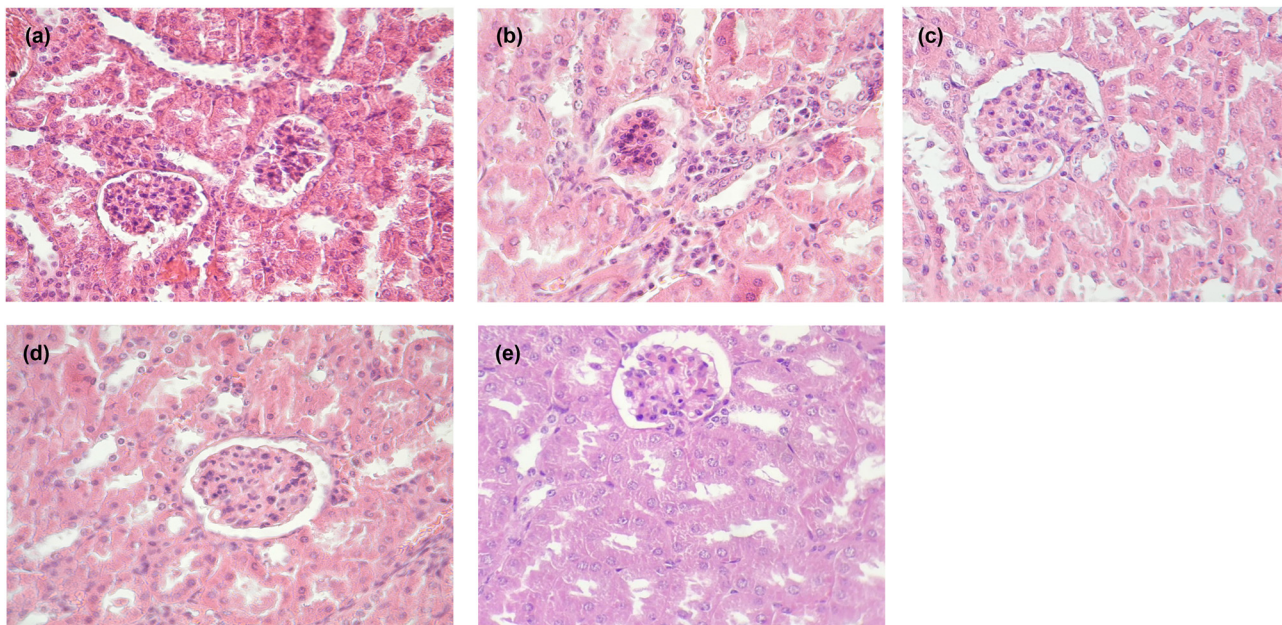


Figure 8: Representative photomicrographs of renal sections (400× magnification). The renal sections from the CP mice (a) and CP + sucrose mice (b) show a loss of the normal renal architecture, with marked infiltration of periglomerular interstitial inflammatory cells. The pretreatment of animals with thistle honey (d) and thyme honey (e) revealed a better preservation similar to the normal group (c).

In addition to the effects on liver tissues, the kidney sections revealed significant glomerular inflammation in mice treated with CP (Figure 8a) and CP combined with sucrose (Figure 8b), characterized by numerous zones of inflammatory infiltrates compared to those in the negative control group (Figure 8c). This inflammation indicates that an immune response likely triggered by the cytotoxic effects of CP. However, when CP was combined with thistle or thyme honey, there was a marked reduction in the level of these inflammatory infiltrates (Figure 8d and e).

Several studies have demonstrated the effect of CP on renal function. Santos *et al.* [34] suggested that CP treatment leads to inflammation due to disturbances in the levels of antioxidant enzymes. A study by Jiang *et al.* [35] reported that CP increases the levels of blood urea nitrogen, CREA, and nuclear factor κ B signaling pathways, which are responsible for the expression and generation of inflammatory cytokines.

However, Rehman *et al.* [36] indicated that toxicity indicators such as blood urea nitrogen decreased in the ellagic acid-pretreated group during CP toxicity, and kidney architecture was restored. Indeed, ellagic acid is an effective dietary antioxidant found in a variety of food sources, such as honey [37], which may partly explain the protective effect observed in the thistle- and thyme honey-pretreated groups.

Histopathological studies have provided additional evidence for the use of serum biochemical parameters, as shown in the photomicrographs. The key results of the present study were that pretreatment with thistle or thyme honey influenced the recovery of kidney and liver damage induced by CP. These results are supported by the literature, which highlights the hepatoprotective and nephroprotective properties of honey. For instance, it has been reported that honey can modulate oxidative stress markers and enhance the activity of endogenous antioxidant enzymes, leading to improved histopathological outcomes [38].

Moreover, the anti-inflammatory properties of honey, through the inhibition of pro-inflammatory cytokines and the enhancement of anti-inflammatory mediators, further support tissue repair and recovery [39]. The observed improvement in kidney and liver architecture in the honey-pretreated groups suggested that thistle and thyme honeys not only mitigated biochemical alterations but also promoted histological recovery, making them promising adjuncts in the management of CP-induced toxicity.

3.4 ORAC assay of serum

The serum ORAC of different groups was assayed. This method, which incorporates fluorescein as a fluorescence probe, inhibits peroxyl-radical-induced oxidation [40].

