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RESEARCH ARTICLE

Hepatoprotective potential of the *n*-butanol extract of *Moricandia arvensis* from Algeria against doxorubicin induced toxicity in *Wistar albino* rats

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Abstract

Doxorubicin (DOX) is a potent anticancer drug; its use has been limited by its hepatotoxicity, which is due to free radicals generation. This work aims to investigate whether the *n*-butanol soluble part of the 70% methanol extract of the aerial parts of *Moricandia arvensis*, alleviates doxorubicin-induced hepatotoxicity. According to the literature data, *Moricandia arvensis* (Brassicaceae) is renowned for its richness in flavonoid and phenolic acid glycosides. In this work, we have rightly assessed the total phenolic and flavonoid contents of the studied extract (*n*-butanol extract). The results obtained (TPC: 86.25 ± 0.00 µg GAE/mg; TFC: 22.54 ± 0.01 µg QE/mg) encouraged us to continue our investigations. *Wistar albino* rats were orally administered with *n*-butanol extract of *M. arvensis* (50 mg/kg and 100 mg/kg body weight) or vitamin E as a standard antioxidant (100 mg/kg) for 10 days; and DOX (15 mg/kg on the 8th day that was intraperitoneally injected. At the end of the experiment, blood and liver samples were analyzed for biomarker levels and histopathological changes. Liver homogenates were used to determine oxidative stress parameters such as malondialdehyde (MDA), glutathione peroxidase (GP_X) and glutathione (GSH) activity. DOX-administered rats significantly increased different levels of the serum biochemical parameters, increased TBARS level, decreased GPx activity and GSH level in the liver. In addition, *M. arvensis* (50 mg/kg and100 mg/kg) *n*-butanol extract treatments significantly decreased the level of TBARS, increased GSH level and GPx activity compared to the DOX-treated rats ($p < 0.01$). The histological study revealed the hepatoprotective effects of the tested extract on DOX-induced toxicity. This was demonstrated by the preservation of hepatic architecture as well as a reduction in structural and functional changes in the liver. The obtained results indicate a protective action of *n*-butanol extract of *M. arvensis* that could be the result of the inhibition of reactive oxygen species (ROS) generation. This may be the result of the presence of phenolic compounds in *M. arvensis* plant.

Keywords Doxorubicin · *Moricandia arvensis* · Hepatoprotective · Flavonoids

Introduction

The liver, one of the human body's critical organs, is responsible for endogenous and exogenous agent's metabolism. This organ has a key function in the elimination and detoxification of drugs. Even though, the liver has a self-regeneration ability, parasitic and viral infections, autoimmune diseases, drug-induced hepatotoxicity, alcoholic fatty liver diseases can increase the prevalence of hepatic failure (Ahmed et al. 2019). The anthracycline doxorubicin

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(DOX), whose important effects have been proved on a wide range of cancer diseases such as sarcomas, carcinomas and hematological malignancies, is usually used in chemotherapy (Carvalho et al. 2009). The DOX antimalignancies activity has been reported in previous studies for the treatment of transplantable leukemia, solid tumors and lymphomas as well (Kabel 2018; Zhu et al. 2016).

Different DOX action mechanisms have been proposed. DOX binds with the DNA by intercalation causing the inhibition of macromolecules biosynthesis via the inhibition of topoisomerase II progression, which is an enzyme that relaxes DNA supercoils for transcription. DOX ensures the topoisomerase II complex stability after breaking DNA chains for replication, preventing the double DNA helix from being resealed and thus halting the replication process (Rivankar 2014). On the other hand, DOX can generate reactive oxygen species (ROS) and apoptosis induction (Ramos et al. 2011). These free radicals are basic to the DOX-mediated cytotoxicity, including cardiotoxicity (Omobowale et al. 2018), genotoxicity, neurotoxicity(Utomo et al. 2018), nephrotoxicity, testicular toxicity (Aksu et al. 2019) and hepatotoxicity (Mansouri et al. 2017).

The increased production of ROS, due to oxidative stress in the liver, can be the result of two pathways: the common one is that of DOX's semiquinone interaction with O_2 producing the superoxide anion radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). The other one occurs in hepatocytes through the main extra-mitochondrial ROS producers which are NADPH oxidases (Carvalho et al. 2009). The main challenge that encounters researchers is to look for the protective effects against free radicals mediated injuries. Therefore,it is highly desirable to explore effective strategies for DOX complications, while keeping its therapeutic effects. Researchers have reported that many natural products have the ability to neutralize the DOX negative effects and other anthracycline antibiotics (Afsar et al. 2019).

Herbal medicines are usually considered as harmless and without side effects with an estimation of about 7500 plants can be used in the local health traditions. The scientific community and the local population know very little about many plants that are commonly used (Paudel et al. 2020).

Saharan plants that are known for their adaptation to hard environmental conditions can constitute a large reservoir of new, safe and effective natural products that can show different biological activities (Berreghioua et al. 2016).

The Brassicaceae (Cruciferae) family, one of the most important groups having 338 genera and 3709 species worldwide, includes several economically important crop plants grown as vegetables, fodder, condiment and oil source (Gidik et al. 2019). Five species of *Moricandia* are spread in the North Africa, South Europe and Western Asia (Skandrani et al. 2010). *Moricandia arvensis* has been used in traditional remedies by the decoction of its leaves and stems in the treatment of syphilis and scurvy, Moreover, this plant is used in traditional cooking (Skandrani et al. 2017) .

