Formulation a modern chemotherapeutic drug of Ziziphora clinopodides Lam leaf aqueous extract conjugated cadmium nanoparticles and analysis of their anti-acute myeloid leukemia potentials in comparison to Daunorubicin in leukemic mouse and cellular models: Antidiabetic and Anticholinergics Potentials

Behnam Mahdavi (✉ b.mahdavi@hsu.ac.ir)

Hakim Sabzevari University

Mohammad Mahdi Zangeneh (✉ m.mehdizangeneh@yahoo.com)

Razi University

Sogand Paydarfar

Hakim Sabzevari University

Esmail Rezaeiseresht

Hakim Sabzevari University

Akram Zangeneh

Razi University

Nastaran Sadeghian

Ataturk Universitesi

Parham Taslimi

Bartın Universitesi

Fatih Şen (✉ fatihsen1980@gmail.com)

Kutahya Dumlupınar Üniversitesi <https://orcid.org/0000-0001-6843-9026>

Ismail Mert Alkac

Kutahya Dumlupınar Üniversitesi

Research

Keywords: Acute myeloid leukemia, Cadmium nanoparticles, Clinopodioides Lam, Daunorubicin, Ziziphora new chemotherapeutic drug

Posted Date: May 29th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-28385/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Formulation a modern chemotherapeutic drug of *Ziziphora clinopodides* Lam leaf aqueous extract conjugated cadmium nanoparticles and analysis of their anti-acute myeloid leukemia potentials in comparison to Daunorubicin in leukemic mouse and cellular models: Antidiabetic and Anticholinergics Potentials

Behnam Mahdavi^{*,a}, Mohammad Mahdi Zangeneh^{*,b,c}, Sogand Paydarfar ^a, Esmail Rezaei-Seresht ^a, Akram Zangeneh ^{b,c}, Nastaran Sadeghian ^d, Parham Taslimi ^e, İsmail Mert Alkac ^f, Fatih Sen^{*f}

^a Department of Chemistry, Hakim Sabzevari University, Sabzevar, Iran.

^b Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.

^c Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran.

^d Department of Chemistry, Faculty of Sciences, Ataturk University, 25240, Erzurum, Turkey

^e Department of Biotechnology, Faculty of Science, Bartın University, 74100 Bartın, Turkey

^f Sen Research Group, Biochemistry Department, Faculty of Arts and Science, Dumlupınar University, Evliya Celebi Campus, 43100, Kutahya, Turkey

***Corresponding author:** b.mahdavi@hsu.ac.ir; m.mehdizangeneh@yahoo.com; fatihsen1980@gmail.com.

ABSTRACT

In this study, the anti-acute myeloid leukemia (AML) effects of *Ziziphora clinopodioides* Lam leaf aqueous extract conjugated cadmium nanoparticles (CdNPs) were compared with daunorubicin as a common chemotherapeutic drug. To synthesize CdNPs, *Z. clinopodioides*

aqueous extract was combined with $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. For analyzing CdNPs, SEM, UV-Vis. spectroscopy, X-ray powder diffraction and Energy dispersing X-ray spectroscopy, Fourier Transform Infrared techniques were used. In FE-SEM images of CdNPs, the diameter of particle dimension was on means of 26.78 nm. To survey the cytotoxicity and anti-AML effects of $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodioides*, Daunorubicin, CdNPs and MTT assay was used on the human AML cell lines. The IC₅₀ of the cadmium nanoparticles was 168, 205 and 210 $\mu\text{g/mL}$ against Murine C1498, 32D/FLT3-ITD and Human HL-60/vcr cell lines, respectively. In vivo study, CdNPs like daunorubicin ameliorated importantly ($P \leq 0.01$) the biochemical, WBC, RBC, inflammatory, platelet, stereological, histopathological and cellular-molecular parameters compared to the other groups. As mentioned, the cadmium nanoparticles had significant anti-AML effects. After approving the above eventuate in the clinical trial studies, these cadmium nanoparticles could be used in humans as a chemotherapeutic medicament for the treatment of AML. Additionally, the novel nanoparticles ($\text{Cd}(\text{NO}_3)_2$ and CdNPs) were good inhibitors of the α -glycosidase, and cholinesterase enzymes.

KEYWORDS: Acute myeloid leukemia; Cadmium nanoparticles; *Clinopodioides* Lam; Daunorubicin; *Ziziphora* new chemotherapeutic drug

1 INTRODUCTION

Metallic nanoparticles have achieved notable consideration in the field of medicine. Recent studies include promising aids for metal-based nanoparticles, bacterial disease and cancer treatments that differ in their physicochemical properties [1,2]. To produce metallic nanoparticles; I) Physical, II) Chemical, III) Biological method [3]. Chemical or physical synthesis methods are expensive. These methods cause time loss. It also consumes energy and is not environmentally friendly. In biological nanoparticle synthesis, several microbes, algae, enzymes and especially plants have been used and served as a suitable alternative method to physical and chemical procedures with high antimicrobial potentials[4]. These nanoparticles

51 have different antibacterial effects on microbial cell functions, including electrostatic
 52 interaction between the particle, Gram-negative (-) bacterial cell wall and denaturation of
 53 intracellular proteins. Through the cell membrane, nanoparticles harmful to the essential
 54 enzymes of bacteria can be the basis of the antibacterial properties of these nanoparticles
 55 [5,6]. Also, the metallic nanoparticles have excellent potentials in the treatment of several
 56 cancers especially leukemia[7–11]. In this regard, in the work of Hemmati *et al.* (2019)
 57 proved that metallic nanoparticle-chitosan composite had excellent anti-acute myeloid
 58 leukemia in the *in vivo* and *in vitro* conditions. In the previous study, metallic nanoparticle-
 59 chitosan composite significantly removed the AML cell lines i.e., 32D/FLT3-ITD (Isoform
 60 containing ITD mutations), Murine C1498 and Human HL-60/vcr and regulated biochemical
 61 and hematological parameters in the animals[8]. Also, Zangeneh (2019) introduced a
 62 chemotherapeutic drug formulated by metallic nanoparticles containing natural compounds
 63 for the treatment of AML[9]. In addition to the above studies, the other studies researched the
 64 anticancer properties of cadmium nanoparticles. In a study, Hossain and Mukherjee (2013)
 65 presented the excellent potential of cadmium nanoparticles in removing human cervical
 66 adenocarcinoma cells (HeLa)[10]. Also, in the other study, Heidari (2016) indicated the
 67 significant role of cadmium nanoparticles in combination with UV radiation in the removing
 68 of tissue tumors[11]. Recently, scientists have used the anti-acute leukemia effects of
 69 medicinal plants for synthesizing the metallic nanoparticles containing natural compounds. So
 70 far, the anti-acute leukemia effects of *Tinospora cordifolia*, *Sophora subprostrata*, *Euphoria*
 71 *hirta*, *Barleria prionitis*, *Lubinus perennis*, *Maytenus boaria*, *Cephaelis acuminata*,
 72 *Phyllanthus niruri*, *Solanum seaforthianum*, *Boswellia serrate*, *Lavendula officinalis*, and
 73 *Cephalotaxus harringtonia* drupacea have been proved[12].
 74 One of the well-known traditional medicine in all of the world is Iranian traditional medicine.
 75 In Iranian traditional medicine and many sub-traditional medicines are there that the most

well-known is Kurdish traditional medicine in the west of Iran. Every year many medicinal supplements and drugs are formulated and produced from the plants of Kurdish conventional medicine[13,14]. One of these plants is *Ziziphora clinopodioides* Lam. *Z. clinopodioides* is used in conventional medicament of Iran for the cure of inflammations, blood and gastrointestinal disorders, cutaneous wounds, and the common cold[15,16]. Many potentials such as anti-inflammatory[17,18], antifungal[17], antibacterial[16,18,19], and antioxidant[18,20] have been revealed as the therapeutic properties of this plant. It has chemical antioxidant matters including pulegone, α and β pinen, flavonoids, terpenoides, piperitenone, sis-isopulegone, thymol and cineol[16,18,21,22]. Presumably, the remedial properties of this species are related to the above compounds.

The pathophysiology of Alzheimer's disease (AD) is related to the depletion of neurotransmitter acetylcholine (ACh) due to acetylcholinesterase (AChE) activity. One of the curative strategies (possible) is to increase the concentration of ACh in brain cells using AChE inhibitors. BChE enzyme is an enzyme used in cholinergic mechanism and is actively used in this system[23,24]. Cholinesterase inhibitors (ChEIs) are extensively used in medicine pharmacological correction of cholinergic neurotransmission pathology. In humans, α -glycosidase is secreted by the epithelial cells in small intestine which hydrolysis carbohydrate molecules into glucose units[25,26].

2 EXPERIMENTAL

2.1 Material

Phosphate buffer solution (PBS), Sabouraud Dextrose Agar, Sabouraud Dextrose Medium, Muller Hinton Agar, Mueller Hinton Medium, carbazole reagent, 4-(Dimethylamino) benzaldehyde, Dulbecco's Modified Eagle Medium (DMED), Ehrlich solution, dimethyl sulfoxide (DMSO), hydrolysate, decamplmaneh fetal bovine serum, borax-sulphuric acid

mixture, 2,2-diphenyl-1-picrylhydrazyl (DPPH), antimycotic and antibiotic solution overall were obtained from Sigma-Aldrich company, United States.

2.2 Synthesis of CdNPs

CdNPs (obtained by green synthesis) was started with a reaction mix of 50 mL of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ in the concentration of 0.15 M and 20 mL of aqueous extract solution of *Z. clinopodioides* leaf (100 $\mu\text{g/mL}$) in the proportion 1:10 in a conical flask (Figure 1). The reaction mixture was kept under magnetic stirring for 12 h at room temperature. After the reaction is complete, the yellow colored colloidal solution of Cd was formed. The compound was centrifuged at 10000 rpm for 15 minutes. The pellet was washed with distilled water in three replicates and centrifuged again for 15 minutes. Common organic chemistry techniques are used to analyze CdNPs. These analysis methods include FT-IR (Fourier Transform Infrared) and UV-Vis. spectroscopy is SEM, XRD (X-ray powder diffraction) and EDS (Energy-dispersive X-ray spectroscopy)[27].

