Original Article

Neurobehavioral effects and toxicological profiles of Pistacia lentiscus essential oil in mice: possible mechanism of action involved

Chafik Terrafe[1,*], Majda Badaoui[2], Fatimazahra Agourram[3], Ismail bouargalne[4], Rachida aboufatima[5], Abderrahman Chait[6]

ABSTRACT

Background

Pistacia lentiscus essential oil has been shown to have antidepressant like effect, gastro-protective effect.

Objectives

This study aims to evaluate for the first time the toxicological profiles and neurobehavioral effects of Pistacia lentiscus (PLEO) in single or triple administration using several animal models. Furthermore, the possible mechanisms of action involved in antidepressant like effects were investigated.

Methods

To this end, mice were randomly divided into the negative control group, treated groups (12.5; 25 and 50mg/kg of PLEO), positive control groups (Diazepam 2 mg/kg; Fluoxetine 10 mg/kg; Omeprazole 25 mg/kg). Concerning the toxicological profile, animals were treated daily for 28 days with PLEO or vehicle, and at the end of the experiment, mice were assessed in a battery of tests. Then, were sacrificed by cervical dislocation for histopathological examinations and estimation of biochemical parameters.

Results

Our outcomes demonstrate that, single administration of PLEO induced a potential anxiolytic like effect. However, triple administration of PLEO provokes a stronger antidepressant like effect more than single administration; this effect was reversed when mice were pretreated with yohimbine, olanzapine, and cyproheptadine. Daily treatment with PLEO has no significantly effect on body weight. Histopathological examination of target organs has not any tissue alterations; in addition, biochemical parameters of treated groups are within normal ranges in comparison to the negative control. Regarding locomotor and exploratory behavior, daily treatment with PLEO has no effect on muscular tone. It is also demonstrate that administration 3 times per day possesses a strong gastro-protective effect.

Conclusion

The findings of this investigation revealed for the first time that, PLEO essential oil possesses a potential anxiolytic and antidepressant like effects. It was also demonstrated that PLEO does not modify exploratory and locomotor activity. The pretreatment with PLEO prevents gastric ulcers.

Keywords

anxiolytic; antidepressant; sub-acute toxicity; neurobehavioral assays.

INTRODUCTION

Traditional pharmacopeian Moroccan culture is rich; a timeless traditional knowledge and practices are transferred from generation to the next. Among the most evident example. The medical use of *Pistacia lentiscus* due to its cardio-tonic, antidiabetic and digestive disorders[1]. Since the antiquity, this plant has been widely used and consumed for different therapeutic purposes [(2-6)]. From that moment, the use of *Pistacia lentiscus* has become more significant in market for herbal medicines. However, descriptions of its pharmacological activities on general health in literature studies

1. Chafik Terrafe, Laboratory of Pharmacology, Neurobiology, Anthropology and Environment, Department of Biology, Faculty of Sciences Semlalia, University Cadi Ayyad , BP 2390 40080 Marrakech, Morocco.
2. Majda Badaoui, Laboratory of Pharmacology, Neurobiology, Anthropology and Environment, Department of Biology, Faculty of Sciences Semlalia, University Cadi Ayyad , BP 2390 40080 Marrakech, Morocco.
3. Fatimazahra Agourram Laboratory of Pharmacology, Neurobiology, Anthropology and Environment, Department of Biology, Faculty of Sciences Semlalia, University Cadi Ayyad , BP 2390 40080 Marrakech, Morocco.
4. Ismail bouargalne, Faculty of sciences, Department of biology, University Mohamed 5, Rabat.
5. Rachida aboufatima, Laboratory of Genie Biologic, Sultan Moulay Slimane University, Faculty of Sciences and Technics, BeniMellal, Morocco.
6. Abderrahman Chait, Laboratory of Pharmacology, Neurobiology, Anthropology and Environment, Department of Biology, Faculty of Sciences Semlalia, University Cadi Ayyad , BP 2390 40080 Marrakech, Morocco.

Correspondence

Chafik Terrafe, Laboratory of Pharmacology, Neurobiology, Anthropology and Environment, Department of Biology, Faculty of Sciences Semlalia, University Cadi Ayyad, BP 2390 40080 Marrakech, Morocco. E mail:Chafik.terrafe@edu.uca.ac.ma

DOI: https://doi.org/10.3329/bjms.v24i1.78732

primarily rely on public disclosures [7,8].