Moricandia arvensis is specifically rich in sulfur compounds, glucosinolates and isothiocyanates (Fahey et al. 2001). Moreover, an indole derivative and three glucosinolates have been reported from this species (Belkhiri and Lockwood 1990). In addition to that, a number of studies have underlined that cruciferous vegetables may have anticarcinogenic activity. On the other hand, glucosinolates are biologically active secondary metabolites and are commonly found in Brassicaceae and vegetables related families. We can also confirm the dietetic properties of *Moricandia arvensis,* as it showed an important antioxidant activity and therefore represent a source of various products, including polyphenols (Zeraib et al. 2011; Braham et al. 2005; Marrelli et al. 2018).

Therefore, the current study aims to evaluate the total phenolic and flavonoid contents of the *n*-butanol soluble part (*n*-butanol extract) of the 70% methanol extract of the aerial part of *Moricandia arvensis* and to investigate its ability to prevent DOX-induced hepatocellular damage.

Materials and methods

Plant materials and extraction procedure

The plant used in this study was collected in May 2015 from Mogheul (Béchar province) in western Algeria, (latitude: N 32°1′ 23.69'', longitude W 2°13′3.06'') and authenticated by Mohamed Benabdelhakem, director of the nature preservation agency, Bechar on the basis of Quezel and Santa (1962). A voucher specimen (MAB0515-MOG-ALG-70) has been deposited at the VARENBIOMOL Research Unit Herbarium, Université Frères Mentouri Constantine 1.

Air-dried and powdered aerial parts (2100 g) of *Moricandia arvensis* (L.) DC. (Brassicaceae), were macerated with methanol–water (70:30, v/v) at room temperature for 48 h. The operation was repeated three times. After filtration, the three alcoholic solutions were combined and concentrated under reduced pressure (up to 35 °C) to reach a volume for around 1000 mL. The remaining solution was diluted with distilled water (840 mL) under magnetic stirring and then kept at 4 °C for one night to precipitate a maximum of chlorophylls. After filtration the resulting aqueous solution was successively extracted with solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate and *n*-butanol. The organic phases were dried with anhydrous sodium sulfate (Na_2SO_4), filtered and concentrated in vacuum (up to 35 °C) to obtain the corresponding extracts: petroleum ether (0.19 g), chloroform (5.7 g), ethyl acetate (1.5 g) and

n-butanol (23.36 g) which were kept in the freezer until further analysis.

Total phenolic content (TPC) determination

The Folin–Ciocalteau assay was conducted to perform the colorimetric analysis, as was defined by Singleton et al. (1999). A 20 µL of the sample was blended with 100µL of Folin–Ciocalteau and 1580µL of distilled water. The resulting mixture was shaken and held for 8 min then; 300µL of 20% Na_2CO_3 solution has been added. After 2 h of incubation in darkness, the optical density was measured spectrophotometrically at 765 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid (GA) has been used as a standard, and TPC concentration was expressed as µg GA equivalent per mg of extract. Tests were carried out in triplicate.

Total flavonoid content (TFC) determination

TFC was determined using the method described by Wang et al. (2008). Briefly 0.5 mL of the sample was mixed with 0.5 mL of 2% aluminum chloride ($AlCl_3$). The mixture was left for 1 h, and then the optical density was estimated at 420 nm. Quercetin (QE) was used as a standard. The standard cuve is prepared using (0, 5, 10, 15, 20 and 30 µg/mL) of QE dissolved in methanol. The TFC concentration was expressed as µg of QE equivalent per mg of extract. Tests were carried out in triplicate.

Animals and treatment

This study was carried out on healthy male *Wistar albino* rats weighing (190–250 g). They were brought from Pasteur Institute (Algiers, Algeria). The rats were maintained under laboratory conditions at an ambient temperature of (25 ± 1 °C) with an ordinary light/dark cycle of 12 h. They were provided with free food and water. The doses used of vitamin E and plant extract, were chosen according to prior in vivo experiments that were performed in our laboratory (Djebbari et al. 2017). The DOX dose was selected as it has been used previously to induce acute hepatotoxicity in male albino rats (Mecheri et al. 2018). These treatments were administered to the animals that were split into 7 groups of 7 animals each.

Group 1 Represent the untreated group

Group 2 Group of rats received a single injection of 15 mg/kg, i.p. of DOX (injected on the 8th day).

Group 3 Group of rats received oral administrations of *n*-butanol extract of *M. arvensis* (50 mg/kg b.w) for 10 days.

Group 4 Group of rats received oral administrations of *n*-butanol extract of *M. arvensis* (100 mg/kg b.w) for 10 days.

Group 5 Group of rats received oral administrations of *n*-butanol extract of *M. arvensis* (50 mg/kg b.w) for 10 days and single injection of 15 mg/kg, i.p. of DOX (injected on the 8th day).

Group 6 Group of rats received oral administrations of *n*-butanol extract of *M. arvensis* (100 mg/kg b.w) for 10 days and single injection of 15 mg/kg, i.p. of DOX (injected on the 8^{th} day).

Group 7 Group of rats received oral administrations of vitamin E (100 mg/kg) for 10 days and single injection of 15 mg/kg, i.p. of DOX (injected on the 8th day).

At the end of this experiment (after 24 h of the last dose of extract or vitamin E treatments and 72 h after DOX administration), the rats were anesthetized and portal vein blood that was used for the biochemical analysis was collected on heparin tubes.

Additionally, rats were sacrificed and livers were removed and examined histopathologically as well as for the measurement of antioxidant enzymes activities and MDA.

The obtained livers were used to make 20% homogenate after dipping in cold KCl (1.15%). Then, the obtained homogenate was cold centrifuged for 15 min at 3000 rpm. All antioxidant parameters were analyzed using these supernatants.

