ASIAN J TROP BIOTECHNOL
Volume 21, Number 2, December 2024
Pages: 68-74

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c210202

Hepatoprotective effect of *Ocimum basilicum* aqueous extract against doxorubicin-induced hepatic damage in albino rats

ISLAM BOULAARES[1,2], SAMIR DEROUICHE[1,2,♥], IMANE YOUSRA GUEMARI[1]

[1]Department of Cellular and Molecular Biology, Faculty of the Sciences of Nature and Life, El-Oued University. El-Oued 39000, Algeria.
Tel.: +213-552285234, ♥email: dersamebio@gmail.com

[2]Laboratory of Biodiversity and Application of Biotechnology in the Agricultural Field, Faculty of the Sciences of Nature and Life, University of El Oued. El-Oued 39000, Algeria

Manuscript received: 9 August 2024. Revision accepted: 7 November 2024.

Abstract. *Boulaares I, Derouiche S, Guemari IY. 2024. Hepatoprotective effect of* Ocimum basilicum *aqueous extract against doxorubicin-induced hepatic damage in albino rats. Asian J Trop Biotechnol 21: 68-74.* This investigation aimed to evaluate the effect of *Ocimum basilicum* L. aqueous extract (ObE) on hepatotoxicity induced by doxorubicin in rats. Standard procedures were used to extract bioactive compounds and to analyze phytochemical compounds quantitatively and qualitatively. The in-vivo study was performed using 15 female albino rats that were grouped into 3 groups (n=5): a control group, a doxorubicin-treated group (DOX), and a group co-treated with doxorubicin and *O. basilicum* aqueous extract (DOX + ObE). Various biochemical, enzymatic, and liver oxidative stress markers were analyzed. Phytochemical results demonstrated that the ObE contained highly phenolic compounds. The results of the in vivo study showed that the treatment of doxorubicin caused a significant decrease of WBC ($P<0.01$), lymphocyte ($P<0.05$), also a significant increase in GOT ($p<0.05$), CPK ($p<0.001$) and LDH ($p<0.05$) activities compared to control group/normal rats. In addition, doxorubicin-treated rats resulted in a significant decrease in GSH ($p<0.01$) and an increase in MDA ($p<0.01$), SOD ($p<0.001$), and GST ($p<0.01$) levels in liver cells compared to the control group. Treatment with ObE partially reversed all of the previously mentioned parameters. This study indicated that basil aqueous extract's antioxidant activity could protect the liver from the harmful effects of doxorubicin or the destructive effects of various liver diseases such as hepatitis or other drug hepatotoxicity.

Keywords: Doxorubicin, hepatotoxicity, *Ocimum basilicum*, oxidative stress, rat

Abbreviations: DOX: Doxorubicin, GSH: Reduced glutathione, MDA: Malondialdehyde, ObE: *Ocimum basilicum* extract, ROS: Reactive Oxygen Species, TFC: Total Flavonoids Contents, TPC: Total Phenolic Contents, WBC: White Blood Cells

INTRODUCTION

Cancer is a group of diseases that cause abnormal cell growth and proliferate beyond control (Golemis et al. 2018). It is a major public health problem worldwide (Siegel et al. 2020) and the second leading cause of death after cardiovascular diseases (Lódi et al. 2019). There are many cancer treatments, the most important of which is chemotherapy, considered the most effective and extensively used for most types of cancer (Ibrahim et al. 2022). Doxorubicin is an anthracycline glycoside antitumor antibiotic used as a first-line drug in a mixture of several chemotherapy drugs for various types of cancer (Choi et al. 2020). However, the use and effectiveness of doxorubicin can be compromised by its hazardous side effects on many organs (Lei et al. 2020), especially the liver. These free radicals could harm the hepatic membranes by phospholipid activation and lipid peroxidation, which increase intracellular Ca^{2+}, produce ALT, and ultimately lead to apoptotic cell death (Boulaares et al. 2024a).

An imbalance in the ratio of pro-oxidants to antioxidants, emphasizing antioxidants, is called oxidative stress (Guemari et al. 2024). Chronic hepatotoxicity is the primary adverse effects that limit the therapeutic use of anthracycline medications (Boulaares et al. 2024b). Several studies have shown a relationship between increased Reactive Oxygen Species (ROS) and oxidative stress and doxorubicin-induced toxicity. The three primary ROS that cause toxicity are superoxide radical (O_2^-), hydroxyl free radical (HO•), and hydrogen peroxide (H_2O_2) (Gasser et al. 2019). Antioxidants are the primary defense against molecular oxidative damage (Acila et al. 2024).

