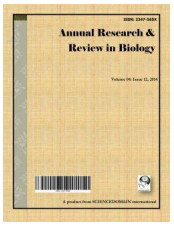

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Effect of Methanol Extract Prepared from Leaf of *Pistacia lentiscus* on Plasma Antioxidant Activity and Biomarkers of Oxidative Stress in Liver Tissue of Healthy Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SD designed the study, performed the statistical analysis. Author Amel Bouaziz wrote the protocol. Author Assia Bentehar wrote the first draft of the manuscript. Authors SK and Abderrahmane Baghiani managed the analyses of the study. Authors SD and SA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of the present study is to estimate the effect of the methanol extract of *Pistacia lentiscus* (PL) on plasma antioxidant capacity and biomarkers of oxidative stress in liver tissue of healthy female rats.
Methodology: The present work assessments oral administration of methanol extract at doses of 100, 200 and 400 mg/kg during 14 days on plasma antioxidant activities using DPPH and reducing

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power tests. Levels MDA, GSH and catalase activity in liver tissue of healthy female rats were estimated.

Results: The doses of 100 and 200 mg/kg during 14 days caused significant elevation of plasma antioxidant capacity using DPPH radical scavenging activity and reducing power assay compared to the control. Also, evaluation of MDA levels revealed that the doses of 100 and 200 mg/kg reduced significantly the lipid peroxidation in liver tissues. Treatment with methanol extract at doses of 100, 200 showed no significant difference in GSH level in the liver when compared with control group. Moreover, the activity of catalase enzyme caused non significantly decreased in 100 and 200 mg/kg treated groups. Highest depletion of the antioxidant activity was reported in post administration of 400 mg/kg. Finally, the dose of 400 mg/kg of the methanol extract for 14 days leads to a decrease of GSH levels and catalase activity. For this reason, medicinal plants need a critical evaluation of dose administration to avoid its side effects.

Keywords: Pistacia lentiscus; MDA; catalase; GSH; liver; in vivo antioxidant.

1. INTRODUCTION

Free radicals are produced either from normal cell metabolisms or from external sources (pollution, cigarette smoke, radiation, medication and their accumulation in the body causes a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative diseases such as cancer, autoimmune disorders, ageing, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [1]. Aerobic organisms have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of reactive oxygen species (ROS). Endogenous antioxidants play a crucial role in maintaining optimal cellular functions. However, under conditions that promote oxidative stress, endogenous antioxidants may not be sufficient. In such cases, dietary antioxidants should be supplied to maintain optimal cellular functions. Some antioxidants can interact with other antioxidants, regenerating their original properties. This process is referred to as the "antioxidant network" [2]. It was reported that the antioxidants were from ''Miracle Molecules'' to ''Marvellous Molecules'' and finally to ''Physiological Molecules'' [3]. There is no doubt that these molecules play a vital role in metabolic pathways and protect cells, but recently conflicting evidence has forced the academic community to discuss the role of antioxidants and pro-oxidants. These latter are defined as chemicals that induce oxidative stress, usually through the formation of reactive species or by inhibiting antioxidant systems [4]. Free radicals are considered prooxidants, but antioxidants which could also act as pro-oxidants in systems that contain redox-active metals [5]. The presence of O_2 and transition metals like iron and copper catalyze the redox cycling of phenolics and may lead to the formation of ROS and phenoxyl radicals which damage DNA, lipids and other biological molecules [6].

Pistacia lentiscus is an evergreen member of the Anacardiaceae family, largely distributed in the Mediterranean region [7], is widely used in phytotherapy for its sedative, antiatherogenic, and antioxidant properties [8]. The aerial parts of *P. lentiscus* were used in folk medicine as stimulants and diuretics, and to treat hypertension, cough, sore throat, eczema, stomach ache kidney stones and jaundice [9]. Other studies found that long-term administration of *Pistacia lentiscus* extract in healthy rats induced hepatic fibrosis and an inflammatory response, mild cholestasis, depletion of reduced GSH associated with an increase in its oxidized form [10]. The present study was undertaken to evaluate the effect of the methanolic extract prepared from the dried leaves of *Pistacia lentiscus* on plasma and liver tissue oxidation biomarkers in healthy rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *P. lentiscus* were collected from Sidi Ibrahim forest (Bordj BouArerridj) in eastern Algeria.The plant was identified by Pr. Hocine Louar from the laboratory of Botany, Faculty of Natural and Life Sciences, University Ferhat Abbas, Setif, Algeria.