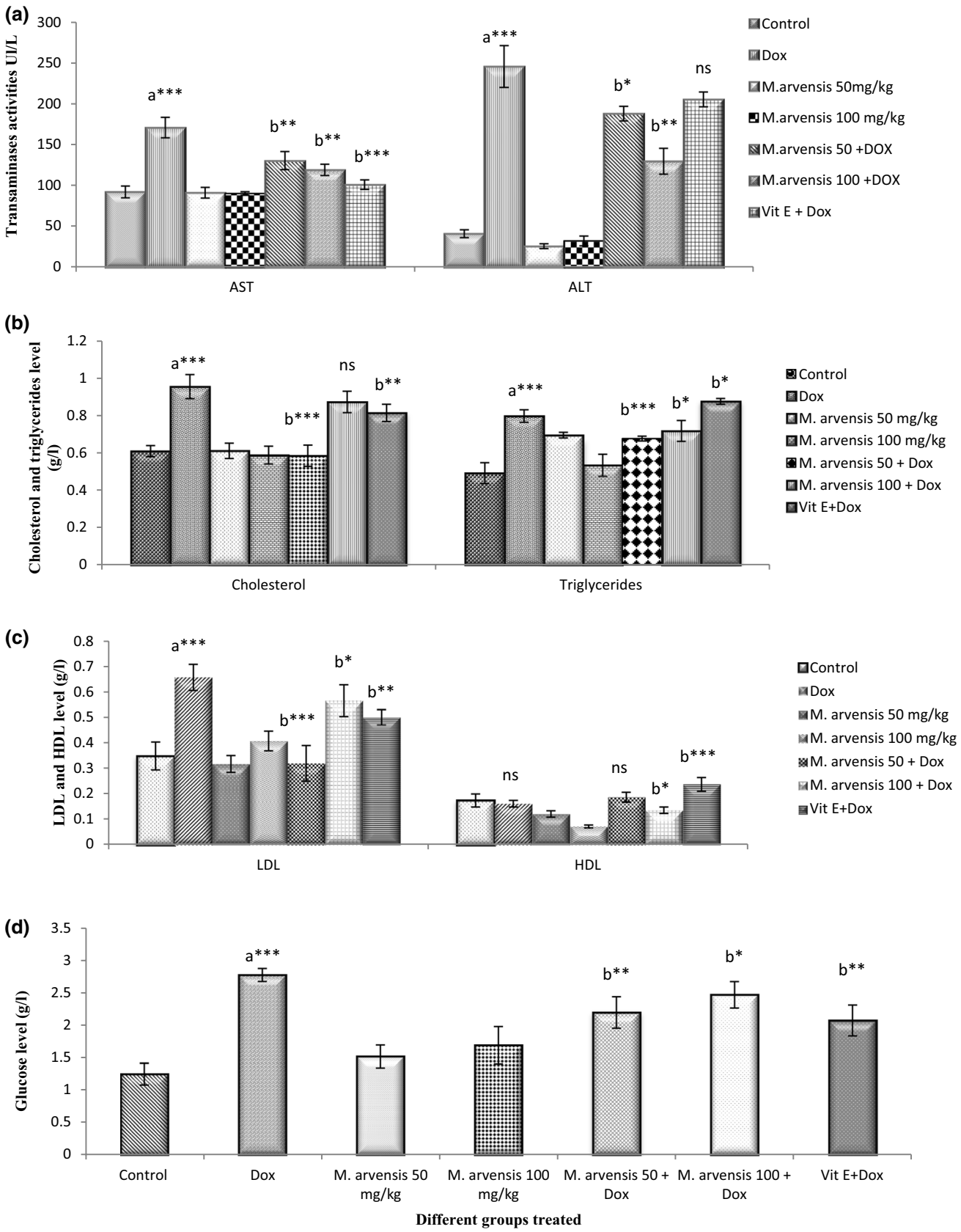
Dosage of biochemical parameters

After blood centrifugation for 10 min at 3000 rpm, the clear serum supernatants were analyzed for AST, ALT, cholesterol, triglyceride, HDL, LDL and glucose using commercial kits (Spinreact, Spain).

Evaluation of tissue antioxidant status

Lipid peroxidation (MDA) assay

Lipid peroxidation was measured in the supernatants of all homogenates using the thiobarbituric acid reactive substances (TBARS), a colorimetric method of Uchiyama and Mihara (1978). The reaction mixture contained the 20% of liver homogenates (0.5 mL), 1% phosphoric acid (3 mL) and 0.67% of thiobarbituric acid (1 ml of TBA). After 45 min of boiling, 4 ml of *n*-butanol was added. Then the mixture was centrifuged and absorbance was measured at 532 nm. All tests were performed in triplicate. The standard curve was constructed using an MDA standard and the TBARS content was given nmol MDA/mg protein.


(a)
Transaminases activities UI/L
300
250
200
150
100
50
0
a***
b**
b**
b***
a***
b*
b**
ns
AST
ALT
Control
Dox
M.arvensis 50mg/kg
M.arvensis 100 mg/kg
M.arvensis 50 +DOX
M.arvensis 100 +DOX
Vit E + Dox
(b)
Cholesterol and triglycerides level (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
a***
b***
ns
b**
a***
b***
b*
b*
Cholesterol
Triglycerides
Control
Dox
M. arvensis 50 mg/kg
M. arvensis 100 mg/kg
M. arvensis 50 + Dox
M. arvensis 100 + Dox
Vit E+Dox
(c)
LDL and HDL level (g/l)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a***
b***
b*
b**
ns
ns
b*
b***
LDL
HDL
Control
Dox
M. arvensis 50 mg/kg
M. arvensis 100 mg/kg
M. arvensis 50 + Dox
M. arvensis 100 + Dox
Vit E+Dox
(d)
Glucose level (g/l)
3.5
3
2.5
2
1.5
1
0.5
0
a***
b**
b*
b**
Control
Dox
M. arvensis 50 mg/kg
M. arvensis 100 mg/kg
M. arvensis 50 + Dox
M. arvensis 100 + Dox
Vit E+Dox
Different groups treated