2.3 Determination of antioxidant activity of cadmium nanoparticles (CdNPs) with DPPH

The DPPH (2,2-diphenyl-1-picrylhydrazyl) molecule has a stable free radical in which delocalization of the spare electron over the molecule, results the molecules do not dimerize. This delocalization results in the deep violet color with a characteristic absorption peak in ethanol solution at 517 nm. When the DPPH solution was mixed with electron donating substance, which leads to the reduced form with color loss. Later, the solution undergoes further reactions and control the stoichiometry, hence reduced (decolorized) the number of molecules of the DPPH by one molecule of the reductant[28].

In this experiment, 3 mL 0.004 % DPPH solution was mixed with several concentrations of $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodides*, CdNPs, and Daunorubicin for determining antioxidant properties. A group containing 3 mL ethanol and 3 mL DPPH was considered as the control group. According to the international standards, the samples absorption rate was determined at 517

nm and the antioxidant potentials of Cd(NO₃)₂, *Z. clinopodioides*, CdNPs, and Daunorubicin were assessed according to the following formula[28]:

$$\text{DPPH free radical scavenging (\%)} = (\text{Control} - \text{Test}/\text{Control}) \times 100$$

2.4 Analysis of cytotoxicity of CdNPs

In this experiment, Cd(NO₃)₂, *Z. clinopodioides*, CdNPs and Daunorubicin were examined for their cytotoxicity activities against 32D/FLT3-ITD, HUVEC, Murine C1498, and Human HL-60/vcr cell sheets. Cell lines (1 x 10⁵ cells / well) were coated separately using 96-well plates in "Dulbecco's Modified Eagle's Medium (DMEM)" with antimycotic antibiotic solution and 10% "Fetal Bovin Serum (FBS)". They were carried out to 96 well plates at a 1×10³ cell concentration and incubated, then treated with several dilutions of Cd(NO₃)₂, *Z. clinopodioides*, CdNPs, and Daunorubicin (0-1000 µg/mL). After 24 h at 37 °C incubation, 0.05 mg/mL of MTT (methylthiazole diphenyl tetrazolium) assay reagent was annexed to all wells. Following 24-hour incubation, MTT reagent was discarded rapidly (37 ° C) and cell lines were washed three times with PBS. Absorbance analysis was performed. Absorbance was examined at 570 nm. In our study, we used the equation given below for cell viability[29]:

$$\text{Cell Viability (\%)} = \frac{\text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

2.5 In vivo design

In the beginning of our study, DMBA injection was applied in 75 animals with AML induction 40 mg / kg twice a week for 6 weeks. One experimental group was selected as healthy control subjects. Normal saline was injected into this group (n=15). Six weeks after our application, 75 DMBA-treated subject mice, subjects without DMBA injection, and four groups that received 1 mg / kg intravenous Cd (NO₃)₂, CdNPs, *Z. clinopodioids* and Daunorubicin injection separately. During this period the mice underwent injection treatment for 24 days. The healthy control group received normal saline. Dosage was repeated for six

cycles of dosing. The subject mice were weighed seven times during the experiment. At the end of 24 days after the animals were anesthetized, blood samples were rapidly taken from the hearts of the animals. In addition, spleens of mice were also collected to analyze biochemical and stereological factors[30].

Central vein, hepatocyte, bile duct, portal vein, sinusoid and hepatic artery volumes in the liver were examined in serological parameters. In addition, white pulp, veins, red pulp, marginal region and follicle volumes in the removed spleen were investigated[13].

“Biochemical (SOD, CAT, GR, GPx, ALP, AST, ALT, GGT, cholesterol, LDL, HDL, triglyceride, total protein, albumin, total and conjugated bilirubin, glucose, urea, creatinine, ferrous, ferritin, and erythropoietin)”, “immunological [anti-inflammatory (IL4, IL5, IL10, IL13, and IFN α) cytokines and Pro-inflammatory (IL1, IL6, IL12, IL18, IFNY, and TNF α)]” parameter concentration was analyzed in serum by ELISA (Enzyme-Linked Immunosorbent Assay) and kits (Ziest-Chem Diagnostics) were determined and studied according to manufacturer's instructions[27,28]. Total in plasma; Automatic hematology analyzer was tested for blast, platelet, lymphocyte, neutrophil, monocyte, eosinophil, basophil, WBC, nRBC and RBC count and HB, PCV, MCV, MCH and MCHC ratios (Sysmex XS 800i)[30]. Analysis of the mRNA expression of S1PR5 and the real-time PCR in blood lymphocytes was performed at the cellular and molecular stages of the study[30].

All stereological, biochemical, immunological, hematological, and cellular and molecular analyses were done in duplicate.

2.6 Statistical analysis

The results were analyzed with SPSS-22 software and analyzed by one-way ANOVA and then "Duncan post-hoc test"(P = 0.01).

2.7. Enzyme studies

The inhibitory effect of novel nanoparticles ($\text{Cd}(\text{NO}_3)_2$ and CdNPs) on AChE and BChE activities was recorded according to spectrophotometric method of Ellman[31] as explained previously[32,33]. α -Glycosidase inhibition effect of novel silver nanoparticles ($\text{Cd}(\text{NO}_3)_2$ and CdNPs) was determined conforming to the assay of Tao et al.[34] The absorbance of samples were recorded at 405 nm as explained previously[35–37].

3 RESULTS AND DISCUSSION

3.1 Investigation of anti-acute (in vitro) myeloid leukemia feature of Cadmium nanoparticles (CdNPs)

In the current experiment, the treated cells with many concentrations of the present $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodioides*, CdNPs and Daunorubicin were examined by MTT test for 48h regarding the cytotoxicity property on normal (HUVEC) and AML cell lines (Figure 2). Absorbance at 570 nm, in the HUVEC cell line $\text{Cd}(\text{NO}_3)_2$; *Z.klinopodioids* showed exceptional vitality up to 1000 $\mu\text{g} / \text{mL}$ for CdNPs and Daunorubicin.

In the case of AML cell lines, the viability of them reduced dose-dependently in the presence of $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodides*, CdNPs, and Daunorubicin. The IC_{50} of $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodides*, CdNPs, and Daunorubicin against Murine C1498 cell line were 735, 380, 197, and 168 $\mu\text{g}/\text{mL}$, respectively, against 32D/FLT3-ITD cell line were 985, 500, 246 and 205 $\mu\text{g}/\text{mL}$, respectively. About the Human HL-60/vcr, the IC_{50} of *Z. clinopodides*, CdNPs, and Daunorubicin were 641, 342, and 210, respectively. The anticancer of cadmium nanoparticles was found to be highly dependent on a range of factors related to their physical properties, such as shape, surface coating, and size. About the size, it has been indicated that cadmium nanoparticles with small size can transfer of cell membrane of tumor cells and kill them. In the larger size, the above ability significantly is confined[38]. As seen in our study in figure 15, cadmium nanoparticles had uniform spherical morphology in size of 26.78 nm. The

size of cadmium nanoparticles in lower than 50 nm is very appropriate for the killing of tumor cell lines[39,40].

3.2 Antioxidant property of CdNPs against DPPH

In our research, *Z. clinopodides* leaf aqueous extract, Daunorubicin, CdNPs similar to BHT showed a considerable concentration-dependent DPPH radical scavenging effect. The IC₅₀ values of *Z. clinopodides* leaf aqueous extract, BHT, Daunorubicin, and CdNPs were 428, 414, 242, and 196, respectively (Figure 3).

The interaction between the *Z. clinopodides* leaf aqueous extract, Daunorubicin, and CdNPs and DPPH might have occurred through the transfer of electrons and hydrogen ions to 2,2-diphenyl-1-picrylhydrazyl radical to form a stable 2,2-diphenyl-1-picrylhydrazine molecule (DPPH)[41–44].

The antioxidant activity exhibited by *Z. clinopodioides* can be attributed to the presence of various phytochemicals that are thought to function synergistically and interactively to neutralize reactive nitrogen species (RNS) and reactive oxygen species (ROS)[45]. In the previous study determined that *Z. clinopodioides* was rich in antioxidant compounds such as pulegone, α and β pinen, flavonoids, terpenoides, sis-isopulegone, piperitenone, thymol and cineol[16,18,21,22]. These bioactive compounds have been shown to protect redox homeostasis by multi-stage antioxidant reaction processes of free radicals[45].

3.3 In vivo anti-acute myeloid leukemia properties of CdNPs

To induce the AML, DMBA was used. DMBA causes many problems in the body such as self-immune, hepatic, splenic, vascular, central nervous system, respiratory, and renal disorders and AML[46]. In details, DMBA-induced AML reduces the platelet, lymphocyte, and RBC counts and increase the WBC, blast, eosinophil, neutrophil, monocyte, and basophil.^[46]

222 The previous studies have been revealed that DMBA as Inflammation inducing agent,
 223 decreases the concentration of anti-inflammatory cytokines and raises the concentration of
 224 pro-inflammatory cytokines[47,48]. As is observed in Figure 4, the anti-inflammatory
 225 cytokines including IFN α , IL4, IL5, IL10, and IL13 reduced significantly ($p \leq 0.01$) and the
 226 pro-inflammatory cytokines including IFN γ , TNF α , IL1, IL6, IL12, and IL18 enhanced
 227 significantly ($p \leq 0.01$) in the DMBA-treated group.

228 Cd(NO₃)₂, *Z. clinopodioides* leaf aqueous extract, CdNPs and Daunorubicin significantly
 229 ($p \leq 0.01$) ameliorated the anti-inflammatory cytokines and pro-inflammatory as compared to
 230 the untreated group. The best results have been seen in the CdNPs-treated group.