Inflammation is another mechanism to induce hepatotoxicity by doxorubicin; the quantities of specific inflammatory chemokines and cytokines, including TNF-α, IL-1β, IL-6, COX-2, and CCL2/MCP-1, are significantly increased by doxorubicin (Guo et al. 2013). Cytokines are essential mediator proteins that facilitate the networking and communication of the immune system. Monocytes or lymphocytes can produce both pro- and anti-inflammatory cytokines. Cytokines with chemotactic action are called chemokines. It is thought that the balance between pro-inflammatory cytokines (IL-1β, IL-2, TNFα, Il-6, IL-8, IFN-γ...) and anti-inflammatory cytokines (IL-10, IL-4, TGFβ) is crucial for immune response homeostasis and inflammation, which are the root causes of many disorders (Chetehouna et al. 2024a).

The liver, an essential organ for digestion and detoxification, is the first and most susceptible to toxins (Zhao et al. (2022). It is a vital organ for metabolism in the

body that secretes bile and converts various nutrients into proteins (Aruna and Gayathiri 2018). The liver transforms, neutralizes, and eliminates toxins through hepatocyte-mediated enzymatic detoxification mechanisms, which is the primary mechanism of tissue detoxification (Allameh et al. 2023).

The plants are traditionally used to treat diseases, and the confirmation of their biological effects has stimulated their therapeutic use. Their active ingredients may cure diseases and relieve symptoms (Belhouala et al. 2021). Nevertheless, research on these plants' bioactive components showed that these compounds are used in traditional and modern medicine (Salmerón-Manzano et al. 2020). *Ocimum basilicum* L., locally known as sweet basil, is a popular medicinal plant. Since ancient times, several species in the genus *Ocimum* have been used to treat different disorders and diseases (Boulaares et al. 2024c). The *O. basilicum* is an important species of the genus *Ocimum* because of its many therapeutic uses (Boulaares et al. 2024d). The *O. basilicum* has shown antioxidant activities due to its bioactive compounds (Derouiche et al. 2020). The antioxidant activity of *O. basilicum* aqueous extracts showed potent free radical scavenging activity (Boulaares et al. 2024b). The objective of the present study is to assess the hepatotoxic effects of doxorubicin and to study the hepato-protective properties of therapeutic systems based on *O. basilicum* in rats.

MATERIALS AND METHODS

Plant materials

The *O. basilicum* was harvested from the region of EL-OUED (Guemar) in August. The leaves were cleaned and dried out by direct sunlight at room temperature. A mechanical grinder powdered the dry leaves until a fine powder was obtained. The *O. basilicum* powder was stored at room temperature in airtight containers until the experiment was performed.

Method of aqueous extract preparation

Aqueous extract was prepared by boiling 10 g of dried leaves powder of *O. basilicum* with 100 mL of distilled water over low heat (50°C) for 2 hours. The extract was then cooled and macerated for 24 hours at room temperature and filtered through Whatman filter paper. Then, it was evaporated using a rotary evaporator and dried using an oven (Chetehouna et al. 2024b).

Phytochemical analysis

Qualitative phytochemical analysis was applied to the aqueous extracts using qualitative characteristics techniques according to the standard method.

Total Phenolic Contents (TPC)

The following protocol carried out the quantitative analysis of total phenols: for 125 µL of the plant extract, add 500 µL of distilled water, then add l25 µL of Folin-Ciocalteu reagent (FCR) and homogenized. After 5 min, 1250 µL of sodium carbonate (Na_2CO_3) at a concentration of 7.5 g/L is added to trigger the oxidation-reduction reaction and completed by adding distilled water to 3 mL. The reaction mixture was stirred and kept in the dark for 2 h at room temperature. The absorbance of each solution is determined at 765 nm using a UV-VIS spectrophotometer (Boizot and Charpentier 2006).

A standard calibration curve was obtained from gallic acid solutions of different concentrations (50 -100 - 150 - 200µg/mL) using the same dosage; all measurements were repeated three times.

Total Flavonoid Contents (TFC)

One mL of each sample and standard (prepared in methanol) is added to 1 mL of the AlCl3 solution (2% dissolved in methanol). After 10 minutes, absorbance was measured relative to the reagent blank prepared at λ max = 430 nanometers (Ahn et al. 2004). The concentration of flavonoids is calculated using a calibration curve of quercetin, ranging from (0.01, 0.03, 0.05, 0.07, 0.09 mg/mL). All the measurements are repeated three times; the results are expressed in mg equivalent to quercetin/g of extract.

Acute toxicity test

The acute toxicity test was performed on albino Wistar rats aged 8 weeks and divided into three groups of five rats each (n=5). Animals were fasting for 12 hours before dosing plant extract (each group of rats was treated with extract doses of 2000 and 5000 mg/kgBW. After administering the extract, animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 h, with particular attention given during the first 4 h, and daily after that, for 14 days. The observed parameters were behavioral and physiological, including skin, fur, eyes, tremors, diarrhea, coma, and sleep. The protocol of the acute toxicity based on the OECD guideline for testing of chemicals (nº 423): Acute Oral Toxicity–Acute Toxic Class Method. 1-14 (2001) with the approval from Islam Boularees no. 03EC/AM/EU2024, February 12, 2024.