◂**Fig. 1** Effect of DOX, plant extract and vitamin E on serum AST, ALT (**a**); Cholesterol, Triglycerides (**b**); LDL, HDL (**c**) and Glucose (**d**) levels in experimental rats.. Data are reported as means ± SD. a: group compared to control group, b: group compared to DOX group. (***$P < 0.001$;*$P < 0.05$; **$P < 0.01$)

Reduced glutathione content measurement

GSH is a co-factor of many enzymes, a powerful antioxidant, and an important scavenger of harmful oxygen radicals, which aids in the maintenance of normal cell functions (Jain et al. 2016). GSH amounts were measured using the Ellman (1959) method. The reaction mixture consisted of 0.5 mL of the obtained homogenates and 0.5 mL of trichloroacetic acid (10%TCA) were centrifuged for 5 min at 2000 rpm. Then, 0.2µL of supernatant was added to 1.8 mL of phosphate buffer solution (pH 8, 0.1 M) and 0.1 µL of Elman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid, called also DTNB). After the development of yellow color, the tubes were immediately read at 412 nm in a spectrophotometer and the GSH content was given in terms of nmol GSH/mg protein. Tests were carried out in triplicate.

Evaluation of glutathione peroxydase (GPx) activity

GPx activity was evaluated using Flohé and Günzler (1984) procedure. In the presence of GSH, GPx reduces the amount of H_2O_2 in the medium. Tissue homogenate (0.2 mL) was added to 0.4 mL GSH (0.1 Mm) and 0.2 mL TBS solution (Tris50mM, NaCl150mM pH 7.4). This mixture was incubated at 25C°. After 5 min, we added 0.2 mL of H_2O_2 (1.3 mM) and let it act for 10 min. Then, 1 mL of 1%TCA was added and the tubes were maintained in an ice bath at 0–5 °C for 30 min to terminate the reaction. Finally, the mixture was centrifuged (3000 rpm and 10 min) and 0.48 mL of supernatant was added to 2.2 mL of TBS solution and 0.32 mL of DTNB (1 mM). The absorbance was read at 412 nm after 5 min and the activity is given in nmol GSH/mg protein. Tests were carried out in triplicate.

Histopathological study

For the histopathological analysis that was conducted at the cyto-anatomo-pathological Laboratory of University Hospital Center of Constantine (Constantine district, Algeria), the liver samples were excised and rinsed with normal saline solution. The organ was then fixed in 10% formalin for 48 h, dehydrated in graded ethanol and embedded in paraffin wax. The obtained liver tubes were cut in 5 µm-thick sections that were deparaffinated in xylene and further stained Harris hematoxylin and eosin. Finally, the obtained slides were observed under the photometric microscope (Leica DM1000, Germany).

Statistical analysis

The obtained data were presented as Mean SD, and statistical significance was determined using Graph Pad Prism 5.01 version 5 for comparison of mean values of control and treated animals, with ($p < 0.05$) considered significant.

Results

Total phenolic content

The TPC of the aerial part extract of *Moricandia arvensis* was determined by the Folin–Ciocalteau procedure using GA as a standard. The *n*-butanol extract possessed a moderate level of the TPC equal to 86.25 ± 0.00 µg GAE/mg.

Total flavonoids content

The TFC of the *n*-butanol extract of *Moricandia arvensis* was measured using the spectrophotometric method that is based on the formation of a complex with $AlCl_3$. The flavonoids content that was expressed in terms of QE that was equal to 22.54 ± 0.01 µg QE/mg.

Effects on biochemical parameters

Serum transaminases (AST and ALT) levels

AST, ALT are sensitive markers of the liver, and their elevated levels are indicative of liver damage. The administration of DOX induced a significant increase ($p < 0.001$) in AST and ALT serum levels, they increased respectively from 92.1 ± 7.16 and 41.24 ± 4.75 to 170.54 ± 12.49 and 245.13 ± 25.48 compared to the control group. Whereas, the oral pre-treatment with both grade doses of *M. arvensis* extract as well as vitamin E lowered serum levels of AST and ALT significantly from 170.54 ± 12.49 and 245.13 ± 25.48 to 130.38 ± 11.07 or 118.95 ± 6.92 or 100.92 ± 5.80 and 187.57 ± 8.9 or 129.66 ± 15.88 or 205.01 ± 9.05in the three co-treated groups DOX + M50, DOX + M100 and DOX + VitE respectively in comparison with DOX-treated group (Fig. 1a).

Cholesterol and triglycerides levels

Cholesterol and triglycerides serum levels in DOX-treated group were increased significantly ($p < 0.001$) when compared to the control group (0.60 ± 0.02 vs 0.95 ± 0.06) and (0.49 ± 0.05 vs 0.79 ± 0.03). However, cholesterol concentration in the serum of co-treated groups DOX + M50 and DOX + VitE were decreased significantly in comparison

with DOX-treated group, they passed from 0.95 ± 0.06 to $0.58 \pm 0.05 (p < 0.001)$ and 0.81 ± 0.04 ($p < 0.01$) respectively. While, the pre-treatment with 100 mg/kg of *M. arvensis* extract induced an insignificant decrease in cholesterol level when compared with DOX-treated group.

The animals of the three co-treated groups DOX + M50, DOX + M100 and DOX + VitE showed a significant decrease in the triglyceride serum level, they were declined from 0.79 ± 0.03 to 0.67 ± 0.01 ($p < 0.001$) or 0.71 ± 0.05 ($p < 0.05$) or 0.87 ± 0.01($p < 0.05$) respectively, when compared with DOX-treated group (Fig. 1b).