2.2 Animals

Healthy albino female rats, weighing 140-190 g were obtained from Pasteur Institute in Algiers, Algeria, and were acclimatized for one week,

prior to experiments. The animals were housed in an air-conditioned animal room and maintained with free access to water and standard diet. The experimental protocol in rats was conducted after the experimental procedures were revised and approved by the Animal Ethics Committee of Institute of Nature and Life Sciences, University Ferhat Abbas, Setif 1, Algeria.

2.3 Preparation of *P. lentiscus* Leaves Extract

The extraction of phenolic compounds was carried out according to Markham [11]. Leaves of *P. lentiscus*(100 g) were dried in shadow, powdered and mixed with one litre of methanol-water solution (85:15 v/v) and kept at room temperature for 3 days. The resulting solution was then filtered and the solvent was evaporated under reduced pressure to get methanol extract (ME).

2.4 Antioxidant Activity *In vivo* of Plant Extract

2.4.1 Animal's treatment

Female rats were divided into 5 groups of 6 animals and treated for 14 days [12]. Group 1 received normal saline (0.9%) and served as control group. Group 2 was given 100 mg/kg of vitamin C. groups 3,4 and 5 were given a methanol extract of *P. lentiscus* (85%) at a dose of 100, 200 and 400 mg/kg, respectively. Drugs dissolved in normal saline (0.9%).

2.4.2 Preparation of plasma

On day 15, all animals were sacrificed. Blood was collected and centrifuged at 3000 rpm for 15 min for determination of plasma antioxidant capacity. The liver was removed and washed with ice-cold saline, blotted with filter paper and kept in plastic vials at -20°C until use [13].

2.4.3 Preparation of liver homogenate

The frozen livers were thawed and cut down into small pieces, placed in 1.15M KCl buffer and homogenized using dounce homogenizer in ice-cold condition to obtain 10 % homogenate. The homogenate thus obtained was centrifuged at 4000 rpm for 15 min and the supernatant collected was used for the determination of MDA, catalase activity, reduced glutathione (GSH) levels [13].

2.5 Plasma Antioxidant Capacity

2.5.1 DPPH radical scavenging assay

The capacity of the plasma to trap the DPPH radical was estimated according to the method of Burits and Bucar [14] with some modifications. Briefly, a volume of plasma was added to DPPH solution (4 mg/100 ml of methanol). After 30 min of incubation at room temperature and centrifugation, the absorbance of the supernatant was measured at 517 nm. Scavenging effect capacity was calculated according to the equation below:

% scavenging activity = [(Abs control - Abs sample) / Abs controls] X 100

$A_{control}$: is the absorbance of the blank solution.

A_{sample} is an absorbance in the presence of the test compound.

2.5.2 Reducing power assay

The reducing power of samples was determined using the method of Chung et al. [15]. The assay medium contained 0.1 ml of plasma was mixed with 0.1ml phosphate buffer (0.2 M, pH 6.6) and 0.1 ml of potassium ferricyanide (1%). After incubation at 50°C for 20 min, 0.25 ml of trichloroacetic acid 1% was added to the mixture followed by centrifugation at 3000 rpm for 10 min. Then, 0.25 ml of the supernatant was mixed with 0.25 ml distilled water and 0.5 ml of 0.1% ferric chloride and the absorbance of the mixture solution was read at 700 nm.