Animal care and experimental design

Thirty female Wistar rats from the Pasteur Institute of Algiers, aged 8 weeks, weighed 184.84±8.48 g. The rats were kept in identical conditions: room temperature, a 15-day adaption period, and a 12-hour light/12-hour dark photoperiod at Echahid University Hamma Lakhdar-El-Oued's Department of Cellular and Molecular Biology. The rats are housed in plastic cages and have free access to water and food by a standard diet. The experiment was conducted for 4 weeks.

Following a time of adaption, the animals were split up into three groups of five for each experiment as follows: (i) Group 1 (Control): were normal rats inoculated with physiological saline (one dose/week, 1.5 mL/kg) for 4 weeks. (ii) Group 2 (DOX): Drug control (Dox) was injected once a week at 1.5 mL/kg) for 4 weeks. (iii) Group 3 (DOX + ObE): Rats were injected with Doxo (once a week, 1.5 mL/kg) and supplemented with ObE (200mg/kg)

for 4 weeks. Doxorubicin dose-induced hepatotoxicity used in this study follows AlQahtani et al. (2019).

Sacrifice, blood sampling, and tissue collection

After 16 h of fasting, the experimental animals were sacrificed under slight anesthesia by chloroform (94%) inhalation. Blood samples were collected into dry tubes at the end of the experiment. Blood samples were centrifugated at 4,000 rpm for 10 min to obtain the serum for enzymatic activity analysis. The liver was carefully sampled, washed with normal saline (NaCl), then weighed and stored at -20°c for oxidative stress analysis.

Enzymatic activities and hematological markers analysis

Commercial kits (Spinreact) were used to assess the enzymatic activity in the serum, and the hematology autoanalyzer (Sysmex) was used for the hematological analysis (FNS).

Oxidative stress parameters

About 1g of liver was homogenized in 9 mL of Tris buffer saline solution (pH=7.4). Homogenates were centrifuged at 4,000 rpm for 20 min + 4°C, the obtained supernatant was used to determine antioxidant activity. MDA was measured using the method described by Sastre et al. (2000). The level of reduced glutathione was determined according to Weckbecker and Cory (1988). The SOD activity testing method uses NBT with superoxide anion (O_2), which is used to detect the presence of SOD using spectrophotometry at 560 nm (Beauchamp and Fridovich 1971). The GSTs were measured according to Habig et al. (1974).

Statistical analysis

The data were expressed as a percentage or an average ± SD (standard deviation), and the Student's t-test of independent samples was used. All data in this study were analyzed using Minitab 13.0 software. $P<0.05$ indicates a statistically significant difference.

RESULTS AND DISCUSSION

Qualitative and quantitative phytochemical analysis of *Ocimum basilicum*

The phytochemical assays of the aqueous extract of *O. basilicum* showed several chemical compounds, such as flavonoids, steroids, phenols, catechic tannin, saponoside, carbohydrates, and alkaloids, but did not contain steroid derivatives. (Table 1). The Total Phenolic and flavonoid compounds were expressed in terms of Gallic acid equivalents (mg of GAE/g sample) and of Quercetin equivalents (mg of QE/g sample), respectively, using the following equation based on the calibration curve: $Y = 0.0045x + 0.009$; $R2 = 0.995$ for phenolic compounds and $Y = 0.0096x + 0.0521$, $R2 = 0.994$ for flavonoids compounds. The total phenolic and flavonoid contents of *O. basilicum* aqueous extract are 77.66±3.27 mg GAE/g and 16.52±0.06 mg of QE/g, respectively (Table 1).

Acute toxicity assays of *O. basilicum* aqueous extract

The results showed no mortality after 24 hours of administration, suggesting the extracts were non-toxic. The other physiological parameters of the rats, i.e., sleep, diarrhea, tremors, and convulsions, were also determined during the experimental period and showed that treatment of aqueous extract of *O. basilicum* caused no adverse effects on the experimental animals during the treatment period (Table 2)

Enzymatic activities

As shown in Table 3, the DOX group increased Glutamate Pyruvate Transaminase (GPT), lactate dehydrogenase (LDH), and creatinine phosphokinase (CPK) significantly; however, Glutamate oxaloacetate transaminase (GOT) was decreased significantly ($p<0.01$) in the DOX than in the control group. Additionally, a significant decrease ($p<0.001$) in the GPT ($p<0.01$), GOT ($p<0.01$), and CPK ($p<0.001$) was observed in the ObE group as compared to the DOX group.