HDL and LDL levels

DOX treatment led to a significant increase ($p < 0.001$) in LDL serum level compared to the control group (0.34 ± 0.05 vs 0.65 ± 0.05). The co-administration of both doses of *M. arvensis* extract and vitamin E showed a significant decrease in LDL serum levels from 0.65 ± 0.05 to 0.32 ± 0.07 ($p < 0.001$) or 0.56 ± 0.06 ($p < 0.05$) or 0.50 ± 0.03($p < 0.01$) respectively, when compared with DOX-treated group.

On the contrary, DOX administration causes an insignificant decrease in serum HDL levels compared to the control group (0.17 ± 0.02 vs 0.16 ± 0.01). However, the three co-treated groups DOX + M50, DOX + M100 and DOX + VitE showed a noticeable increase in HDL serum level, they passed from 0.16 ± 0.01 to 0.18 ± 0.01 or 0.13 ± 0.01or 0.23 ± 0.02 respectively, when compared with DOX-treated group (Fig. 1c).

Glucose level

Glucose serum level were increased significantly ($p < 0.001$) in DOX-treated group (1.36 ± 0.25 vs 2.59 ± 0.20) in comparison with the control group. The concomitant treatment of DOX with the graded doses of *M. arvensis* extract and vitamin E induced a significant decrease in glucose serum concentrations, they were decrease from 2.59 ± 0.20 to $2.44 \pm 0.08 (p < 0.01)$ or 2.56 ± 0.05 ($p < 0.05$) or $2.50 \pm 0.27 (p < 0.01)$ respectively, when compared with DOX-treated group (Fig. 1d).

Evaluation of antioxidant status in tissue samples

Effect on MDA level

The administration of DOX alone induced a significant increase ($p < 0.001$) in hepatic MDA levels when compared to the control group (0.02 ± 0.01 vs 0.10 ± 0.01). However, the oral pre-treatment with the two doses of *M. arvensis* extract and vitamin E decreased MDA level values that were near normal concentration, they passed from 0.10 ± 0.01 to 0.07 ± 0.01 or 0.04 ± 0.01 or 0.02 ± 0.01, thus providing a protective effect against DOX-induced lipid peroxidation in the liver (Fig. 2).

Effect on GSH level

The administration of a single dose of DOX induced a significant decrease ($p < 0.001$) in the GSH level (3.06 ± 0.18 vs 1.64 ± 0.08) in the liver homogenates as compared to the normal group. The pre-treatment with different doses of *M. arvensis n*-butanol extract and vitamin E showed a significant improvement in GSH concentration, they were increase from 1.64 ± 0.08 to $2.75 + 0.09$ or $2.71 + 0.14$or $2.53 + 0.09$ respectively, when compared with DOX-treated group (Fig. 3a).

Effect on GPx activity

The glutathione peroxidase activity in liver tissues of DOX-treated group marked a significant decrease ($p < 0.001$) when compared to the control group (0.54 ± 0.04 vs 0.42 ± 0.04). Whereas, the three co-treated groups DOX + M50, DOX + M100 and DOX + VitE showed a significant elevation in the activity of this enzyme. Indeed, GPx activity values increased from 0.42 ± 0.04 to $0.68 + 0.08$ or $0.59 + 0.07$or $0.78 + 0.08$ respectively, when compared with DOX-treated group (Fig. 3b).

Histopathology examination of liver tissues

A histopathological examination of liver tissues revealed that hepatocytes had a normal histological structure in the control group with rounded nuclei and blood sinusoids (Fig. 4). However, rats treated with DOX exhibited structural alteration in the liver tissue, a hepatic necrosis, ballooning of hepatocytes, disappearance of the nucleus and inflammatory cell infiltrates were observed. While the histoarchitecture of the livertissues treated with plant extract or vitamin E before administration of DOX showed a sinusoidal congestion. In contrast, the liver in groups treated with *Moricandia arvensis* only has a nearly normal liver histology with some minor changes like congestion. In addition, the hepatic architecture was preserved in 50%, 70% and 80% for animals treated with 50 mg/kg, 100 mg/kg of *n*-butanol extract of *Moricandia arvensis* and vitamin E, respectively.