The results of the ORAC assay showed that serum from animals that received an intraperitoneal injection of CP had a low level of antioxidant capacity, as demonstrated by the relative fluorescence decay curves (Figure 9a), where the intensity of the relative fluorescence decreased from the beginning of the reaction. The results for the CP group were similar to those obtained for the CP + sucrose group. In contrast, compared with other pretreatments, the group treated with thistle honey showed the most significant increase in the serum antioxidant activity. In contrast, treatment with thyme honey had fluorescence intensities comparable to those recorded for the serum of the negative control group, as reflected by the ORAC indices (Figure 9b), which were 6.71 ± 0.98 and 6.78 ± 1.35 mM TE for the CP + thyme honey group and the control

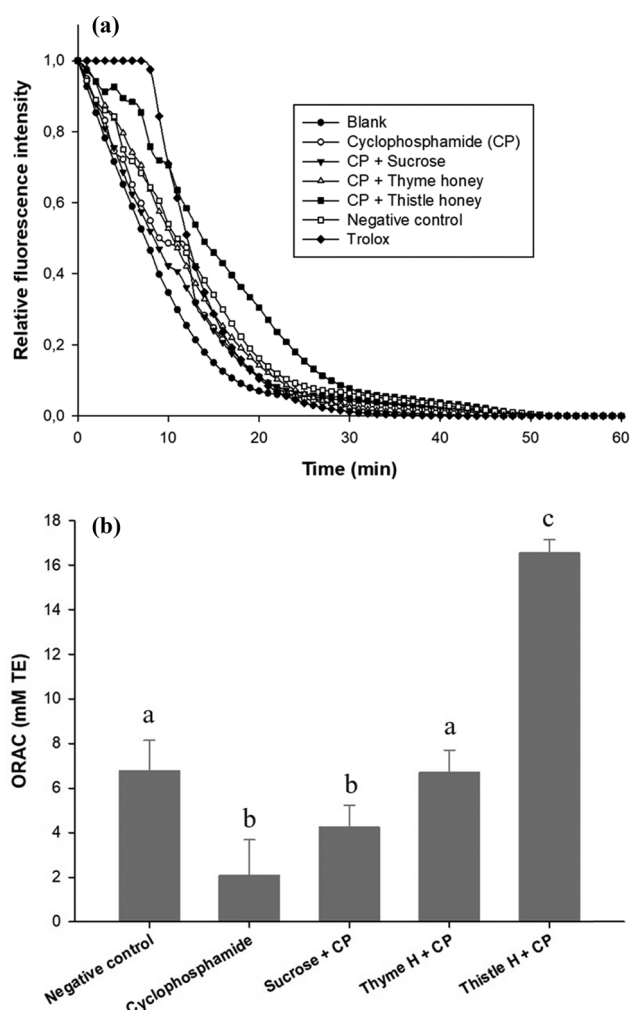


Figure 9: Fluorescence decay curve during the ORAC test. Relative fluorescence intensities (a) and the ORAC indices (b) of serums from different types of treatments. All values are expressed as mean $\pm$ SD; $n = 5$ for each treatment group. Values with different letters are significantly different ($p < 0.05$).

group, respectively. The highest ORAC value was found in the thistle honey group, which had a significantly greater ORAC index compared to other groups (16.55 ± 0.59 mM TE) ($p < 0.05$), which confirmed its high antioxidant capacity.

These findings are consistent with those of Zhao et al. [41], who reported that pretreatment with *Apis cerana* honey (Qinling Mountains) significantly decreases the serum lipoprotein oxidation associated with increased serum radical absorption capacity.

An important consideration is the molecular mechanisms involved during exposure to CP-induced oxidative stress. Mohanty et al. [42] demonstrated that quercetin decreases the expression of neutrophil cytosol factor 1 (p47phox), which regulates NADPH oxidase in rats. Chopra et al. [43] reported that supplementation with quercetin decreased LDL oxidation. Jung et al. [44] reported that naringin enhances antioxidant enzymes in the erythrocytes of individuals with hypercholesterolemia. Based on these studies, we suggest that the enzymatic pathway is essential for attenuating the toxic effects induced by CP and that the effects of phenolic compounds on this pathway are remarkable.

4 Conclusions

The experimental findings in this study further confirm the utility of the micronucleus assay for evaluating chromosomal damage and highlight the significance of the PCE/NCE ratio as a biomarker for cytotoxicity. Specifically, our results demonstrate that CP treatment induces cytotoxicity and significant clastogenic effects, as evidenced by the increased formation of micronucleus and decreased PCE/NCE ratio. Notably, pretreatment with thyme and thistle honey effectively restored the PCE/NCE ratio to normal levels, leading to a significant reduction in the number of micronucleated erythrocytes. Histopathological examinations and serum biochemical analyses also revealed a notable improvement in kidney and liver functions and architecture following CP exposure, further supporting the ameliorative effects of honey pretreatment. These beneficial effects can be attributed to the enhancement of antioxidant status in the experimental animals. In summary, honey emerges as a promising adjuvant therapy alongside CP to mitigate the genotoxic effects associated with CP treatment. These findings offer valuable insights into potential strategies for ameliorating the adverse effects of CP therapy. Further studies are needed to elucidate the impact of honey on the efficacy of CP in cancer treatment. Understanding the interplay between honey supplementation and CP's

antineoplastic activity could provide valuable insights into optimizing therapeutic strategies for enhanced treatment outcomes in cancer patients.

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