Table 1. Qualitative and quantitative phytochemical compounds of *Ocimum basilicum* aqueous extract

Phytochemical compound	The presence/absent
Flavonoids	+
Unsaturated steroids	+++
Steroids derivatives	- - -
Phenols	+++
Catechic Tannin	+++
Saponoside	+++
Carbohydrate	+++
Alkaloid	+++
Total phenols (mg GAE/g extract)	77.66±3.27
Total flavonoids (mg QE/g extract)	16.52±0.06

Note: +: Present, -: Absent

Table 2. Effect of *Ocimum basilicum* aqueous extract on physiological parameters

Parameters	0 mg/kg					2000 mg/kg					5000 mg/kg				
	0h	3h	7h	14h	24h	0h	3h	7h	14h	24h	0h	3h	7h	14h	24h
Death rats	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Note: N: Normal

Table 3. Enzyme activities in control and experimental groups

Parameters	Control	DOX	DOX+ObE
GPT (UI/l)	47.2±7.68	60±24.70*	42.55±3.92^{b}
GOT (UI/l)	146±15.20	132.5±3.51**	121.7±11.23
CPK (UI/l)	182.5±1.51	1337±60.38***	220.12±31.32^{c}
LDH (UI/l)	1133±137.06	1419±140.37*	1320±55.42

Note: Data are expressed as mean ± SD (n=6). *****p<0.05, ******p<0.01, *******p<0,001: Significantly different from control group, ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0,001: Significantly different from DOX group

Table 4. Leukocyte count in control and experimental groups

Parameters	Control	DOX	DOX+ObE
WBC (10^3/ul)	3.43±0.27	2.04±0.44**	4.248±0.396^{c}
Neutophil (10^3/ul)	0.78±0.11	0.55±0.09*	1.358±0.16^{**c}
Lymphocyte (10^3/ul)	1.98±0.18	1.03±0.14*	2.52±0.262^{c}
Monocyte (10^3/ul)	0.04±0.00	0.18±0.06***	0.056±0.0116^{c}
Eosinophil (10^3/ul)	0.10±0.00	0.06±0.009**	0.0625±0.014*
Basophil (10^3/ul)	0.20±0.02	0.04±0.003***	0.22±0.0653^{a}
Platelet (10^3/ul)	896.8±67.4	1232±35.5*	1362.5±48.3$^{***\ a}$

Note: Data are expressed as mean ± SD (n=6). *****p<0.05, ******p<0.01, *******p<0.001: Significantly different from control group, ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001: Significantly different from DOX group

Table 5. Markers of liver oxidative stress in control and experimental groups

Parameters	Control	DOX	DOX+ObE
GSH (µmol/g tissue)	6.21±1.5	5.733±0.82**	2.72±0.36^{***c}
MDA (µmol/g tissue)	16.1±1.32	31.5±5.72**	27.41±2.23^{**a}
SOD (UI/mg of prot)	0.39±0.06	1.52±0.039***	0.72±0.001$^{*\ c}$
GST (nmol/min/mg prot)	0.5±0.003	4.5±0.06**	0.21±0.0032$^{***\ c}$

Note: Data are expressed as mean ± SD (n=6). *****p<0.05, ******p<0.01, *******p<0.001: Significantly different from control group. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001: Significantly different from DOX group

Hematological markers

The results of the hematological markers (Table 4) showed a significant decrease in WBC (P<0.01), lymphocyte(P<0.05), eosinophil (P<0.01), basophil (P<0.001), neutrophil (P<0.05), PLT (P<0.05), and a significant increase in monocyte (P<0.01). The findings also demonstrated a significant amelioration in the level of WBC, neutrophil, lymphocyte, monocyte, and basophil in the treatment group compared to the DOX group. This study's findings demonstrated a reduction of monocytes in ObE groups (p<0.001, p<0.01), respectively. Furthermore, no significant (P>0.05) variation in the eosinophil and platelet parameters exists.

Oxidative stress parameters

Our results (Table 5) showed a significant decrease (p<0.01) in the GSH level of the liver in the DOX group compared to the control. However, the results showed a very significant diminution (p<0.001) of GSH in the ObE group compared to the DOX group. The malondialdehyde (MDA) levels were increased very significantly (p<0.001) in the DOX group compared to the control. The MDA level of the liver treated with ObE was decreased significantly compared to the DOX group. SOD and GST showed a highly significant increase (p<0.01) in the DOX group compared to the control. However, treatment with ObE presented a highly significant decrease (p<0.01) in the SOD and GST activities compared to the DOX group.