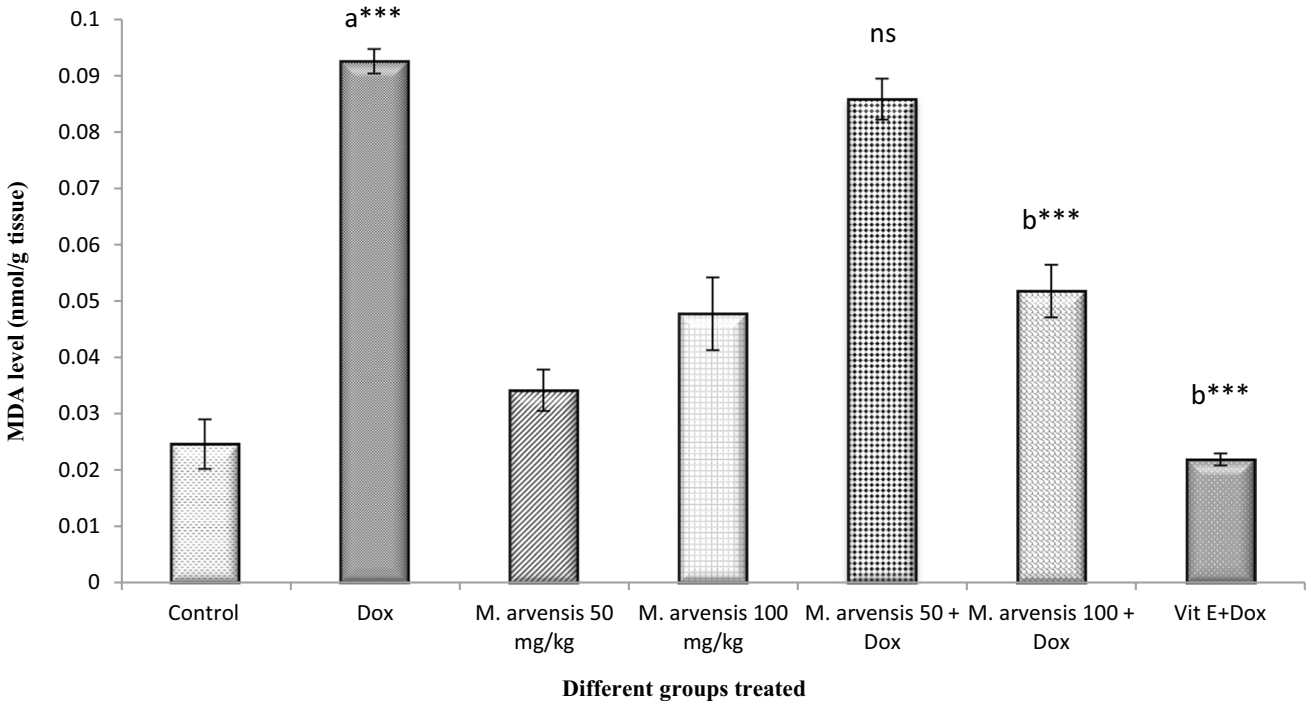


Fig. 2 Effect of DOX, plant extract and vitamin E on lipid peroxidation (TBARs content) in liver. Data are reported as means ± SD. **a**: group compared to control group, **b**: group compared to DOX group. (***$P < 0.001$)

Discussion

DOX is one of the anthracycline drugs that is widely used in cancer chemotherapy in a variety of human neoplasms like breast cancer, lymphomas, sarcomas, leukemias and others (Gu et al. 2018). Despite the wide use and efficiency of this treatment, many authors have reported different side effects that could mainly cause toxicity of several organs like brain, kidney heart and liver (El-Din et al. 2018; Ibrahim Fouad and Ahmed 2021; Siva et al. 2022; Wali et al. 2020).

The liver is an organ that has mainly a role in detoxification which makes it a preferable target of genotoxic compounds and anticancer drugs including DOX. Statistically, around 40% of patients treated by DOX have liver damage that might be due to the presence of a common quinine moiety in the anthracycline ring structure. This may cause both oxidative and reductive biotransformation and can result in oxidative stress generation due to the presence of ROS (Afsar et al. 2019). Thus, natural and/or synthetic antioxidant molecules might have a protective effect against the oxidative stress caused by cytotoxic drugs including DOX (Abdel-Sattar et al. 2012).

Plants bio-products with protective roles against oxidative damage in human body recently attracted immense attention. Phenolic compounds, is ranged as the first class of plant-derived antioxidants, potentially reduces oxidative stress. while, the balance between antioxidants and pro-oxidants is maintained owing to their metal chelating and free radical quenching effects (Gonçalves et al.2017).

Our attention was guided to *Moricandia arvensis* which belongs to the Brassicaceae family that contains phenolic compounds including flavonoids (Cartea et al. 2011). The determination of total phenolic and flavonoid contents of aerial part *M. arvensis n*-butanol extract findings disclosed a moderate level of antioxidant compounds with a TPC of 86.25 ± 0.00 µg GAE/mg and with TFC of 22.54 ± 0.01 µg QE/mg.

The single-dose administration of DOX (15 mg / kg) on rats has caused acute liver damage, which is evidenced by a the significant elevation of serum ALT and AST. The continuous generation of ROS and failure of the antioxidant system may cause hepatotoxicity. The high levels of biochemical biomarkers in the serums after drug-induced hepatic toxicity administration are an indication of liver damage that can be due to the enzyme leakage from hepatocytes (Wali et al. 2020). These results are in accordance with Zhao et al. (2012); Djebbari et al. (2017) and Sikandar et al. (2019).

Our results has shown that the DOX-treated rats significantly exhibited higher cholesterol, triglycerides and LDL levels and, in contrast, lower HDL levels. These findings are in accordance with the results reported by Mansouri et al.