Powder from crushing and grinding *Pistacia lentiscus* commonly known as Derou, essential oil, vegetable oil or infusion mixed with other plants such as *Ceratonia siliqua*, *Trigonella Foenum graecum*. Due to this extensive use in popular cultures and North African countries including Morocco dominance in production and commerce, Derou was added in official pharmacopeia.[9]

Recently, *Pistacia lentiscus* is widely recommended by herbalists to cure gastrointestinal disorders[8]. In human research, the gum of this plant at 350 mg/kg taken 3 times per day for 3 weeks of therapy reduced the symptoms of functional dyspepsia compared to placebo. In the same research, *Pistacia lentiscus* reduced symptoms such as general stomach discomfort, anxiety related stomach pain, a dull upper abdominal soreness and indigestion [10].

Pistacia lentiscus was purchased without any prescription and used on the assumption as safe herbal product. Moreover, the safety character is currently unknown. Regarding the possible side effect of this plant, there is conflicting evidence. Therefore, a further characterization of the side effects of *Pistacia lentiscus* essential oil (PLEO) in animal models is needed since the treatment of gastro-intestinal disorders. In the Mediterranean circuit, the population used up to three doses per day, thus long-term adverse effects.[3,10,11,12]

The present investigation has two objectives. To assess the possible mechanism of action involved on antidepressant and anxiolytic like effects of *Pistacia lentiscus* essential oil in mice after single or triple admiration. In the second part, to examine the PLEO safety on mice. To this end, the toxicological profiles of PLEO were evaluated in female and male mice through a repeated administration for 28 days. We obtained the behavioral finding in the inverted screen test (IST), open field test (OFT), rota rood test (RRT). Lastly, gastro-protective effect of PLEO was evaluated using HCL/Ethanol model.

MATERIEL AND METHODS

Chemical reagent

The antagonists (olanzapine, cyproheptadine, yohimbine and propranolol are used as D2, 5HT2, α2 and β antagonist receptors), were purchased from Novartis, Pharma (Lot: SLBM77593R, SLMZ4129N, 00549LN and KNZ7834O, respectively) Maroc, S.A. References drugs (Diazepam, Fluoxetine and Omeprazole) were purchased from Sotherma and Pharma5 from Morocco. Tween 80 used as vehicle solution was purchased from Sigma-Aldrich (Germany). The alkanes injected into Rtx-5 column were purchased from Sigma-Aldrich, (St, Louis, USA).

Plant material

Collection and identification

Collection of aerial part (fruits) of *Pistacia lentiscus* and its identification was previously described in our team study.[13]

Essential oil isolation and GC-MS analysis

The method of isolation of *Pistacia lentiscus* essential oil and identification of its principal compounds were described in our recent publication.[10]

Animals

The animal material used in this investigation was male Swiss mice weighing 27-33 g from animal house of Faculty of Sciences Semlalia at (12 hours dark/light cycle) in temperature conditions 24± 1 hour. Experimental animals were divided into different groups residing in cages with free access to food and water. One hour before all experiments, animals were acclimatized to the laboratory conditions. All pharmacological assays were accomplished in conformity to the institutional protocols and in accordance with the European Council Directive (86/609/EEC).

Drugs and treatment

The intraperitoneal pathway was used to administer various treatments to all groups. Diazepam (DZP; 2 mg/kg), Fluoxetine (FLX; 10 mg/kg) or Omeprazole (Omz; 30 mg/kg) were used as positive group (30 mg/kg). Which are solubilized in saline solution (9‰). The negative control was treated using saline solution and Tween 80 mixture. PLEO at (12.5, 25 and 50 mg/kg) was used for the treatment of all other groups.

Animals were pretreated with different antagonists such as; cyproheptadine, yohimbine, propanolol, olanzapine as (5HT3, α2, β and D2 receptors). In order to evaluate the possible mechanism of action involved in antidepressant-like effect. Drugs used were purchased from the society Novartis Pharma Maroc.

Experimental procedures

Pistacia lentiscus essential oil effects on antidepressant and anxiolytic like behavior

This aim was achieved by evaluating doses of PLEO that were declared as safe according to[10], in separate groups of male Swiss mice (six each). A prior acute toxicity assessment served as the basis for the selection of doses and acute schedules (single or triple). Six separate groups of animals were created and utilized for the elevated plus maze test. Teen additional independent groups were used for evaluation in the forced swimming and open field tests five minutes later to detect any motor alterations (see figure 1).