231 Also, DMBA is known to induce AML disease mixed from lymphoblastic leukemia,
 232 myelogenous leukemia, thymic leukemia, and hepatic leukemia of erythroblastic stem
 233 cells[38]. In the previous study, it has been shown that DMBA with enhancing the number of
 234 blast counts to 10-15 percent of WBC, induces AML[49].

235 In the present study, the number of total WBC, blasts, eosinophil, neutrophil, monocyte,
 236 basophil and nRBC counts raised significantly ($p \leq 0.01$). The number of RBC, platelet, and
 237 lymphocyte counts and the other RBC parameters including Hb, PCV, MCV, MCH and
 238 MCHC decreased significantly ($p \leq 0.01$) in the DMBA-treated group (Figures 5,6). The above
 239 results show that DMBA successfully induced AML in mice.

240 Cd(NO₃)₂, *Z. clinopodides* leaf aqueous extract, CdNPs, and Daunorubicin ameliorated
 241 significantly ($p \leq 0.01$) the hematological parameters. The best results have been seen in
 242 CdNPs and Daunorubicin-treated group. In the present study, the anti-hemolytic activity of
 243 Cd(NO₃)₂, *Z. clinopodides* leaf aqueous extract, CdNPs, and Daunorubicin may be associated
 244 with their antioxidant potentials. Different studies have reported that antioxidant compounds
 245 omit the free radicals of hematotoxic substances and inhibit their toxic effects[50–52]. Also,

246 many studies similar to our study revealed significant antioxidant effects of cadmium
247 nanoparticles[10,11].

248 In our study, mRNA expression of S1PR1 in lymphocytes increased by 33.2, 8.1, 14.7, 20,
249 and 23.1 folds in control, Cd(NO₃)₂, *Z. clinopodides* leaf aqueous extract, CdNPs and
250 Daunorubicin, respectively compared to the untreated group. Also, the findings of S1PR5
251 mRNA expression revealed that the number of folds increased 36.8, 12.1, 17.3, 24.2, and 26.9
252 in control, Cd(NO₃)₂, *Z. clinopodides* leaf aqueous extract, CdNPs, and Daunorubicin,
253 respectively in comparison to the untreated group. The best results were seen in groups treated
254 with Daunorubicin and CdNP (Figure 7).

255 In this study, DMBA-induced AML significantly ($p \leq 0.01$) increased the concentrations of
256 ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin,
257 glucose, urea, creatinine, ferrous, ferritin, and erythropoietin and decreased HDL, total protein
258 and albumin in serum. Cd(NO₃)₂, *Z. clinopodides* leaf aqueous extract, CdNPs, and
259 Daunorubicin significantly ($p \leq 0.01$) ameliorated the above parameters. There were no
260 significant changes ($p \leq 0.01$) between CdNPs, Daunorubicin, and control groups in GGT,
261 triglyceride, glucose, and creatinine levels. The best results in improving the biochemical
262 parameters were seen in the CdNPs-treated group (Table 1).

263 In the recent study, the levels of the liver, spleen, and serum SOD, CAT, and GPx increased
264 and GR reduced significantly ($p \leq 0.01$) at Cd(NO₃)₂, *Z. clinopodides* leaf aqueous extract,
265 CdNPs, and Daunorubicin-treated mice in comparison to the untreated group. Agreement with
266 our study, in the study of Saravanan and Ponmurugan (2013), it revealed the strong
267 antioxidant effect of medicinal plants with ameliorating the concentrations of SOD, CAT, and
268 GPx[53]. In another study, it was reported that the medicinal plant extracts had excellent
269 antioxidant property, because they raised the concentration of antioxidant enzymes including
270 CAT, SOD, and GPx as compared to the tartrazine-received group (untreated group)[54].

271 Also, in the study of Ogunlade *et al.* (2012), it was revealed that the plant extracts, with
272 raising the degradation of free radicals, increased the concentrations of CAT, GPx, SOD and
273 MDA and decreased glutathione (GSH) in animals with alcohol-induced hepatotoxicity[55].
274 In the present study, the body weight of the untreated group decreased ($p \leq 0.01$) after six
275 weeks of acute myeloid leukemia induction.

276 Cancer cachexia is described as a wasting syndrome involving loss of muscle mass and fat
277 directly caused by tumor factors[56]. But, the administration of $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodides*
278 leaf aqueous extract, CdNPs, and Daunorubicin raised ($p \leq 0.01$) the body weight in
279 comparison to the untreated group (Figure 8).

280 The results analysis of Figures 9-11 indicated that DMBA significantly ($p \leq 0.01$) increase the
281 leukemic myeloblasts infiltrating in the spleen and liver. With this infiltrating, the volumes,
282 weights of spleen, liver and their sub-compartments raised in the untreated as compared to
283 other groups.

284 In detail, DMBA raised significantly ($p \leq 0.01$) the volumes of hepatocyte, central vein, portal
285 vein, sinusoid, bile duct, and hepatic artery in the liver and the volumes of white pulp,
286 marginal zone, and follicle in the spleen.

287 Treatment with $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodides* leaf aqueous extract, CdNPs, and Daunorubicin
288 reduced significantly ($p \leq 0.01$) the leukemic myeloblasts infiltrating in the spleen and liver.
289 The best results were observed in the Daunorubicin-treated and CdNPs groups.

290 About the hepatoprotective activities of plant extracts, it was revealed that they reduced the
291 levels of ALP, AST, and ALT in volume of the mice liver, hepatic artery, central vein and
292 portal vein in diabetic mice[57].

293 Besides, Goodarzi *et al.* (2017) has shown that extracts reduce rised concentrations of hepatic
294 biochemical parameters alike ALT, AST and ALP. Rate of liver, sinusoids and hepatocytes
295 has also been shown to decrease compared to the group treated with CCl_4 [58].

3.4 Characterization of CdNPs

EDS can be used as a semi-quantitative method of synthesis. The result of EDS (Figure 12) shows the composition profile of CdNs obtained by synthesis. In the synthesized nanoparticles as a result of the study, the result of the tests, cadmium and sulfur presence was reported and confirmed with the displayed signals. The peaks at 3.16 keV and 3.34 keV corresponded to the $L\alpha$ and $L\beta$ peaks of cadmium while the peaks at around 2.3-2.5 keV corresponded to the $K\alpha$ and $K\beta$ peaks of sulfur. This study confirmed many previous studies. Signals measured in the study are based on a previous study on the synthesized CdNPs using *C. reinhardtii* extract;[59] The other signals including $Ok\alpha$ and $CK\alpha$ belong to the organic molecules present in *Z. clinopodioides* extract that linked to the surface of CdNPs. Structural crystallinity of CdNPs was analyzed using XRD images. The image showed in Figure 13. As a result of the study, it was determined that there was cadmium sulfide synthesis consistent with FTIR results. The peak location with 2θ values of 26.68, 44.16, 52.47, and 70.65 are indexed as (111), (202), (311), and (313) planes which are in good agreement with those of clinic phase CdS obtained from standard database ICDD PDF card no. 96-900-0068. The crystalline structure of the sample obtained from the diffractogram is evident in the middle. Since the sample was pure in our study, the presence of regular and appropriate peaks was evaluated. Peaks occurring at different rates have been recorded before [60–62]. The approximate crystal size of CdNPs was determined according to the X-ray diffraction theory according to the “Scherrer inference”.

$$D = \frac{k\lambda}{\beta \cos \theta}$$

I-“k is the Scherrer’s constant (k=0.94),”

II-“ β is the full-width at half-maximum of the corresponding plane (in radians),”

III-“ λ is the wavelength of X-ray radiation ($\lambda=0.15406$ nm),”

320 IV-“ θ is the characteristic X-ray radiation.”

321 (CdNPs had an average crystal dimension of 25.65 nm.)

322 When we examine the previous literature, cadmium sulfide and cadmium oxide are the
323 common products of the biosynthesis of cadmium nanoparticles. The researches have
324 reported various crystal sizes for the biosynthetic CdSNPs or CdONPs according to the XRD
325 patterns. Instead of, for CdSNPs the crystal size of 3.42 nm has been reported for the synthetic
326 CdSNPs using *Annona muricata* leaf extract; [22] 6 nm for *Chlamydomonas reinhardtii*; [48]
327 1.47 nm for Banana peel extract; [49] 18 nm and 12 nm for *Calotropis gigantea* leaf
328 extract; [51] 5.67 nm for *Mimosa pudica*; [63–66] 4 nm for *Murraya koenigii* extract [67]. On
329 the other hand, 10.86 nm have been reported for the synthetic CdONPs using *Limonia*
330 *acidissima* leaves extract; [68] 18.5–61.9 nm for *Abelmoschus esculentus*; [69] 22 nm for
331 *Andrographis paniculata* leaf extract; [70] 25 to 50 nm for *Agathosma betulina*; [71] 22.9 and
332 35.0 nm for *Hibiscus sabdariffa* flower extract; [72] 44–56 nm for *Aloe barbadensis*; [73]
333 28.92 to 44.95 nm for *Parkia speciosa* seed extract [74].

334 In FT-IR images, peaks in the region between 500 and 700 cm^{-1} are connected to metal-
335 oxygen vibration. In this study, the cadmium sulfide nanoparticles nascently confirms by the
336 set of peaks at 524, 621, and 678 cm^{-1} belong to the bending vibration of Cd-S. The
337 appearance of these peaks, with a small difference in the number of wave has been reported
338 previously for biosynthetic CdNPs [48,62,63]. The presence of secondary metabolites, which
339 are responsible for the capping and reduction of cadmium nitrate precursor to CdNPs, has
340 been studied using the FT-IR technique. According to the results of our study, *Z. clinopodioides*
341 extract and CdNPs (Figure 14) gave images like FT-IR spectra that biosynthesize CdNPs.
342 The presence of dissimilar-IR bands interested to the existence of various functional groups in
343 *Z. clinopodioides* extract. For example, the peaks in 3371 and 2939 cm^{-1} related to “O-H” and
344 aliphatic “C-H stretching”; the peaks at a range of 1421 to 1749 cm^{-1} correspond to “C=C and

345 C=O stretching”, and the peaks at 1261 and 1081 cm^{-1} could be ascribed to “-C-O and -C-O-C
346 stretching”. The peaks we observe are proof that diversified compounds exist, like as
347 phenolic, flavonoid and carboxylic materials showed in previous studies [64] Ghidan et al.
348 ((2016) reported that biomolecules are bound to CdNPs by hydroxyl and carbonyl as plant
349 extracts, as stabilizing, dispersing and reducing agents [66].