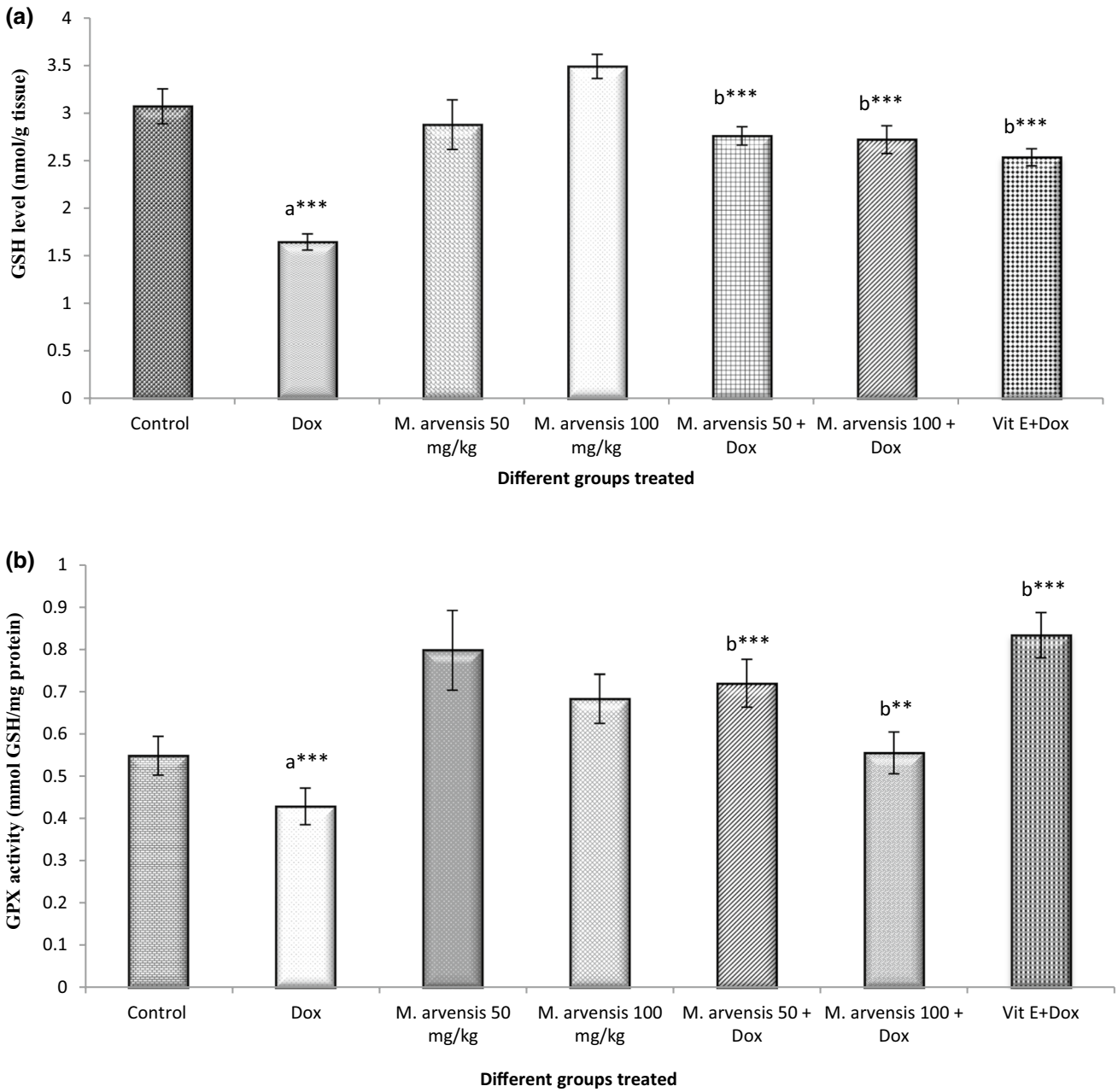


Fig. 3 Glutathion level and Glutathione peroxydase activity in control and experimental groups.. Data are reported as means ± SD. **a**: group compared to control group, **b**: group compared to DOX group. (***$P < 0.001$; **$P < 0.01$)

(2017) and Afsar et al. (2019). These alterations in lipid profile indicated that the DOX administration may interfer with lipid metabolism or biosynthesis (Bilgic and Ozgocmen 2019). In an another study, the authors showed that the DOX administration has the ability to alter the chemical structure and composition as well as the biological membrane functions, particularly in mitochondria. This can be due to the peroxidation of membrane lipids, leading to the release of proteins and cholesterol from the cytosol into the blood stream (Moussa et al. 2020).

The DOX injection has led to the glucose uptake reduction. In normal conditions, glucose is essentially transported across membrane cells inducing the translocation of glucose transporter isoform 4 (GLUT 4) (Biondo et al. 2016). In our study, the DOX administration has induced hyperglycemia ($p < 0.001$). This result, is in accordance with Moussa et al. (2020). Based on the study of Renu et al. (2019), DOX administration has caused a down-regulation in the expression of both GLUT1 and GLUT4 causing an attenuation of glucose transportation into adipocytes. However, the oral