Elevated plus maze test (EPMT)

The apparatus of plus maze consists of four arms: two elevated close and two open arms above the floor surface (h=40 cm). Five groups (n=six each) had been treated with Pistacia essential oil at (12.5, 25 and 50 mg/kg), vehicle and Diazepam as negative and positive group respectively. Thirty minutes later, animals of all groups were placed on central intersection of apparatus; two parameters were recorded such as time spent in each arm and number of entries during five min.[13] (figure1)

Forced swimming test (FST)

The possible antidepressant like effect of *Pistacia lentiscus* essential oil was carried out in two experimental methods. Firstly, five independent groups received a single injection of PLEO at (12.5, 25 and 50 mg/kg). Thirty minutes later, they were evaluated by assessing the forced swimming test pre-session; other groups negative and positive were administered with vehicle and fluoxetine (2.5 mg/kg) respectively. In second series, other five groups were received three administrations of PLEO doses over a 24h period (21h, 7h and 60 min before 3 min test session. Positive control group treated with three administrations of fluoxetine (2.5 mg/kg), but 30 minutes before the 3 min of test, the third treatment was administered. Animals were placed separately into cylindrical apparatus (Ø=30cm, H=59 cm). Mice were allowed to swim for 6 minutes then dried and transported back to their cages. Immobility time and dynamic swimming were recorded.[14] (figure 2)

Sub-acute toxicity (28 days) assessment of Pistacia lentiscus essential oil in female and male mice

A toxicological investigation was carried out using the repeated dose 28-day oral toxicity protocol for rodents a specified by the Organization for Economic Cooperation and Development (OECD 2008). PLEO at 12.5, 25 and 50 mg/kg was administered daily to various groups (5 male and 5 female each). Animals have been extensively monitored on the first day during the first 2 hours post treatment, as well as at eight and twelve hours post administration (60 min per observation). Animals were monitored once daily for one hour on the next day to determine possible toxicological signs such as, lethargy, piloerection, coma, locomotor alterations and abdominal constrictions. The body weight variation was recorded daily for 28 day. Animals were assessed using a battery of locomotor assays on the 29th day. Following that, animals were scarified by cervical dislocation to examine the possible biochemical and macroscopic alterations on target organs.[(15-17)](Schematic time line)

Behavioral side effects of Pistacia lentiscus essential oil in locomotor activity assays

On the 29th post treatment with PLEO, animals were examined in a battery of assays. The test order was started by open field test before the inverted screen test and rotarod assay. This succession did not interfere with individual behavior of each mice obtained in each assay. The open field, inverted screen and rotarod tests respectively, motor coordination, neuromuscular function and motor activity impairments were assessed (Schematic time line).

Open field test

Exploration and mobility were carried out using open field test. Each individual animal was placed in the center of apparatus five minutes on the test day. The number of crossings, immobility duration and redress behavior are recorded.[18]

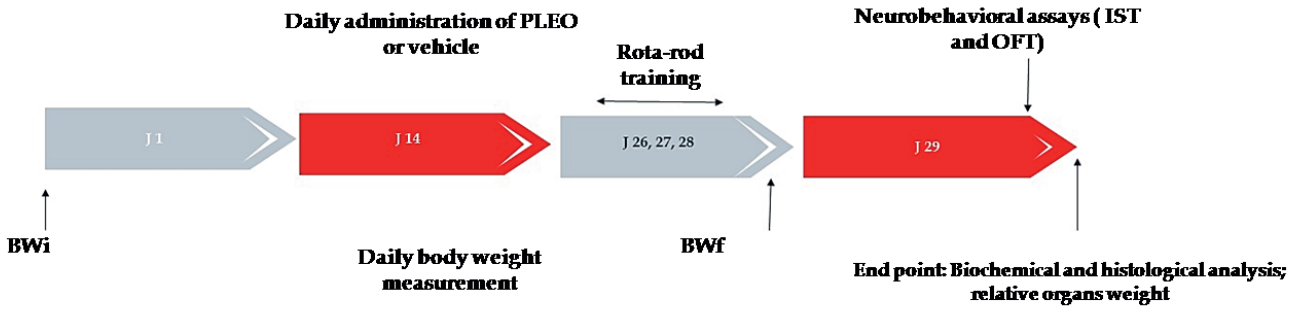
Inverted screen test

A (30 cm^2 square-wire mesh, 25 mm2 holes) screen was reversed over two seconds period with a mousse's head dangling in the center of this screen. A firm cushioned platform was used to maintain the screen consistently 20 to 30 cm above the floor. The parameter recorded was latency for failing was scored by blinded to different treatments. Two minutes constitute the cut-off time of test. However, the test may be finished after occurrence of first fall in two minutes assay for each sequence. The results are presented as percentages according to the following formula:

%= First fall latency (sec) * 100/cut-off time of test (2 minutes).[16]

Rotarod assessment

To investigate the eventual impairment of motor activity, rotarod test was carried out. During the 26th 27th and



28th days post treatment period, animals were trained to remain for 5 min while 22rpm is fixed as speed rotating. In the 29 day, animals were assessed in the test session during 5 minutes. For each mouse, the first fall latency from the apparatus was recorded.[16]

Schematic timeline of sub-acute toxicity (28 day) protocol

Relative organs weight

In the end of experiment, animals were scarified by cervical dislocation to remove the involved organs in toxicity evaluation such as kidneys, spleen and liver. Then, organs were placed in saline solution (0.9%). The relative weight was calculated using this formula: RW= weight of organ (g) / body weight on sacrifice day (g).