350 When the surface morphology and size of CdNPs are examined in detail, and for that using
351 FE-SEM, it was reported Figure 15 (a-d). The figure depicts a successful synthesis of CdSNPs
352 with spherical morphology that was reported previously [75]. As understood from the graphs,
353 it shows that the homogeneity of the synthesized CdNPs is well distributed. A trend towards
354 aggregation is reported for synthesized NPs. This property of metallic nanoparticles
355 synthesized by green synthesis has been previously reported (for example, CdNP, AuNP,
356 AgNP and TiNP can be given) [48,55,76,77]. The average diameter of the nanoparticle size in
357 CdNPs was reported as 26.78 nm. This dimension is very similar to the result obtained from
358 XRD analysis.

359 In the academic studies we examined for our study, different sizes have been analyzed for the
360 green synthesis CdSNPs or CdONPs using herbal extracts. In Instance, 3 to 5 nm has been
361 reported for synthetic CdSNPs using tea leaf extract;[78] 4 nm for *A. muricata*:[23] 2-7 nm
362 for *C. reinhardtii*:[48] 1.48 nm for banana peel extract;[49] 5-20 nm for Fungus of *Fusarium*
363 *oxysporum*:[50] 20 nm for *C. gigantea* leaf extract;[51] and 20 nm for *M. Koenigii* [56]. On
364 the contrary, bigger sizes have been reported for biosynthetic CdONPs: 10-30 nm for *Limonia*
365 *acidissima* leaves extract;[57] 78 nm for *A. esculentus* fruit extract;[58] 164 nm for *A.*
366 *paniculata* leaf extract;[59] 100 nm for *A. betulina*:[60] 113 nm for *H. Sabdariffa* flower
367 extract;[61] 97-129 nm for *A. Barbadosensis*:[62] 20-50 nm for *P. speciosa* seed extract;[63]
368 and 36-57 nm for *Leucaena leucocephala*.

UV-Vis. The spectra of CdNPs produced by green synthesis with the extract of *Z. clinopodioids* are as shown in Figure 16. Optical features taken from UV-Vis showed that CdNPs were synthesized by spectroscopy. A wide absorbance of 390-450 nm, synthetic CdNPs are included. In addition to these data, due to the reduction in granul dimension, a blue change consist of compared to the stack CdS (515 nm). This observation overlaps with the previously reported data on biosynthesized CdNps [48,50]. This band is also characteristic of CdS particles in the quantum size range [50].

3.5. Enzyme results

3.5.1. Cholinesterase enzymes inhibition results

All of novel nanoparticles ($\text{Cd}(\text{NO}_3)_2$ and **CdNPs**) had remarkably forward AChE restrictively effect than of control AChE inhibitor compound such as Tacrine. I When we evaluate, the K_i values of these innovative nanoparticles and standard compound (tacrin) synthesized are clearly seen in Table 2. These novel nanoparticles effectively inhibited AChE, with K_i values of 7.22 ± 1.23 and 6.80 ± 1.30 mM. Then, all these novel nanoparticles had almost similar inhibition profiles. The most active **CdNPs** showed K_i value of 6.80 ± 1.30 mM. For AChE, IC_{50} values of TAC as positive control and novel nanoparticles were studied in this study the following order: **CdNPs** (8.64 mM, r^2 : 0.9839) < $\text{Cd}(\text{NO}_3)_2$ (11.38 mM, r^2 : 0.9344) < **TAC** (19.53 mM, r^2 : 0.9703). For BChE, IC_{50} values of TAC as positive control and novel nanoparticles the following order: **CdNPs** (14.22 mM, r^2 : 0.9361) < $\text{Cd}(\text{NO}_3)_2$ (18.32 mM, r^2 : 0.9328) < **TAC** (25.54 mM, r^2 : 0.9342). Additionally, the novel nanoparticles effectively inhibited BChE, with K_i values of 12.68 ± 1.67 and 10.94 ± 2.32 mM, respectively. However, all of these novel nanoparticles had almost similar inhibition profiles. The most active **CdNPs** effectively inhibited BChE, with K_i value of 10.94 ± 2.32 mM.

3.5.2. α -Glycosidase inhibition results

Novel nanoparticles (**Cd(NO₃)₂** and **CdNPs**) have IC₅₀ values of 3.51 and 0.76 μM and Ki values of 5.87±0.48 and 1.74±0.13 mM (Table 2) (for enzyme glycosidase). The results obtained have shown that all of the newly synthesized compounds show inhibitory effects of the α-glycosidase enzyme acarbose (IC₅₀: 5.37 mM) as a control glycosidase inhibitor. Indeed, the most effective Ki value of **CdNPs** was 1.74±0.13 mM, respectively. For this metabolic enzyme, IC₅₀ values and new nanoparticles are used in the following order when ACR is used as a control: **CdNPs** (0.76 mM, r²: 0.9788) < **Cd(NO₃)₂** (3.51 mM, r²: 0.9359) < **ACR** (5.37 mM, r²: 0.9816). α-Glycosidase inhibitors can delay carbohydrate digestion and thereby helping to lower postprandial blood sugar levels. These inhibitors such as voglibose, acarbose and miglitol, have been used as first-line therapies for type 2 diabetes in the clinical setting [79,80].

4 CONCLUSION

In the present experiment, *Z. clinopodioides* leaf aqueous extract-based CdNPs were successfully obtained. UV-Vis, FE-SEM, FT-IR, EDS and XRD were analyzed in the closest way to perfection. *In vivo* area, CdNPs importantly ($p \leq 0.01$) coordinated the chapters of biochemical, immunological, histopathological, hematological cellular-molecular parameters like as daunorubicin. In the *in vitro* area, CdNPs have a low cell viability due to cell lines determined without cytotoxicity in the HUVEC cell line. In the light of these reported results, it appears that CdNPs may be applied for the treatment of AML in humans. AD can be stabilized by inhibition of cholinesterases (i.e. it can be kept under control); however, BChE is rare and localized in peripheral tissues. Thus, the discovery of new selective AChE inhibitors with environmental inhibition determined can be considered the therapeutic effect for the treatment of AD. Nanoparticles can be specified as good suppressors for the treatment of the disease. On the other hand, these compounds are antidiabetic and anticholinergic; due to these situations, it is hoped that it can be used in important drug applications for the future.

418 **Abbreviations**

419 AML: anti-acute myeloid leukemia

420 CdNPs : conjugated cadmium nanoparticles

421 FTIR: Fourier-Transform Infrared Spectroscopy,

422 U.Vis.: Ultraviolet-Visible Spectroscopy

423 TEM: Transmission Electron Microscopy.

424 DPPH: 2,2-diphenyl-1-picrylhydrazyl

425 HUVECs: Human Umbilical Vein Endothelial Cells

426 **Acknowledgements**

427 Not applicable.

428 **Funding**

429 This study is not supported by any financial support.

430 **Author Information**

431 **Affiliations**

432 Department of Chemistry, Hakim Sabzevari University, Sabzevar ,Iranian,

433 **Behnam Mahdavi**

434 Department of Chemistry, Hakim Sabzevari University, Sabzevar ,Iranian,

435 **Sogand Paydarfar**

436 Department of Chemistry, Hakim Sabzevari University, Sabzevar ,Iranian,

437 **Esmail Rezaeiseresht**

438 Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University,

439 Kermanshah, Iran and Biotechnology and Medicinal Plants Research Center, Ilam University

440 of Medical Sciences, Ilam, Iran.

441 **Akram Zangeneh & Mohammad Mahdi Zangeneh**

442 Department of Chemistry, Faculty of Sciences, Ataturk University, 25240, Erzurum, Turkey,

443 **Nastaran Sadeghian**

444 Department of Biotechnology, Faculty of Science, Bartın University, 74100 Bartın, Turkey,

445 **Parham Taslimi**

446 Sen Research Group, Department of Biochemistry, Faculty of Arts and Science, Dumlupınar

447 University, Evliya Çelebi Campus, 43100, Kütahya, Turkey,

448 **Fatih Sen & Ismail Mert Alkac,**

449 **Authors' contributions**

450 B.M., M.M.Z. and F.S. organized all experiments and wrote the manuscript.; S.P., E.R-S.,

451 A.Z., N.S., P.T. and I.M.A. performed all experiments. They have also drawn the figures.

452 **Corresponding author**

453 Correspondence to Behnam Mahdavi, Mohammad Mahdi Zangeneh and Fatih Sen

454 **Availability of data and materials**

455 All data generated or analyzed during this study are included in this article.

456 **Ethics Declarations**

457 **Ethics approval and consent to participate**

458 Not applicable for this study.

459 **Consent for publication**

460 We give our consent for the manuscript to be published in Journal of Nanobiotechnology.

461 **Competing interests**

462 The authors declare that they have no competing interests.

463 ***Compliance with ethical standards***

464 This study complies with ethical standards.

465

466

467

REFERENCES

- [1] Raut RW, Kolekar NS, Lakkakula JR, Mendhulkar VD, Kashid SB. Extracellular synthesis of silver nanoparticles using dried leaves of *pongamia pinnata* (L) pierre. Nano-Micro Lett 2010;2:106–13. <https://doi.org/10.1007/bf03353627>.
- [2] Varma RS. Greener approach to nanomaterials and their sustainable applications. Curr Opin Chem Eng 2012;1:123–8. <https://doi.org/10.1016/j.coche.2011.12.002>.
- [3] Arunachalam KD, Annamalai SK, Hari S. One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from *Memecylon umbellatum*. Int J Nanomedicine 2013;8:1307–15. <https://doi.org/10.2147/IJN.S36670>.
- [4] Sintubin L, De Windt W, Dick J, Mast J, Van Der Ha D, Verstraete W, et al. Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Appl Microbiol Biotechnol 2009;84:741–9. <https://doi.org/10.1007/s00253-009-2032-6>.
- [5] Ball V. Polydopamine nanomaterials: Recent advances in synthesis methods and applications. Front Bioeng Biotechnol 2018;6:109. <https://doi.org/10.3389/fbioe.2018.00109>.
- [6] Zangeneh MM, Bovandi S, Gharehyakheh S, Zangeneh A, Irani P. Green synthesis and chemical characterization of silver nanoparticles obtained using *Allium saralicum* aqueous extract and survey of *in vitro* antioxidant, cytotoxic, antibacterial and antifungal properties. Appl Organomet Chem 2019;33. <https://doi.org/10.1002/aoc.4961>.
- [7] Singh P, Pandit S, Mokkapati VRSS, Garg A, Ravikumar V, Mijakovic I. Gold Nanoparticles in Diagnostics and Therapeutics for Human Cancer. Int J Mol Sci 2018;19:1979. <https://doi.org/10.3390/ijms19071979>.