2.6 Determination of Antioxidant Status in the Liver Tissue

2.6.1 Lipid peroxidation

MDA is an important parameter for lipid oxidation, which was determined by the method of Okhawa et al. [16]. Each MDA molecule interacts with two molecules of thiobarbitiric acid (TBA) in an acidic medium and an elevated temperature to form a pink compound that can be measured at a 532 nm. Briefly, 0.5 ml of liver homogenate was mixed with 0.5 ml of TCA (20%) and 1ml TBA (0.67%). The mixture was boiled for 15 min. Then, 2 ml of *n*-butanol was added, and the solution was centrifuged at 3000 rpm for 15 min and read at 532 nm. The concentration of MDA was determined from a standard curve of 1, 1, 3,3 tetraethoxypropane in the same conditions and it was expressed as n mol/g tissue.

2.6.2 GSH level

Reduced glutathione (GSH) level was estimated using the method of Zerargui et al. [17]. This method is based on the reduction of 5,50-dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent) by sulfhydryl groups to form 2-nitro-5-mercaptobenzoic acid, which was estimated at a 412 nm. Briefly, 50 µl of homogenate was diluted in 10 ml of phosphate buffer (0.1 M, pH 8). Then, 20µl of DTNB (0.01 M) was mixed with 3 ml of the mixture of dilution. The yellow color developed was read at 412 nm after 5 min. A series of standards GSH (0.4-20 µmol/ml) were treated in a similar manner. GSH was expressed as µmol/ g tissue.

2.6.3 Enzymatic activity of catalase

The activity of the catalase enzyme in the liver tissue was measured using the method of Bentahar et al. [18], which was based on the decrease of absorbance at the 240 nm caused by H_2O_2 fragmentation into water and oxygen by the enzyme. In brief, 50 µl of the homogenate was mixed with 2.95 ml of H_2O_2 (19 mM) diluted in the phosphate buffer (0.1 M, pH = 7.4). The change in absorbance was monitored for two minutes. The enzymatic activity was expressed by the number of units per gram of tissue according to this formula:

U/g tissue = (2.3033/ T) × (log A1/A2) /g tissue.

A1: Absorbance at t0
A2: Absorbance at t1.
T: Interval of time (minute).

2.7 Statistical Analysis

Data obtained are expressed as mean ± SEM. Differences between the control and the treatments in these experiments were tested for significance using analysis of variance followed by Dunnet's test. A probability P value less than 0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity of the Plasma

Several methods have been developed to measure the antioxidant activity of plasma, including the DPPH and ABTS radical scavenging tests and the iron-retractable potential using FRAP method [19]. The relationship between these methods was their easiness to use and reliability in estimating the oxidative stress of plasma or organs [20].

DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. It is based on that a hydrogen donor is an antioxidant or radical scavenger. The results are shown in Fig. 1 showed that the administration of methanol extract of *P. lentiscus* at doses of 100 and 200 mg/kg in rats increased significantly the plasma antioxidant capacity with values of 25.09 % and 25.96% respectively compared to control group (09.92%).However, the administration of the doses of 400 mg/ kg had no significant effect (14.02%) compared with control group (P> 0.05).

Antioxidants, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Results showed (Fig. 2) that the two groups treated with methanol extract of *P. lentiscus* at 100 and 200 mg/kg had the highest reducing power with values of 1.703 ± 0.38 and 1.947 ± 0.0.04 respectively (P <0.001).

These obtained results showed no association between the concentration of *P. lentiscus* and plasma antioxidant capacity using DPPH scavenging assay and reducing power. Inappropriate concentrations, natural compounds act as antioxidants by giving electrons to modify free radicals. But at high concentration, these compounds become pro-oxidants. Several studies showing controversial results of exogenous antioxidants, debating that the type, dosage and matrix of these antioxidants may be determining factors impacting the balance between beneficial and deleterious effects of these natural compounds [21]. There are also some proofs that they act as pro-oxidants, under certain conditions, such as high doses or the presence of metal ions [22]. The antioxidant or pro-oxidant activity intimately depends on their concentration [21]. The consequences of pro-oxidant activity could be the possible damage to the biomolecules such as DNA, proteins and lipids, and the consequent cellular death.

3.2 Antioxidant Activity in Liver Tissue

The liver plays a key role in the biosynthetic and metabolic processes of the body. In particular,

since the liver is an important organ for detoxification.