Discussion

As an anthracycline chemotherapeutic drug, doxorubicin (DOX) (Adriamycin) is used alone or in combination with other treatments/medications to treat various cancer types (Ajzashokouhi et al. 2019). As such, doxorubicin (DOX) is a broad-spectrum anticancer drug with high efficacy in numerous hematological malignancies and solid tumors, but its clinical use is restricted due to its cardiotoxicity (Abboud et al. 2018)

The result of phytochemical screening confirmed the presence of flavonoids, unsaturated steroids, phenols, catechic tannin, saponoside, carbohydrates, and alkaloids in aqueous extracts of the leaves of *O. basilicum* is considered a high source of secondary metabolites, such as glycosides, tannins, phenolic compounds, saponins, flavonoids, and terpenes (Tariq et al. 2016). Terpenes exhibit anti-inflammatory activity (Chetehouna et al. 2024b). Flavonoids exert anti-oxidative effects as free radical scavengers and possess therapeutic potential for osteoporosis and cancer. Moreover, consuming phenolic compounds reduces the risk of liver disease (Rahman et al. 2021). The O. *basilicum* has shown antioxidant activities through its bioactive compounds like flavonoids and

phenolic compounds (Derouiche et al. 2020). The antioxidant activity of *O. basilicum* aqueous extracts showed very strong free radical scavenging activity, and it is a widely used medicinal plant (Boulaares et al. 2024c). Another study by Pistelli et al. (2020) showed that basil contains phenolic antioxidant compounds.

In the present study, doxo-induced hepato-toxicity increases enzymes LDH, CPK, and GPT activities, known as liver function markers. DOX induces immunosuppression by reducing leukocyte levels (WBC, lymphocyte, eosinophil, and basophil). Bioaccumulation of doxorubicin generates free radicals that trigger membrane degradation and liver disruption, leading to elevations of LDH, CPK, ALT, and AST (Boulaares et al. 2020). Doxorubicin, as a chemotherapy treatment, possesses several side effects, including cardiotoxic and hepatotoxic. It suppresses the immune system by decreasing the expression of IL-2, production of the γ-interferon, Natural Killer (NK) cells, proliferation of lymphocytes, and ratio CD4+/CD8+ (Shaty et al. 2019). The levels of pro-inflammatory cytokines, such as IL-1 and TNFα, were significantly increased following doxorubicin administration. Doxo-induced hepatotoxicity is also related to increases in oxidative stress and production of ROS (Gasser et al. 2019). The primary types of cardiotoxicity ROS are superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl free radical (HO) (Hajare et al. 2016).

The treatment of the aqueous extract of *O. basilicum* has resulted in decreased LDH, CPK, and GPT activities compared to the doxorubicin-treated group. The bioactive phytochemical compounds (flavonoids, tannins, and phenolic compounds) may explain their efficacy in cardioprotective capacity (Zhakipbekov et al. 2024). Flavonoids have effectively reduced the increase of CPK activity induced by doxo. The combination of flavonoids with doxorubicin has been proven to reduce the toxicity of doxorubicin (Syahputra al. 2022). Flavonoids have a high antioxidant capacity, allowing them to mitigate oxidative stress's adverse effects (Derouiche et al. 2022). These results could be due to bioactive compounds that have liver-protective effects. Flavonoids reduced the high level of CPK activity caused by doxorubicin. Therefore, it has been demonstrated that combining flavonoids with doxo could lower doxo cardiotoxicity (Sadzuka et al. 1997). The ability of almost all flavonoid groups as antioxidants has the potential to be applied in various fields. Our phytochemical analysis of *O. basilicum* extract revealed high phenolic and flavonoid concentrations, suggesting potential health benefits. Flavonoids, with their anti-inflammatory and antioxidant properties, could be utilized for developing new drugs or supplements to scavenge free radicals and improve human health through beneficial pharmacological effects (Hozayen and Seif 2011; Derouiche 2020).

In our study, the level of reduced glutathione (GSH) in the liver of the group treated with doxorubicin was significantly decreased compared to the control. These findings align with the study of Antonucci et al. (2021), who stated that Doxo generates free radicals and reduces the ability to detoxify ROS. Also, our results indicate an increase of MDA, SOD, and GST in the liver of the group treated with doxorubicin. Doxorubicin reduces nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P-450 reductase enzyme by one electron, which raises malonaldehyde levels (Anber 2018). MDA was the end product of lipid peroxidation (Acila et al. 2024). Doxo-induced oxidative stress is confirmed by the elevation of oxidized lipids (MDA) (Shosha et al. 2023); the pathway involves the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of the doxo to the corresponding doxo semiquinone. In the presence of oxygen, redox cycling of doxo-derived quinone–semiquinone yields superoxide radicals (Boulaares et al. 2024a). The application of ObE in the doxorubicin treatment group resulted in a high decrease of MDA in liver homogenate compared to the doxo group. The plant extract decreases the MDA due to the content of phenolic and flavonoid compounds with high antioxidant activities. Flavonoids suppress doxo-induced lipid peroxidation in the heart and liver (Hasnat et al. 2024). The results of our previous phytochemical analysis of *O. basilicum* show the presence of high phenols and flavonoids. It has been demonstrated that the polyphenolic flavonoid protects the liver from various cardiovascular illnesses. Its antioxidant and anti-inflammatory properties, which may control several cellular signaling pathways, are known to be associated with its function (Ma et al. 2017). Furthermore, one of the primary phenolic compounds in *O. basilicum*, Rosmarinic Acid (RA), possesses pharmacological and antioxidant qualities. It was shown that rosmarinic acid inhibits these apoptotic traits by restoring the potential of the mitochondrial membrane and lowering the production of intracellular Reactive Oxygen Species (ROS) (Ferreira et al. 2014).