- 493 [8] Hemmati S, Joshani Z, Zangeneh A, Zangeneh MM. Biosynthesis and chemical
494 characterization of polydopamine-capped silver nanoparticles for the treatment of acute
495 myeloid leukemia in comparison to doxorubicin in a leukemic mouse model. Appl
496 Organomet Chem 2020;34. <https://doi.org/10.1002/aoc.5277>.
- 497 [9] Zangeneh MM. Green synthesis and formulation a modern chemotherapeutic drug of
498 *Spinacia oleracea* L. leaf aqueous extract conjugated silver nanoparticles; Chemical
499 characterization and analysis of their cytotoxicity, antioxidant, and anti-acute myeloid
500 leukemia properties in comparison to doxorubicin in a leukemic mouse model. Appl
501 Organomet Chem 2020;34. <https://doi.org/10.1002/aoc.5295>.
- 502 [10] Hossain ST, Mukherjee SK. Toxicity of cadmium sulfide (CdS) nanoparticles against
503 *Escherichia coli* and HeLa cells. J Hazard Mater 2013;260:1073–82.
504 <https://doi.org/10.1016/j.jhazmat.2013.07.005>.
- 505 [11] Heidari A. Anti-Cancer Effect of UV Irradiation at Presence of Cadmium Oxide (CdO)
506 Nanoparticles on DNA of Cancer Cells: A Photodynamic Therapy Study. Arch Cancer
507 Res 2016;4. <https://doi.org/10.21767/2254-6081.100061>.
- 508 [12] Soni A, Krishnamurthy R. Plants-The Next Generation Treatment Of Leukemia. Indian
509 J Plant Sci 2013;2:117–25.
- 510 [13] Hagh-Nazari L, Goodarzi N, Zangeneh MM, Zangeneh A, Tahvilian R, Moradi R.
511 Stereological study of kidney in streptozotocin-induced diabetic mice treated with
512 ethanolic extract of *Stevia rebaudiana* (bitter fraction). Comp Clin Path 2017;26:455–
513 63. <https://doi.org/10.1007/s00580-016-2398-7>.
- 514 [14] Ahmeda A, Zangeneh A, Zangeneh MM. Green synthesis and chemical
515 characterization of gold nanoparticle synthesized using *Camellia sinensis* leaf aqueous
516 extract for the treatment of acute myeloid leukemia in comparison to daunorubicin in a
517 leukemic mouse model. Appl Organomet Chem 2020;34.

- 518 <https://doi.org/10.1002/aoc.5290>.
- 519 [15] Naghibi F, Mosaddegh M, Mohammadi Motamed M, Ghorbani A. Labiatae Family in
520 folk Medicine in Iran: from Ethnobotany to Pharmacology. Iran J Pharm Res IJPR
521 2005;4:63–79. <https://doi.org/10.22037/ijpr.2010.619>.
- 522 [16] Behravan J, Ramezani M, Hassanzadeh MK, Eskandari M, Kasaian J, Sabeti Z.
523 Composition, Antimycotic and Antibacterial Activity of *Ziziphora clinopodioides* Lam.
524 Essential Oil from Iran. J Essent Oil-Bearing Plants 2007;10:339–45.
525 <https://doi.org/10.1080/0972060X.2007.10643565>.
- 526 [17] Sonboli A, Mirjalili MH, Hadian J, Ebrahimi SN, Yousefzadi M. Antibacterial activity
527 and composition of the essential oil of *Ziziphora clinopodioides* subsp. *bungeana* (Juz.)
528 Rech. f. from Iran. Zeitschrift Fur Naturforsch - Sect C J Biosci 2006;61:677–80.
529 <https://doi.org/10.1515/znc-2006-9-1011>.
- 530 [18] Salehi P, Sonboli A, Eftekhari F, Nejad-Ebrahimi S, Yousefzadi M. Essential oil
531 composition, antibacterial and antioxidant activity of the oil and various extracts of
532 *Ziziphora clinopodioides* subsp. *rigida* (BOISS.) RECH. f. from Iran. Biol Pharm Bull
533 2005;28:1892–6. <https://doi.org/10.1248/bpb.28.1892>.
- 534 [19] Chen Z, Yuan Y, Zhou H, Wang X, Gan Z, Wang F, et al. 3D Nanocomposite
535 architectures from carbon-nanotube-threaded nanocrystals for high-performance
536 electrochemical energy storage. Adv Mater 2014;26:339–45.
537 <https://doi.org/10.1002/adma.201303317>.
- 538 [20] Ghafari H, Yasa N, Mohammadirad A, Dehghan G, Zamani MJ, Nikfar S, et al.
539 Protection by *Ziziphora clinopoides* of acetic acid-induced toxic bowel inflammation
540 through reduction of cellular lipid peroxidation and myeloperoxidase activity. Hum
541 Exp Toxicol 2006;25:325–32. <https://doi.org/10.1191/0960327105ht626oa>.
- 542 [21] Mahdavi B, Paydarfard S, Zangeneh MM, Goorani S, Seydi N, Zangeneh A.

- Assessment of antioxidant, cytotoxicity, antibacterial, antifungal, and cutaneous wound healing activities of green synthesized manganese nanoparticles using *Ziziphora clinopodioides* Lam leaves under *in vitro* and *in vivo* condition. Appl Organomet Chem 2020;34. <https://doi.org/10.1002/aoc.5248>.
- [22] Belyaev NF, Demeubaeva AM. Chromatographic study of the composition of the essential oil of *Ziziphora clinopodioides*, a vicarious form of *Origanum vulgare*. Chem Nat Compd 1999;35:52–4. <https://doi.org/10.1007/BF02238209>.
- [23] Erdemir F, Celepci DB, Aktaş A, Gök Y, Kaya R, Taslimi P, et al. Novel 2-aminopyridine liganded Pd(II) N-heterocyclic carbene complexes: Synthesis, characterization, crystal structure and bioactivity properties. Bioorg Chem 2019;91:103134. <https://doi.org/10.1016/j.bioorg.2019.103134>.
- [24] Boztas M, Taslimi P, Yavari MA, Gulcin I, Sahin E, Menzek A. Synthesis and biological evaluation of bromophenol derivatives with cyclopropyl moiety: Ring opening of cyclopropane with monoester. Bioorg Chem 2019;89:103017. <https://doi.org/10.1016/j.bioorg.2019.103017>.
- [25] Genç Bilgiçli H, Kestane A, Taslimi P, Karabay O, Bytyqi-Damoni A, Zengin M, et al. Novel eugenol bearing oxypropanolamines: Synthesis, characterization, antibacterial, antidiabetic, and anticholinergic potentials. Bioorg Chem 2019;88:102931. <https://doi.org/10.1016/j.bioorg.2019.102931>.
- [26] Bursal E, Aras A, Kılıç Ö, Taslimi P, Gören AC, Gülçin İ. Phytochemical content, antioxidant activity, and enzyme inhibition effect of *Salvia eriophora* Boiss. & Kotschy against acetylcholinesterase, α -amylase, butyrylcholinesterase, and α -glycosidase enzymes. J Food Biochem 2019;43:e12776. <https://doi.org/10.1111/jfbc.12776>.
- [27] Durga B, Raziya S, Rajamahanti SG, Govindh B, Raju KV, Annapurna N. Synthesis and Characterization of Cadmium Sulphide Nanoparticles Using *Annona Muricata*

568 Leaf Extract as Reducing/Capping Agent 2016;5:1035–41.
 569 <https://doi.org/10.7598/cst2016.1291>.

570 [28] Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the
 571 antiradical efficiency of polyphenols. *J Sci Food Agric* 1998;76:270–6.
 572 [https://doi.org/10.1002/\(SICI\)1097-0010\(199802\)76:2<270::AID-JSFA945>3.0.CO;2-](https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9)
 573 9.

574 [29] Arulmozhi V, Pandian K, Mirunalini S. Ellagic acid encapsulated chitosan
 575 nanoparticles for drug delivery system in human oral cancer cell line (KB). *Colloids*
 576 *Surfaces B Biointerfaces* 2013;110:313–20.
 577 <https://doi.org/10.1016/j.colsurfb.2013.03.039>.

578 [30] Kabeel MM, Ghoneim AM, Mansy SE. Anti-leukemic activity of a four-plant mixture
 579 in a leukemic rat model. *J Basic Appl Zool* 2018;79:1–12.
 580 <https://doi.org/10.1186/s41936-018-0019-5>.

581 [31] Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric
 582 determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;7:88–95.
 583 [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9).

584 [32] Cetin A, Türkan F, Taslimi P, Gulçin İ. Synthesis and characterization of novel
 585 substituted thiophene derivatives and discovery of their carbonic anhydrase and
 586 acetylcholinesterase inhibition effects. *J Biochem Mol Toxicol* 2018;33:e22261.
 587 <https://doi.org/10.1002/jbt.22261>.