3.2.1 Estimation of lipid peroxidation

Fig. 3 showed that the extract of *P. lentiscus* had the ability to inhibit lipid peroxidation in liver tissue and thus reduce the production of MDA compounds. The group treated with a dose of 200 mg/kg showed a greater ability to reduce MDA levels (60.77 nmol / g of tissue) compared to the control group (81.64 nmol / g tissue) ($P < 0.01$). Also, vitamin C which was used as a positive standard reduced MDA levels with value of 36.35 nmol / g of tissue.

Lipid peroxidation alters the physiological functions of cell membranes and plays an important role in cellular membrane damage. Peroxidation is believed to be involved in cellular ageing and in various diseases, such as Parkinson's and Alzheimer's disease as well as schizophrenia, atherosclerosis, inflammatory

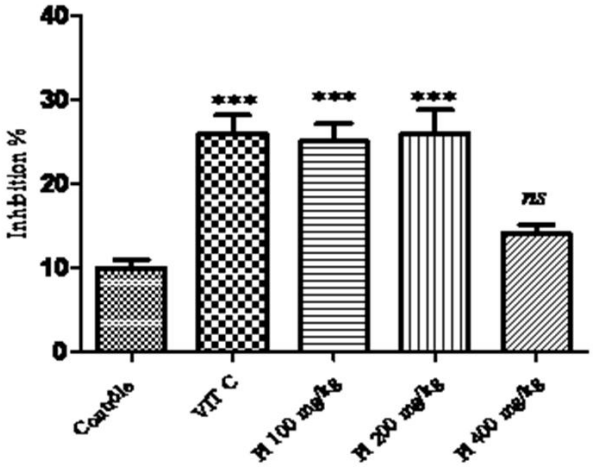

Fig. 1. Effect of methanol extract of *Pistacia lentiscus* (PI) on plasma antioxidant capacity using DPPH radical scavenging activity

*Data were presented as % means ± SEM (n = 6). (ns: no significant difference; *** p < 0.001) compared to control group*

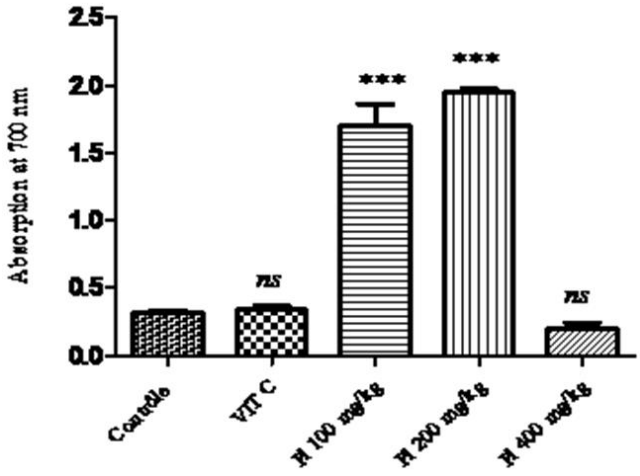

Fig. 2. Effect of methanol extract of *Pistacia lentiscus* (PI) on antioxidant capacity using reducing power assay

*Data were presented as means ± SEM (n = 6). (ns: no significant difference; *** p < 0.001) compared to control group*

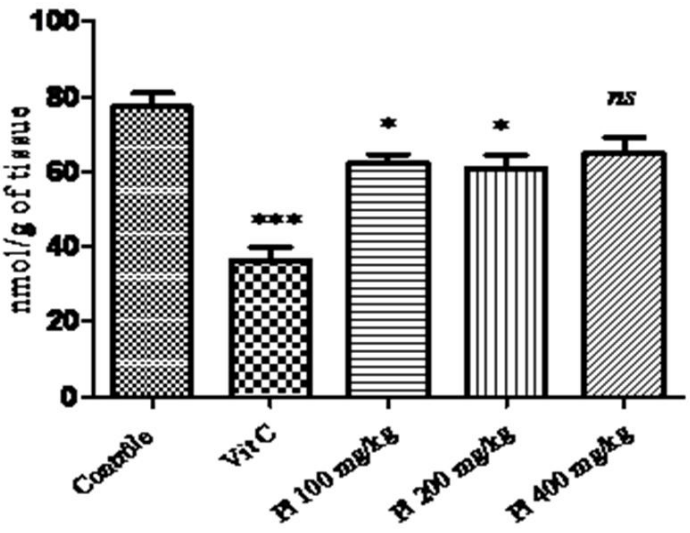

Fig. 3. Effect of methanol extract of *Pistacia lentiscus* (PI) on MDA level in liver tissue of rats
*Values are given as means ± SEM (n=6). (ns: no significant difference; * p < 0.05; ** p < 0.01; *** p < 0.001) compared to control group*