In conclusion, *O. basilicum* is highly efficient in limiting the risk of doxorubicin in healthy tissues and thus reducing its toxicity, especially in the liver. Treating *O. basilicum* aqueous extract improves enzymatic and hematological markers and reduces oxidative stress. It confirms the effectiveness of these compounds in protecting the body's organs from the side effects of doxorubicin, especially the liver.

ACKNOWLEDGEMENTS

We thank the Faculty of Natural and Life Sciences laboratory staff of El-Oued University, Algeria, for their basic instruction, which was essential to the successful execution of this study.

REFERENCES

Abboud SH, Hadi NR, Assad HC, Shaker RA. 2018. Cardioprotective effects of erythropoietin against doxorubicin-induced cardiotoxicity in rats. World Heart J 9 (3): 212-230.

Acila S, Derouiche S, Allioui N. 2024. Embryo growth alteration and oxidative stress responses in germinating *Cucurbita pepo* seeds

exposed to cadmium and copper toxicity. Sci Rep 14: 8608. DOI: 10.1038/s41598-024-58635-1.

Ahn MR, Kumazawa S, Hamasaka T, Bang KS, Nakayama T. 2004. Antioxidant activity and constituents of propolis collected in various areas of Korea. J Agric Food Chem 52 (24): 7286-7292. DOI: 10.1021/jf048726s.

Ajzashokouhi AH, Bostan HB, Jomezadeh V, Hayes AW, Karimi G. 2019. A review on the cardioprotective mechanisms of metformin against doxorubicin. Human Exp Toxicol 39 (3): 237-248. DOI: 10.1177/0960327119888277.

Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. 2023. Oxidative stress in liver pathophysiology and disease. Antioxidants 12 (9): 1653. DOI: 10.3390/antiox12091653.

AlQahtani AA, Osman A-MM, Damanhouri ZA, Al-Kreathy HM, Al-Malky HS, Ramadan WS, Alharthi SE, Kamel FO. 2019. Cardioprotective effect of marine astaxanthin on doxorubicin-induced cardiotoxicity in normal rats. J Pharm Res Intl 27 (3): 1-11. DOI: 10.9734/jpri/2019/v27i330170.

Anber ZNH. 2018. Effect of doxorubicin and cyclophosphamide regimen versus taxane on liver enzymes in Iraqi women with breast cancer. Biomed Res 29 (21): 3869-3873. DOI: 10.4066/biomedicalresearch.29-18-1140.

Antonucci S, Di Sante M, Tonolo F. 2021. The determining role of mitochondrial reactive oxygen species generation and monoamine oxidase activity in doxorubicin-induced cardiotoxicity. Antioxid Redox Signal 34 (7): 531-550. DOI: 10.1089/ars.2019.7929.

Aruna P, Gayathiri NM. 2018. Cardioprotective activity of telmisartan, metformin and its combination against doxorubicin induced myocardial infarction in rat model. Res J Pharm Technol 11 (12): 5293-5296. DOI: 10.5958/0974-360X.2018.00964.2.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44 (1): 276-287. DOI: 10.1016/0003-2697(71)90370-8.

Belhouala K, Benarba B. 2021. Medicinal plants used by traditional healers in Algeria: A multiregional ethnobotanical study. Front Pharmacol 12: 760492. DOI: 10.3389/fphar.2021.760492.

Boizot N, Charpentier JP. 2006. Rapid method for evaluating the content of phenolic compounds in the organs of a forest tree. The Cahier des Techniques of INRA, Paris.

Boulaares I, Derouiche S, Guemari IY 2024a. Impact of doxorubicin chemotherapy on oxidative stress status in heart and liver: An experimental study on rats. Pharm Sci Anal Res J 6 (3): 180067.

Boulaares I, Derouiche S, Guemari IY. 2024b. Protective effect of ObE against doxorubicin-induced immunosuppression and cardiotoxicity in rats. Res J Pharm Technol 17 (4): 1839-1843. DOI: 10.52711/0974-360X.2024.00292.