588 [33] Turkan F, Cetin A, Taslimi P, Karaman M, Gulçin İ. Synthesis, biological evaluation
 589 and molecular docking of novel pyrazole derivatives as potent carbonic anhydrase and
 590 acetylcholinesterase inhibitors. *Bioorg Chem* 2019;86:420–7.
 591 <https://doi.org/10.1016/j.bioorg.2019.02.013>.

592 [34] Tao Y, Zhang Y, Cheng Y, Wang Y. Rapid screening and identification of α -

glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. Biomed Chromatogr 2013;27:148–55. <https://doi.org/10.1002/bmc.2761>.

[35] Eruygur N, Koçyiğit UM, Taslimi P, Ataş M, Tekin M, Gülçin. Screening the in vitro antioxidant, antimicrobial, anticholinesterase, antidiabetic activities of endemic *Achillea cucullata* (Asteraceae) ethanol extract. South African J Bot 2019;120:141–5. <https://doi.org/10.1016/j.sajb.2018.04.001>.

[36] Demir Y, Taslimi P, Ozaslan MS, Oztaskin N, Çetinkaya Y, Gulçin İ, et al. Antidiabetic potential: *In vitro* inhibition effects of bromophenol and diarylmethanones derivatives on metabolic enzymes. Arch Pharm (Weinheim) 2018;351:1800263. <https://doi.org/10.1002/ardp.201800263>.

[37] Huseynova M, Taslimi P, Medjidov A, Farzaliyev V, Aliyeva M, Gondolova G, et al. Synthesis, characterization, crystal structure, electrochemical studies and biological evaluation of metal complexes with thiosemicarbazone of glyoxylic acid. Polyhedron 2018;155:25–33. <https://doi.org/10.1016/j.poly.2018.08.026>.

[38] Martin JP, Dailey M, Sugarman E. Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. Arch Biochem Biophys 1987;255:329–36. [https://doi.org/10.1016/0003-9861\(87\)90400-0](https://doi.org/10.1016/0003-9861(87)90400-0).

[39] Ho YS, Magnenat JL, Bronson RT, Cao J, Gargano M, Sugawara M, et al. Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. J Biol Chem 1997;272:16644–51. <https://doi.org/10.1074/jbc.272.26.16644>.

[40] Namvar F, Rahman HS, Mohamad R, Baharara J, Mahdavi M, Amini E, et al. Cytotoxic effect of magnetic iron oxide nanoparticles synthesized via seaweed aqueous extract. Int J Nanomedicine 2014;9:2479–88. <https://doi.org/10.2147/IJN.S59661>.

- 618 [41] Kalita P, Tapan BK, Pal TK, Kalita R. ESTIMATION OF TOTAL FLAVONOIDS
619 CONTENT (TFC) AND ANTI OXIDANT ACTIVITIES OF METHANOLIC
620 WHOLE PLANT EXTRACT OF BIOPHYTUM SENSITIVUM LINN. J Drug Deliv
621 Ther 2013;3:33–7. <https://doi.org/10.22270/jddt.v3i4.546>.
- 622 [42] Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay.
623 J Food Sci Technol 2011;48:412–22. <https://doi.org/10.1007/s13197-011-0251-1>.
- 624 [43] Matthäus B. Antioxidant activity of extracts obtained from residues of different
625 oilseeds. J Agric Food Chem 2002;50:3444–52. <https://doi.org/10.1021/jf011440s>.
- 626 [44] Aliev G, Palacios HH, Lipsitt AE, Fischbach K, Lamb BT, Obrenovich ME, et al.
627 Nitric oxide as an initiator of brain lesions during the development of Alzheimer
628 disease. Neurotox Res 2009;16:293–305. <https://doi.org/10.1007/s12640-009-9066-5>.
- 629 [45] Ishino K, Wakita C, Shibata T, Toyokuni S, Machida S, Matsuda S, et al. Lipid
630 peroxidation generates body odor component trans-2-nonenal covalently bound to
631 protein in vivo. J Biol Chem 2010;285:15302–13.
632 <https://doi.org/10.1074/jbc.M109.068023>.
- 633 [46] Nishimura H, Akiyama T, Monobe Y, Matsubara K, Igarashi Y, Abe M, et al.
634 Expression of sphingosine-1-phosphate receptor 1 in mantle cell lymphoma. Mod
635 Pathol 2010;23:439–49. <https://doi.org/10.1038/modpathol.2009.194>.
- 636 [47] Abdalla DR, Rocha Aleixo AA, Murta EFC, Michelin MA. Innate immune response
637 adaptation in mice subjected to administration of DMBA and physical activity. Oncol
638 Lett 2014;7:886–90. <https://doi.org/10.3892/ol.2013.1774>.
- 639 [48] Yusuf N, Nasti TH, Meleth S, Elmetts CA. Resveratrol enhances cell-mediated immune
640 response to DMBA through TLR4 and prevents DMBA induced cutaneous
641 carcinogenesis. Mol Carcinog 2009;48:713–23. <https://doi.org/10.1002/mc.20517>.
- 642 [49] Sugiyama T, Osaka M, Koami K, Maeda S, Ueda N. 7,12-DMBA-induced rat

- 643 leukemia: A review with insights into future research. *Leuk Res* 2002;26:1053–68.
 644 [https://doi.org/10.1016/S0145-2126\(02\)00045-0](https://doi.org/10.1016/S0145-2126(02)00045-0).
- 645 [50] Korotkova EI, Misini B, Dorozhko E V., Bukkel M V., Plotnikov E V., Linert W.
 646 Study of OH● Radicals in Human Serum Blood of Healthy Individuals and Those with
 647 Pathological Schizophrenia. *Int J Mol Sci* 2011;12:401–9.
 648 <https://doi.org/10.3390/ijms12010401>.
- 649 [51] Vladimirov YA, Lyubitsky O, Babenkova IV, teselkin Yu.O.klabenov G.I. Inhibition
 650 by serum antioxidants of luminol oxidation in the presence of hemoglobin and
 651 hydrogen peroxide. *Probl Med Chem* 1997;43:87–93.
- 652 [52] Kluk MJ, Ryan KP, Wang B, Zhang G, Rodig SJ, Sanchez T. Sphingosine-1-phosphate
 653 receptor 1 in classical Hodgkin lymphoma: Assessment of expression and role in cell
 654 migration. *Lab Invest* 2013;93:462–71. <https://doi.org/10.1038/labinvest.2013.7>.
- 655 [53] Saravanan G, Ponmurugan P. S-allylcysteine improves streptozotocin-induced
 656 alterations of blood glucose, liver cytochrome P450 2E1, plasma antioxidant system,
 657 and adipocytes hormones in diabetic rats. *Int J Endocrinol Metab* 2013;11.
 658 <https://doi.org/10.5812/ijem.10927>.
- 659 [54] Moradi R, Hajialiani M, Salmani S, Almasi M, Zangeneh A, Zangeneh MM. Effect of
 660 aqueous extract of *Allium saralicum* R.M. Fritsch on fatty liver induced by high-fat diet
 661 in Wistar rats. *Comp Clin Path* 2019;28:1205–11. [https://doi.org/10.1007/s00580-018-](https://doi.org/10.1007/s00580-018-2834-y)
 662 [2834-y](https://doi.org/10.1007/s00580-018-2834-y).
- 663 [55] Ogunlade B, Saalu C, Ogunmodede S, Akunna GG, Adeeyo OA, Ajayi GO. The
 664 salutary role of allium cepa extract on the liver histology, liver oxidative status and
 665 liver marker enzymes of rabbits submitted to alcohol-induced toxicity. *Am J Biochem*
 666 *Mol Biol* 2012;2:67–81. <https://doi.org/10.3923/ajbmb.2012.67.81>.
- 667 [56] Argilés JM, Busquets S, Stemmler B, López-Soriano FJ. Cancer cachexia:

668 Understanding the molecular basis. *Nat Rev Cancer* 2014;14:754–62.
 669 <https://doi.org/10.1038/nrc3829>.

670 [57] Goodarzi N, Zangeneh MM, Zangeneh A, Najafi F, Tahvilian R. مقاله پژوهشی پزشد علوم
 671 دانشگاه مجله کی رفسنجان دوره 16 ، خرداد 1396 ، 238-227 سورانه الکلی عصاره حفاظتی اثرات . vol.
 672 16. *Journal of Rafsanjan University of Medical Sciences*; 2017.

673 [58] Zangeneh MM, Goodarzi N, Zangeneh A, Tahvilian R, Najafi F. Amelioration of renal
 674 structural changes in STZ-induced diabetic mice with ethanolic extract of *Allium*
 675 *saralicum* R.M. Fritsch. *Comp Clin Path* 2018;27:861–7.
 676 <https://doi.org/10.1007/s00580-018-2674-9>.

677 [59] Rao MD, Pennathur G. Green synthesis and characterization of cadmium sulphide
 678 nanoparticles from *Chlamydomonas reinhardtii* and their application as photocatalysts.
 679 *Mater Res Bull* 2017;85:64–73. <https://doi.org/10.1016/j.materresbull.2016.08.049>.

680 [60] Zhou GJ, Li SH, Zhang YC, Fu YZ. Biosynthesis of CdS nanoparticles in banana peel
 681 extract. *J Nanosci Nanotechnol* 2014;14:4437–42.
 682 <https://doi.org/10.1166/jnn.2014.8259>.

683 [61] Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, et al. Enzyme
 684 mediated extracellular synthesis of CdS nanoparticles by the fungus, *Fusarium*
 685 *oxysporum*. *J Am Chem Soc* 2002;124:12108–9. <https://doi.org/10.1021/ja027296o>.

686 [62] Ayodhya D, Veerabhadram G. One-pot green synthesis, characterization,
 687 photocatalytic, sensing and antimicrobial studies of *Calotropis gigantea* leaf extract
 688 capped CdS NPs. *Mater Sci Eng B Solid-State Mater Adv Technol* 2017;225:33–44.
 689 <https://doi.org/10.1016/j.mseb.2017.08.008>.