diseases, and cardiac ischemia-reperfusion injury [23]. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of this peroxidation [24]. Some phytochemical constituents in the extract as polyphenols and flavonoids may be responsible for the antioxidant activity. Flavonoids are a ubiquitous group of phenolic compounds which are present in most plants, concentrated in seeds, peel, and flowers [25]. Numerous studies have shown that flavonoids possess potent antioxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals [26]. Shahidi et al. [27] attributed the pharmacological activities such as anti-inflammatory, antiviral, antibacterial, antiulcer, antiosteoporotic, antiallergic, and antihepatotoxic actions of flavonoids to their potent antioxidant activity.Abdelwahhed et al. [28] reported that phenolic compounds derived from the fruit of *P. lentiscus* extract showed that the presence of gallic acid and its derivatives (1, 2, 3, 4, 6-pentagalloylglucose) played an important role in protection against lipid peroxidation induced by H_2O_2.

3.2.2 Levels of GSH in the liver

The amount of glutathione (GSH) in liver tissue was measured to determine the effect of plant extracts on a non-enzymatic antioxidant. It was observed that treatment (Fig. 4) with *P. lentiscus* methanol extract at dose 200 mg/kg increased the level of GSH value (19.95 µmol/g tissue) compared to the control group (17.25 µmol/g tissue)) (p> 0.05). While their level decreased in the group treated with 400 mg/kg.

Glutathione (GSH) is a tripeptide found in most of the tissues, especially in high concentrations in the liver, and plays an extremely important role in protecting hepatocytes, erythrocytes, and other cells against toxic injury. It is involved in enzymatic and nonenzymatic reactions. Nonenzymatically, it acts as a low-molecular-weight scavenger of reactive electrophilic xenobiotics and competes with DNA, RNA, and proteins in capturing electrophiles [29]. In this study, ME of *P. lentiscus* administered at dose of 400 mg/kg decreased the hepatic reduced glutathione. This finding is consistent with that of Ljubuncic et al. [10] who demonstrated that long-term administration (five weeks) of *P. lentiscus* extract in healthy rats induced hepatic fibrosis and an inflammatory response, mild cholestasis, depletion of reduced GSH associated with an increase in its oxidized form. Galati et al. [30] investigated that excessive intake of phenolic compounds can be transformed by peroxidase into an oxidized form (phenoxyl radicals), which in some cases is sufficiently effective for oxidation of GSH and NADH associated with significant oxygen consumption and thus the formation of active oxygen species. Others studies have shown that the hepatocyte's incubation with polyphenols with multiple rings leads to partial oxidation of GSH to GSSG in these cells [31].

3.2.3 Catalase activity

The three major antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) catalyze the decomposition of ROS. CAT is an essential enzyme in the decomposition of intracellular H_2O_2. It promotes the breakdown of H_2O_2 into water and oxygen without producing free radicals. As shown in Fig. 5, daily administration of methanolic extract of *P. lentiscus* at doses of 100 and 200 mg/kg caused no-significant reduction in CAT activity (37.09 and 37.12 U/ g tissue respectively) compared to control group (43.30 U/ g tissue). However, he treatment with the same extract at a dose of 400 mg/kg reduced significantly the activity of this enzyme (27.25 U/ g tissue) ($p < 0.01$).