Boulaares I, Derouiche S, Niemann J. 2024c. HPLC-Q-TOF-MS analysis of phenolic compounds, in vitro biological activities and in vivo acute toxicity evaluation of *Ocimum basilicum* L. Fresenius Environ Bull 33 (2): 73-82.

Boulaares I, Derouiche S, Niemann J. 2024d. *Ocimum basilicum* L.: A systematic review on pharmacological actions and molecular docking studies for anticancer properties. J Biochem Technol 15 (1): 12-18. DOI: 10.51847/TlkGjN14PM.

Boulaares I, Guemari IY, Derouiche S. 2020. Analysis of some hematological and biochemical markers in women cancer patients receiving doxorubicin chemotherapy. J Pharm Biosci 8: 29-34. DOI: 10.20510/UKJPB/8/I1/1581327899.

Chetehouna S, Derouiche S, Atoussi O. 2024a. Identification of new alkaloids from algerian purslane by HPLC-QTOF-MS and beneficial effect of purslane enriched with zinc on experimental alzheimer disease in rats. Curr Trends Biotechnol Pharm 18 (1): 1595-1607. DOI: 10.5530/ctbp.2024.1.8.

Chetehouna S, Derouiche S, Réggami Y, Boulaares I, Frahtia A. 2024b. Gas chromatography analysis, mineral contents and anti-inflammatory activity of *Sonchus maritimus*. Trop J Nat Prod Res 8 (4): 6787-6798. DOI: 10.26538/tjnpr/v8i4.7.

Choi WG, Kim DK, Shin Y, Park R, Cho YY, Lee JY, Kang HC, Lee HS. 2020. Liquid chromatography-tandem mass spectrometry for the simultaneous determination of doxorubicin and its metabolites doxorubicinol, doxorubicinone, doxorubicinolone, and 7-deoxydoxorubicinone in mouse plasma. Molecules 25 (5): 1254. DOI: 10.3390/molecules25051254.

Derouiche S, Chetehouna S, Atoussi W. 2022. The effects of aqueous leaf extract of *Portulaca oleracea* on haemato-biochemical and histopathological changes induced by sub-chronic aluminium toxicity in male wistar rats. Pharm Res-Mod Chin Med 4: 100101. DOI: 10.1016/j.prmcm.2022.100101.

Derouiche S, Guemari IY, Boulaares I. 2020. Characterization and acute toxicity evaluation of the MgO nanoparticles synthesized from aqueous leaf extract of *Ocimum basilicum* L. Alger J Biosci 1 (1): 1-6. DOI: 10.5281/zenodo.4041021.

Derouiche S. 2020. Current review on herbal pharmaceutical improve immune responses against COVID-19 infection. Res J Pharm Dos Forms Technol 12 (3): 181-184. DOI: 10.5958/0975-4377.2020.00031.2.

Ferreira TS, Moreira CZ, Cária NZ, Victoriano G, Silva WF, Magalhães JC. 2014. Phytotherapy: an introduction to its history, use and application. Rev Bras Plant Med 16 (2): 290-298. DOI: 10.1590/S1516-05722014000200019.

Gasser A, Chen YW, Audebrand A, Daglayan A, Charavin M, Escoubet B, Karpov P, Tetko I, Chan MWY, Cardinale D, Désaubry L, Nebigil CG. 2019. Prokineticin Receptor-1 signaling inhibits dose- and time-dependent anthracycline-induced cardiovascular toxicity via myocardial and vascular protection. JACC CardioOncol 1 (1): 84-102. DOI: 10.1016/j.jaccao.2019.06.003.

Golemis EA, Scheet P, Beck TN. 2018. Molecular mechanisms of the preventable causes of cancer in the United States. Genes Dev 32 (13-14): 868-902. DOI: 10.1101/gad.314849.118.

Guemari IY, Boulaares I, Derouiche S. 2024. Study of the role of oxidative stress in pathophysiology of cardiovascular diseases. Collect J Cardiovasc Med 1 (1): ART0014. DOI: 10.70107/collectjcardiovasc-ART0014.

Guo RM, Xu WM, Lin JC, Mo LQ, Hua XX 2013. Activation of the p38 MAPK/NF-κB pathway contributes to doxorubicin-induced inflammation and cytotoxicity in H9c2 cardiac cells. Mol Med Rep 8 (2): 603-608. DOI: 10.3892/mmr.2013.1554.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249 (22): 7130-7139. DOI: 10.1016/S0021-9258(19)42083-8.

Hajare AA, Powar Trupti A, Bhatia Neela M, More Harinath N. 2016. Development and validation of RP-HPLC method for determination of doxorubicin hydrochloride from vacuum foam dried formulation. Res J Pharm Techol 9 (9): 1352-1356. DOI: 10.5958/0974-360X.2016.00259.6.