690 [63] Venugopal K, Rather HA, Rajagopal K, Shanthi MP, Sheriff K, Illiyas M, et al.
 691 Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and
 692 A549 lung cell lines) of the crude extract of *Syzygium aromaticum*. *J Photochem*

693 Photobiol B Biol 2017;167:282–9.
 694 <https://doi.org/10.1016/J.JPHOTOBIOL.2016.12.013>.

695 [64] Oganessian GB, Galstyan AM, Mnatsakanyan VA, Paronikyan R V., Ter-Zakharyan
 696 YZ. Phenolic and flavonoid compounds of *Ziziphora clinopodioides*. Chem Nat Compd
 697 1991;27:247. <https://doi.org/10.1007/BF00629776>.

698 [65] Tian S, Shi Y, Yu Q, Upur H. Determination of oleanolic acid and ursolic acid contents
 699 in *Ziziphora clinopodioides* Lam. by HPLC method. Pharmacogn Mag 2010;6:116–9.
 700 <https://doi.org/10.4103/0973-1296.62898>.

701 [66] Ghidan AY, Al-Antary TM, Awwad AM. Green synthesis of copper oxide
 702 nanoparticles using *Punica granatum* peels extract: Effect on green peach Aphid.
 703 Environ Nanotechnology, Monit Manag 2016;6:95–8.
 704 <https://doi.org/10.1016/j.enmm.2016.08.002>.

705 [67] Kanude KR, Jain P. Biosynthesis of CdS nanoparticles using *Murraya Koenigii* leaf
 706 extract and their biological studies. Int J Sci Res Res Pap Multidiscip Stud E 2017;3:5–
 707 10.

708 [68] Dhanasekaran K, Devendiran MP, Kolandasamy N, Nazeer AA, Deepa S.
 709 Phytochemical Reduction of Heavy Metal Ion Cd ²⁺ to Stable CdO Using Ethanolic
 710 Extract of *Limonia acidissima* Leaves and Antimicrobial Investigation Thereof. SSRN
 711 Electron J 2019. <https://doi.org/10.2139/ssrn.3370136>.

712 [69] Somasundaram G, Rajan J. Effectual Role of *Abelmoschus esculentus* (Okra) Extract
 713 on Morphology, Microbial and Photocatalytic Activities of CdO Tetrahedral Clogs. J
 714 Inorg Organomet Polym Mater 2018;28:152–67. [https://doi.org/10.1007/s10904-017-](https://doi.org/10.1007/s10904-017-0695-5)
 715 0695-5.

716 [70] Karthik K, Dhanuskodi S, Gobinath C, Prabukumar S, Sivaramakrishnan S.
 717 *Andrographis paniculata* extract mediated green synthesis of CdO nanoparticles and its

718 electrochemical and antibacterial studies. J Mater Sci Mater Electron 2017;28:7991–
 719 8001. <https://doi.org/10.1007/s10854-017-6503-8>.

720 [71] Thema FT, Beukes P, Gurib-Fakim A, Maaza M. Green synthesis of Montepomite CdO
 721 nanoparticles by Agathosma betulina natural extract. J Alloys Compd 2015;646:1043–
 722 8. <https://doi.org/10.1016/j.jallcom.2015.05.279>.

723 [72] Thovhogi N, Park E, Manikandan E, Maaza M, Gurib-Fakim A. Physical properties of
 724 CdO nanoparticles synthesized by green chemistry via Hibiscus Sabdariffa flower
 725 extract. J Alloys Compd 2016;655:314–20.
 726 <https://doi.org/10.1016/j.jallcom.2015.09.063>.

727 [73] Somasundaram G, Rajan J, Sangaiya P, Dilip R. Phytochemicals and Morphological
 728 Influence of Aloe Barbadensis Miller Extract Capped Biosynthesis of CdO Nanosticks.
 729 J Inorg Organomet Polym Mater 2019;29:1862–73. [https://doi.org/10.1007/s10904-](https://doi.org/10.1007/s10904-019-01147-7)
 730 019-01147-7.

731 [74] Permana YN, Yulizar Y. Potency of Parkia speciosa Hassk seed extract for green
 732 synthesis of CdO nanoparticles and its characterization. IOP Conf. Ser. Mater. Sci.
 733 Eng., vol. 188, Institute of Physics Publishing; 2017. [https://doi.org/10.1088/1757-](https://doi.org/10.1088/1757-899X/188/1/012018)
 734 899X/188/1/012018.

735 [75] Shivaji K, Mani S, Ponmurugan P, De Castro CS, Lloyd Davies M, Balasubramanian
 736 MG, et al. Green-Synthesis-Derived CdS Quantum Dots Using Tea Leaf Extract:
 737 Antimicrobial, Bioimaging, and Therapeutic Applications in Lung Cancer Cells. ACS
 738 Appl Nano Mater 2018;1:1683–93. <https://doi.org/10.1021/acsanm.8b00147>.

739 [76] Baghayeri M, Mahdavi B, Hosseini-Mohsen Abadi Z, Farhadi S. Green synthesis of
 740 silver nanoparticles using water extract of *Salvia leriifolia* : Antibacterial studies and
 741 applications as catalysts in the electrochemical detection of nitrite. Appl Organomet
 742 Chem 2018;32:e4057. <https://doi.org/10.1002/aoc.4057>.

- [77] Seydi N, Mahdavi B, Paydarfard S, Zangeneh A, Zangeneh MM, Najafi F, et al. Preparation, characterization, and assessment of cytotoxicity, antioxidant, antibacterial, antifungal, and cutaneous wound healing properties of titanium nanoparticles using aqueous extract of *Ziziphora clinopodioides* Lam leaves. *Appl Organomet Chem* 2019;33:e5009. <https://doi.org/10.1002/aoc.5009>.
- [78] Neuberger N, Adidharma H, Fan M. Graphene: A review of applications in the petroleum industry. *J Pet Sci Eng* 2018;167:152–9. <https://doi.org/10.1016/j.petrol.2018.04.016>.
- [79] Taslimi P, Aslan HE, Demir Y, Oztaskin N, Maraş A, Gülçin İ, et al. Diarylmethanon, bromophenol and diarylmethane compounds: Discovery of potent aldose reductase, α -amylase and α -glycosidase inhibitors as new therapeutic approach in diabetes and functional hyperglycemia. *Int J Biol Macromol* 2018;119:857–63. <https://doi.org/10.1016/j.ijbiomac.2018.08.004>.
- [80] Zengin M, Genc H, Taslimi P, Kestane A, Guclu E, Ogutlu A, et al. Novel thymol bearing oxypropanolamine derivatives as potent some metabolic enzyme inhibitors – Their antidiabetic, anticholinergic and antibacterial potentials. *Bioorg Chem* 2018;81:119–26. <https://doi.org/10.1016/j.bioorg.2018.08.003>.

Figure Captions:

FIGURE 1.The image of *Ziziphora clinopodides* Lam.

FIGURE 2.Percent viability measured on HUVEC, 32D-FLT3-ITD, Human HL-60/vcr, and Murine C1498 after treatment with present $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodides* leaf aqueous extract, CdNPs, and Daunorubicin.

FIGURE 3.Antioxidant activity of $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodides* leaf aqueous extract, CdNPs, Daunorubicin, and BHT.

768 **FIGURE 4.**The levels of immunological parameters in tested groups. A: Anti-inflammatory
769 cytokines. B: Pro-inflammatory cytokines. Different letters indicate the significant difference
770 ($p \leq 0.01$) between the experimental groups.

771 **FIGURE 5.**The number of platelet, RBC, nRBC, and WBC in the experimental groups.
772 Non-identical letters reveal a notable shift between the experimental groups ($p \leq 0.01$).

773 **FIGURE 6.**The levels of RBC parameters in tested groups.
774 Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

775 **FIGURE 7.**Expression fold change of S1PR1 and S1PR5 in control, Cd(NO₃)₂, *Z.*
776 *clinopodides* leaf aqueous extract, CdNPs, and Daunorubicin groups in comparison to the
777 untreated group.

778 **FIGURE 8.**The weights of the body in tested groups.

779 **FIGURE 9.**The percentage of leukemic myeloblasts infiltrating to spleen and liver in tested
780 groups.

781 Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

782 **FIGURE 10.**The weights and volumes of liver and spleen in tested groups.

783 Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

784 **FIGURE 11.**The levels of stereological parameters of liver and spleen in tested groups. A:
785 Spleen. B: Liver. Different letters indicate the significant difference ($p \leq 0.01$) between the
786 experimental groups.

787 **FIGURE 12.**EDS pattern of CdNPs.

788 **FIGURE 13.**XRD data of CdNPs.

789 **FIGURE 14.**FT-IR spectra of CdNPs.

790 **FIGURE 15.**FE-SEM image of CdNPs.

791 **FIGURE 16.**UV–vis absorption spectra of CdNPs.

792 **Table Captions:**

793 **Table 1.** The levels of biochemical parameters in tested groups.

794 **Table 2.** Inhibition results of nanoparticles on some metabolic enzymes (IC₅₀ ve Ki values)

795

796

797 **Figure 1.** The image of *Ziziphora clinopodides* Lam.

Figure 2. Percent viability measured on HUVEC, 32D/FLT3-ITD, Human HL-60/vcr, and Murine C1498 after treatment with present Cd(NO₃)₂, *Z. clinopodides* leaf aqueous extract, CdNPs, and Daunorubicin.

Figure 3. Antioxidant activity of Cd(NO₃)₂, *Z. clinopodioides* leaf aqueous extract, CdNPs, Daunorubicin, and BHT.

FIGURE 4: The levels of immunological parameters in tested groups. A: Anti-inflammatory cytokines. B: Pro-inflammatory cytokines. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 5. The number of platelet, RBC, nRBC, and WBC in the experimental groups. Non-identical letters reveal a notable shift between the experimental groups ($p \leq 0.01$).