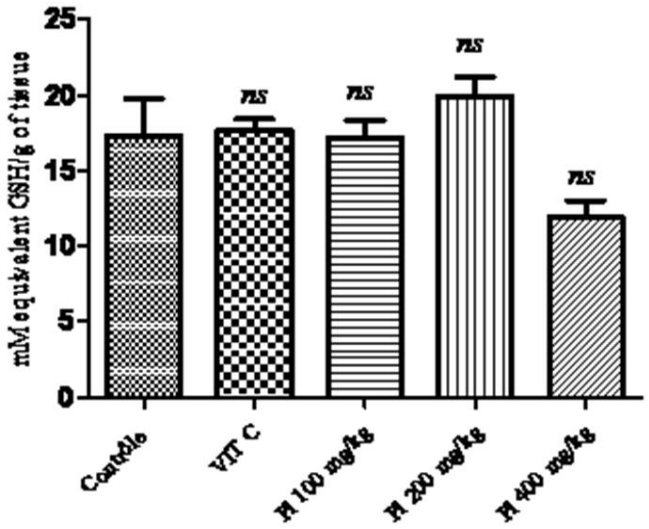

Fig. 4. Effect of methanol extract of *Pistacia lentiscus* (PI) on reduced glutathione level (GSH) in liver tissue of rats

Values are given as means ± SEM (n=6). (ns: no significant difference) compared to control group

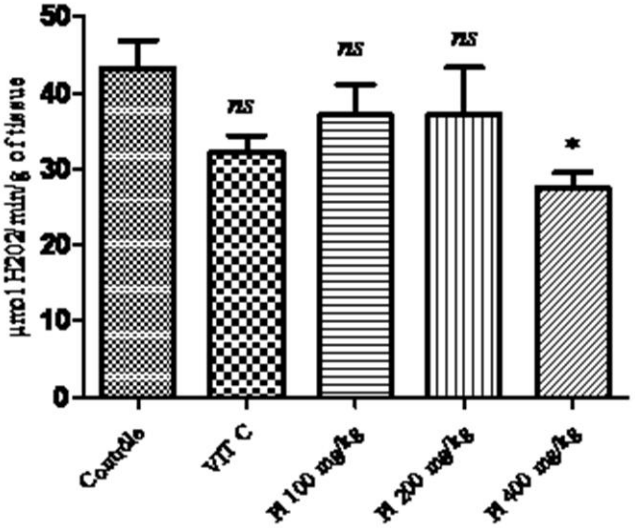

Fig. 5. Effect of methanol extract of *Pistacia lentiscus* (PI) on CAT activity in liver tissue of rats

Values are given as means ± SEM (n=6). (ns: no significant difference;
** p < 0.05) compared to control group*

The activity of catalase increases when the level of oxidative stress rises or when the activity of glutathion peroxidase decreases. Catalase enzyme plays an active role in the treatment of oxidative stress by cells. In this study, the treatment rats with *P. lentiscus* at the doses of 100 and 200 mg/kg didn't affect enzyme activity. However, the administration of high concentration of ME of *P. lentiscus* decreased significantly catalase activity. Other results demonstrated that leaves of *P. lentisus* contain toxic phytochemicals [10]. Our results contradict the experimental findings of Janakat and AlMerie [32] whose reported that a single dose of a decoction of *Pistacia lentiscus* was beneficial (1.946 g/4ml/kg) for three days. This effect is seemingly time-dependent because a single oral dose of the decoction was beneficial. This discrepancy obviously warrants further investigation in future. As is known, the leaves of *Pistacia lentiscus* contain condensed tannins which were proven as hepatotoxins at higher doses [33,34]. Therefore, we can only speculate that long-term treatment with *Pistacia lentiscus* could be induced hepatotoxicity due to the activity of these tannins.

4. CONCLUSION

Extract prepared from *Pistacia lentiscus* is commonly used traditionally to treat the gastrointestinal disorders in Algeria. We could conclude that the administration of methanol extract of the plant leaves caused an increase in the total plasma antioxidant capacity and an improvement of antioxidant status and decreasing lipid peroxidation by lowering MDA level in liver tissues at doses of 100 and 200 mg/kg of rats during 14 days. Whereas this extract exhibited a toxic effect when administered daily for 2 weeks at dose of 400 mg/kg of rat. These results will be supported by future studies on toxic effect of methanol extract for confirming the use of plant in treatment of diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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