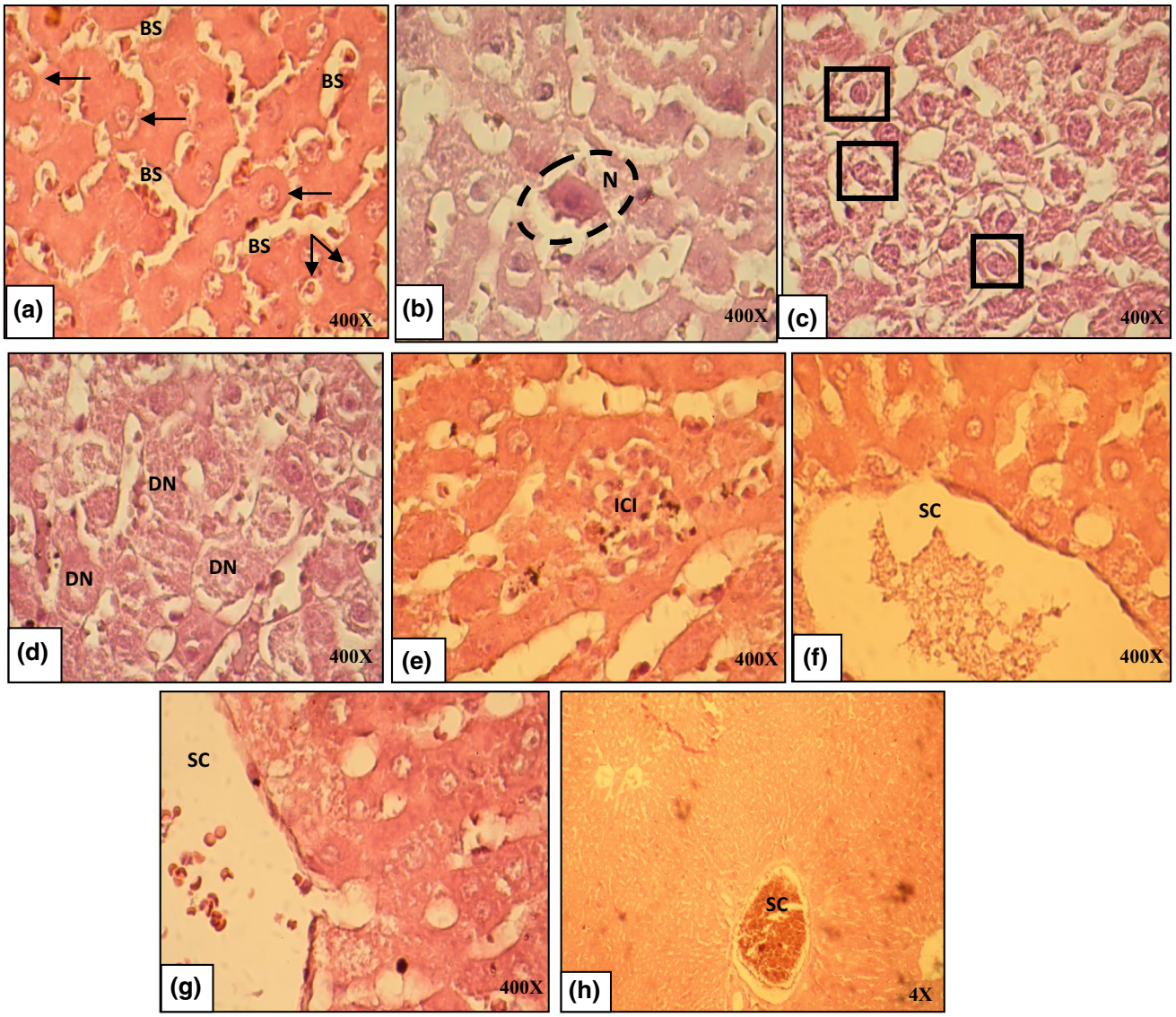


Fig. 4 Histopathologic results of rat liver **a**: normal hepatic architecture with normal blood sinusoids (BS) and rounded nuclei (arrow) **b**-**e**: Histology of liver treated with DOX b: hepatocellular necrosis (N), **c** ballooning of hepatocytes, **d** disappearance of the nucleus (DN), **e** inflammatory cell infiltrates (ICI); **f** and **g** liver of treated groups *n*-butanol extract 50 mg/kg and 100 mg/kg respectively showed Sinusoidal congestion (SC); **h** Histology of the liver treated with DOX + extract 50 mg/kg, 100 mg/kg and vitamin E showed Sinusoidal congestion (SC)

treatment of *n*-butanol extract of *Moricandia arvensis* has shown a significant amelioration of biochemical parameters in the DOX-treated groups.

Changes in the liver biochemical markers were followed by an increase of LPO in the DOX-treated rats. The increase of MDA, a major oxidation product of polyunsaturated fatty acids, is a key indicator of lipid peroxidation (Ahmed et al. 2019; Kalender et al. 2005).The obtained results showed that MDA levels were increasing in DOX-treated rats, which is consistent with the findings of Omobowale et al.(2018) and Al-Oanzi et al. (2020). The high levels of MDA after DOX injection could be attributed to the production of oxygen free radicals that caused multiple tissue mutilations, countering with membrane proteins, lipids and nucleic acids (Afsar et al. 2017).

Glutathione (GSH) is a tripeptide that has a major role in the detoxification of free radicals an antioxidant molecule. When the GSH levels decrease in tissues, it may result in peroxidative injuries and damage of cell defense against ROS. In our study, GSH levels decreased in the DOX-treated rats compared to the control group. In addition to being a direct free radical scavenger, GSH acted as a substrate for GPx. DOX treatment has led to a decrease in the GPx activity that could be caused by the unavailability of GSH

(Rashid et al. 2013).Our results $ agree with those obtained by several authors such as Fathy et al. (2017) and Gu et al. (2018). However, in our study, *Moricandia arvensis,* the treated rats has shown a decrease in the liver tissue MDA levels with an increase in the GSH levels and GP_X activity compared to the DOX- intoxicated group which confirms the protective effect on liver tissues. Natural bioactive compounds extracted from plants may have high antioxidant activity that could be due to the phenolics scavenged free radicals ability for example(El-Din et al. 2018).

The histopathology study for rats liver, administered with DOX, indicated the presence of lobular hepatic necrosis, ballooning of hepatocytes, disappearance of the nucleus and inflammatory cell infiltration. These findings are in accordance with those obtained by Mecheri et al. (2018). Other studies have confirmed that the intraperitoneal injection of 20 mg/kg of DOX dose presented parenchymal mononuclear cell infiltration, pyknotic nuclei in hepatocytes and macroand microvesicular steatosis (Bilgic and Ozgocmen 2019). Therefore, the pretreatment with *Moricandia arvensis* extract has restored the normal architectural structure of the liver. This observation correlates with the results of biochemical and oxidative stress parameters.

Conclusion

In conclusion, the present study revealed that *n*-butanol extract of *Moricandia arvensis* reduced the effect of DOX on the biochemical and histological liver injury in rats. We can say that *Moricandia arvensis* extract is a potential treatment for induced oxidative damage as a consequence of oxidative stress, including DOX- induced hepatotoxicity.

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Declarations

Ethical statement The University's Ethics Committee approved all experimental assays, which were carried out in accordance with national guidelines for the care and use of laboratory animals.

Conflict of interest Meriem Laraba has no conflict of interest. Sana Hazar Tachour has no conflict of interest. Hanene Belbache has no conflict of interest. Nassima Boubekri has no conflict of interest. Radja Djebbari has no conflict of interest. Fadila Benayache has no conflict of interest. Samir Benayache has no conflict of interest. Djamila Zama has no conflict of interest.

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