Figure 6. The levels of RBC parameters in tested groups. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 7. Expression fold change of S1PR1 and S1PR5 in control, Cd(NO₃)₂, *Z. clinopodioides* leaf aqueous extract, CdNPs, and Daunorubicin groups in comparison to the untreated group.

Figure 8: The weights of the body in tested groups.

Figure 9. The percentage of leukemic myeloblasts infiltrating to spleen and liver in tested groups. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 10. The weights and volumes of liver and spleen in tested groups. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 11. The levels of stereological parameters of liver and spleen in tested groups. A: Spleen. B: Liver. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 12: EDS pattern of CdNPs.

846

Figure 13. XRD data of CdNPs.

847

848

Figure 14. FT-IR spectra of CdNPs.

849

Figure 15. FE-SEM image of CdNPs.

Figure 16. UV-vis absorption spectra of CdNPs.

870 **Table 1.** The levels of biochemical parameters in tested groups.

Parameters		Groups (n=10)					
		Control	Untreated	Daunorubicin	Cd(NO ₃) ₂	<i>Z.clinopodioides</i>	CdNPs
Serum	ALP (IU/L)	176.3±10.1 ^a	569.2±18.4 ^d	323.6±13.2 ^c	347.4±14.7 ^c	317.8±15.1 ^c	229.7±12.8 ^b
	AST (IU/L)	99.6±8.3 ^a	401.5±14.2 ^c	197.8±11.4 ^b	220±14.2 ^b	192.4±13 ^b	185.3±12.1 ^b
	ALT (IU/L)	31.5±2.5 ^a	69.4±4.7 ^d	48.6±3.9 ^b	56.2±4.3 ^c	46.3±3.6 ^b	36.5±3 ^a
	GGT (IU/L)	7.2±0.8 ^a	18.6±1.2 ^c	8.6±0.8 ^a	12.8±0.9 ^b	8.5±0.7 ^a	8±0.9 ^a
	Cholesterol (mmol/L)	4.8±0.4 ^a	7.1±0.6 ^d	5.7±0.4 ^b	6.5±0.3 ^c	5.6±0.4 ^b	5.5±0.3 ^b
	LDL (mmol/L)	3.2±0.2 ^a	5.9±0.5 ^d	4.5±0.3 ^b	5.2±0.2 ^c	4.5±0.4 ^b	4.2±0.3 ^b
	HDL (mmol/L)	5.9±0.5 ^a	3±0.2 ^d	4.5±0.2 ^b	3.8±0.3 ^c	4.6±0.4 ^b	5±0.3 ^b
	Triglyceride (mmol/L)	0.6±0.0 ^a	2.2±0.1 ^b	1±0.0 ^a	1.1±0.0 ^a	1±0.0 ^a	0.8±0.0 ^a
	Total protein (g/dL)	8.1±0.9 ^a	4.2±0.5 ^e	6.3±0.6 ^c	5.3±0.5 ^d	6.5±0.5 ^c	7.3±0.4 ^b
	Albumin (g/dL)	3.2±0.2 ^a	1.4±0.1 ^c	2.3±0.2 ^b	2±0.1 ^b	2.3±0.2 ^b	2.9±0.3 ^a
	Total bilirubin (mg/dL)	0.41±0.0 ^a	0.86±0.0 ^c	0.6±0.0 ^d	0.65±0.0 ^c	0.6±0.0 ^c	0.5±0.0 ^b
	Conjugated bilirubin (mg/dL)	0.09±0.0 ^a	0.23±0.0 ^c	0.15±0.0 ^b	0.16±0.0 ^b	0.14±0.0 ^b	0.10±0.0 ^a
	Glucose (g/dL)	76.7±7.5 ^a	124.3±10.7 ^b	85.6±9.2 ^a	88.7±10 ^a	84.2±8.3 ^a	80.3±8.6 ^a
	Urea (mg/dL)	12.1±0.9 ^a	35.4±2.6 ^d	21.4±1 ^b	26.4±1.3 ^c	21.7±0.9 ^b	19.7±0.8 ^b
	Creatinine (mg/dL)	0.6±0.0 ^a	1.9±0.0 ^b	0.8±0.0 ^a	1±0.0 ^a	0.8±0.0 ^a	0.8±0.0 ^a

	Ferrous (µg/dL)	196.5±11.5 ^a	395.6±14.3 ^d	256.6±12.5 ^b	315.3±13.2 ^c	300.2±12 ^c	243.5±9.6 ^b
	Ferritin (µg/g protein)	165.7±11.4 ^a	419.6±15.2 ^d	285.3±11.7 ^b	343.2±12.9 ^c	327.6±11.2 ^c	259.5±12.4 ^b
	Erythropoietin (mU/mL)	18.7±1.4 ^a	43.7±3.2 ^d	26.5±2 ^b	30.5±1.6 ^c	26.8±1.4 ^b	24.3±1.9 ^b
	SOD (µ/mg protein)	9.6±0.8 ^a	3.8±0.4 ^c	6.4±0.6 ^c	5.5±0.6 ^d	6.3±0.5 ^c	7.3±0.7 ^b
	CAT (µ/mg protein)	3.1±0.3	0.9±0.1 ^c	2.8±0.2 ^a	2.3±0.1 ^b	2.9±0.1 ^a	3.1±0.2 ^a
	GR (U/ml protein)	5.4±0.4 ^a	14.6±1.2 ^c	8±0.7 ^c	10.1±0.7 ^d	8.2±0.8 ^c	6.6±0.3 ^b
	GPx (U/ml protein)	1.8±0.1 ^a	0.5±0.0 ^b	1.6±0.0 ^a	1.5±0.0 ^a	1.6±0.0 ^a	1.7±0.0 ^a
Spleen	SOD (µ/mg protein)	31.6±2.9 ^a	9±0.8 ^d	19.9±0.8 ^b	14.8±0.9 ^c	15.8±1.2 ^c	18.7±0.7 ^b
	CAT (µ/mg protein)	17.8±1.5 ^a	6.7±0.8 ^c	15.6±1.1 ^a	11.7±0.8 ^b	12.4±0.7 ^b	15.2±0.9 ^a
	GR (U/ml protein)	3.8±0.3 ^a	15.7±0.9 ^d	6.1±0.5 ^b	9.8±0.7 ^c	8.8±0.6 ^c	6.6±0.7 ^b
	GPx (U/ml protein)	1.7±0.1 ^a	0.3±0.0 ^b	1.5±0.0 ^a	1.4±0.0 ^a	1.4±0.0 ^a	1.5±0.0 ^a
Liver	SOD (µ/mg protein)	44.2±3.6 ^a	19.8±1.2 ^d	36.2±2.3 ^b	26.8±1.7 ^c	28.6±1.9 ^c	34±1.7 ^b
	CAT (µ/mg protein)	25.8±1.4 ^a	8.9±0.7 ^d	19.8±0.9 ^b	15.1±1.1 ^c	15.7±0.8 ^c	19.5±1 ^b
	GR (U/ml protein)	7.1±0.7 ^a	20.4±1.5 ^c	9.2±1 ^b	14.2±1.1 ^d	14±0.9 ^d	11.3±0.7 ^c
	GPx (U/ml protein)	2.2±0.2 ^a	0.8±0.0 ^b	2.1±0.1 ^a	1.8±0.1 ^a	1.8±0.1 ^a	2±0.1 ^a

Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Table 2. Inhibition results of nanoparticles on some metabolic enzymes (IC₅₀ ve Ki values)

Cd(NO₃)₂	Enzymes	α-Gly (mM)	AChE (mM)	BChE (mM)
	IC₅₀	3.51	11.38	18.32
	r²	0.9359	0.9344	0.9328
	Ki+std	5.87±0.48	7.22 ± 1.23	12.68±1.67
CdNPs	Enzymes	α-Gly (mM)	AChE (mM)	BChE (mM)
	IC₅₀	0.76	8.64	14.22
	r²	0.9788	0.9839	0.9361
	Ki+std	1.74±0.13	6.80± 1.30	10.94±2.32
Standards (Acarbose for α -Gly, tacrine for AChE and BChE)	Enzymes	α-Gly (mM)	AChE (mM)	BChE (mM)
	IC₅₀	5.37	19.53	25.54
	r²	0.9816	0.9703	0.9342
	Ki+std	6.28±1.24	12.32±2.51	17.52±4.41

Figures

Figure 1

The image of *Ziziphora clinopodides* Lam.

Figure 2

Percent viability measured on HUVEC, 32D/FLT3-ITD, Human HL-60/vcr, and Murine C1498 after treatment with present Cd(NO₃)₂, Z. clinopodides leaf aqueous extract, CdNPs, and Daunorubicin.

Figure 3

Antioxidant activity of $\text{Cd}(\text{NO}_3)_2$, *Z. clinopodioides* leaf aqueous extract, CdNPs, Daunorubicin, and BHT.

Figure 4

The levels of immunological parameters in tested groups. A: Anti-inflammatory cytokines. B: Pro-inflammatory cytokines. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 5

The number of platelet, RBC, nRBC, and WBC in the experimental groups. Non-identical letters reveal a notable shift between the experimental groups ($p \leq 0.01$).

Figure 6

The levels of RBC parameters in tested groups. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 7

Expression fold change of S1PR1 and S1PR5 in control, Cd(NO3)2, Z. clinopodides leaf aqueous extract, CdNPs, and Daunorubicin groups in comparison to the untreated group.

Figure 8

The weights of the body in tested groups.

Figure 9

The percentage of leukemic myeloblasts infiltrating to spleen and liver in tested groups. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 10

The weights and volumes of liver and spleen in tested groups. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 11

The levels of stereological parameters of liver and spleen in tested groups. A: Spleen. B: Liver. Different letters indicate the significant difference ($p \leq 0.01$) between the experimental groups.

Figure 12

EDS pattern of CdNPs.

Figure 13

XRD data of CdNPs.

Figure 14

FT-IR spectra of CdNPs.

Figure 15

FE-SEM image of CdNPs.

Figure 16

UV-vis absorption spectra of CdNPs.