Hasnat H, Shompa SA, Islam MM et al. 2024. Flavonoids: A treasure house of prospective pharmacological potentials. Heliyon 10 (6): e27533. DOI: 10.1016/j.heliyon.2024.e27533.

Hozayen WG, Seif HSA. 2011. Protective effects of rutin and hesperidin against doxorubicin-induced lipodystrophy and cardiotoxicity in albino rats. Am J Sci 7 (12): 765-775.

Ibrahim YS, Akram A, Rajab WJ, Ahmed MMA, Mahmood MA. 2022. Assessment of some biochemical parameters among pleural liquid and serum during platinum-based chemotherapy against ovarian cancer. Res J Pharm Technol 15 (12): 5665-1. DOI: 10.52711/0974-360X.2022.00955.

Lei J, Wang H, Zhu D, Wan Y, Yin L. 2020. Combined effects of avasimibe immunotherapy, doxorubicin chemotherapy, and metal-organic frameworks nanoparticles on breast cancer. J Cell Physiol 235 (5): 4814-4823. DOI: 10.1002/jcp.29358.

Lódi M, Priksz D, Fülöp GÁ et al. 2019. Advantages of prophylactic versus conventionally scheduled heart failure therapy in an experimental model of doxorubicin-induced cardiomyopathy. J Transl Med 17: 1-16. DOI: 10.1186/s12967-019-1978-0.

Ma Y, Yang L, Ma J, Lu L, Wang X, Ren J, Yang J. 2017. Rutin attenuates doxorubicin-induced cardiotoxicity via regulating autophagy and apoptosis. Biochim Biophys Acta Mol Basis Dis 1863 (8): 1904-1911. DOI: 10.1016/j.bbadis.2016.12.021.

Pistelli L, Ascrizzi R, Giuliani C, Cervelli C, Ruffoni B, Princi E, Pistelli L. 2020. Growing basil in the underwater biospheres of Nemo's Garden®: Phytochemical, physiological and micromorphological analyses. Sci Hortic 259: 108851. DOI: 10.1016/j.scienta.2019.108851.

Rahman MM, Rahaman MS, Islam MR et al. 2021. Role of phenolic compounds in human disease: Current knowledge and future prospects. Molecules 27 (1): 233. DOI:10.3390/molecules27010233.

Sadzuka Y, Sugiyama T, Shimoi K, Kinae N, Hirota S. 1997. Protective effect of flavonoids on doxorubicin-induced cardiotoxicity. Toxicol Lett 92: 1-7. DOI: 10.1016/s0378-4274(97)00028-3.

Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. 2020. Worldwide Research Trends on Medicinal Plants. Intl J Environ Res Public Health 17 (10): 3376. doi:10.3390/ijerph17103376.

Sastre J, Pallardó FV, García de la Asunción J, Viña J. 2000. Mitochondria, oxidative stress and aging. Free Radic Res 32 (3): 189-198. DOI: 10.1080/10715760000300201.

Shaty MH. Al-Ezzi MI. Arif IS, Basil D. 2019. Effect of metformin on inflammatory markers involved in cardiotoxicity induced by doxorubicin. Res J Pharm Technol 12: 5815-5821. DOI: 10.5958/0974-360X.2019.01007.2.

Shosha MI, El-Ablack FZ, Saad EA. 2023. Glycine protects against doxorubicin-induced heart toxicity in mice. Amino Acids 55 (5): 679-693. DOI: 10.1007/s00726-023-03261-w.

Siegel RL, Miller KD, Jemal A. 2020. Cancer statistics, 2020. CA Cancer J Clin 70 (1): 7-30. DOI: 10.3322/caac.21590.

Syahputra RA, Harahap U, Dalimunthe A, Nasution MP, Satria D. 2022. The role of flavonoids as a cardioprotective strategy against doxorubicin-induced cardiotoxicity: A review. Molecules 27 (4): 1320. DOI: 10.3390/molecules27041320.

Tariq J, Shumaila S, Riaz S, Afzal S, Hussain S, Riaz S, Uzair M, Chaudhary BA. 2016. Phytochemical and biological evaluation of *Ocimum basilicum* roots. Intl J Pharm Pharm Res Human 2: 292-301.

Weckbecker G, Cory JG. 1988. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer Lett 40 (3): 257-264. DOI: 10.1016/0304-3835(88)90084-5.

Zhakipbekov K, Turgumbayeva A, Akhelova S. 2024. Antimicrobial and other pharmacological properties of *Ocimum basilicum*, Lamiaceae. Molecules 29 (2): 388. DOI: 10.3390/molecules29020388.

Zhao J, Wang P, Pan Y. 2022. Predicting liver disorder based on machine learning models. J Eng 2022: 978-984. DOI: 10.1049/tje2.12184.