Histological examination and biochemical analysis

The protocol used to accomplish this study was previously described in our recent publication[10].

Gastro-protective effect of Pistacia lentiscus essential oil

With few adjustments, the test was carried out as described by Matsuda. Male swiss mice were divided into 5 groups (n=6) and given intraperitoneal injection of PLEO, vehicle (10 ml/kg) or Omeprazole (30 mg/kg) after being fasted for 24 hours. All groups were received 0.2 ml of 0.3 M HCL/60% Ethanol solution orally to induce stomach ulcers after 60 min. Then, animals were sacrificed by cervical dislocation to measure stomach injury.[19] The ulceration index was calculated according to the following formula;

Lesion scores:

Degree 1: lesion <1 mm

Degree 2: lesion between 1 and 2 mm

Degree 3: lesion >2 mm

Ulceration index = (1× (number of lesions degree 1) + 2× (number of lesions degree 2) + 3× (lesions degree 3))/10

Statistical analysis

ANOVA one way was used to compare all statistical analysis presented as mean±SEM. Then, Kruskal-Wallis or Tukey's tests were performed followed by post-hoc test.

RESULTS

Chemical profile of Pistacia lentiscus essential oil

The essential oil of *Pistacia lentiscus* obtained from fruits had a distinct odor, clear and yellowish color. According to the GC-MS analysis, this oil contains 31 volatile compounds corresponding to 97.69%. The major compounds are α-myrcene (22.98%), α-pinene (15.89%) and terpineol (24.12%). All results are mentioned in Table 1.

Acute effects of Pistacia lentiscus essential oil on behavior

Anxiolytic and antidepressant-like effects of PLEO in forced swimming and plus maze tests

Concerning anxiolytic like effect, single or triple administration of PLEO produce a significant effect by increasing the time spent in the open arms of the plus maze and decreasing of number of entries in open arms in comparison to the control group. Obtained results are similar to those reported by diazepam utilized as reference drug; suggesting a possible anxiolytic like effect (Figure.4 A-B).

As shown in figure 4C, the acute administration of PLEO or fluoxetine (single administration) did not induced a significant reduce of immobility time during test period. Therefore, triple administration of PLEO produced a remarkable decrease in immobility time at

all pharmacological doses. Particularly, antidepressant like effect of PLEO at 50 mg/kg provoked similar and comparable effect to those recorded by reference drug. In order to investigate the possible mechanism involved in antidepressant like effect, mice were pretreated with different antagonists such as, yohimbine (2 mg/kg; α2 adrenergic receptor antagonist), olanzapine (2 mg/kg; dopaminergic receptor antagonist), propranolol (5 mg/kg;β adrenergic receptor antagonist) and cyproheptadine (3 mg/kg; 5HT2 receptor antagonist). The obtained outcomes demonstrates that, pretreatment with olanzapine and cyproheptadine reverse the antidepressant like effect of PLEO at high pharmacological dose (50 mg/kg). This indicates the possible involvement of dopaminergic and 5HT2 receptors (Figure 4C).

Repeated dose 28-day toxicity profile in female and male mice

Changes in body weight variation induced by Pistacia lentiscus essential oil

Results shown at (Figure 5) illustrate the change in body weight during the 28 days of experimental period. About female groups, no doses of treatment affect significantly the change of the parameter cited above. The graph present normal body weight fluctuations that do not deviate from that of control group.

At the first fifteen days of experiment, male groups of different doses of treatment by *Pistacia lentiscus* essential oil present normal changes in body weight comparing with those of control group, however, during the second fifteen days, treated groups show body weight changes slightly less than those of control group. This last is responsible of an increase in the cited parameter but still not significant in comparison with all treated groups.

Relative organs weight and histopathological effect of PLEO

The (Table 2) present ROW index of relative organs weight of experimental groups of animals; it is evident from the table that the treatment by different doses of *Pistacia lentiscus* essential oil (12.5, 25 and 50mg/kg) do not affect the index described above. Right and left kidneys, liver, stomach and spleen of male and female experimental groups present approximatively close values of ROW index. In addition, macroscopic examination of target organs demonstrates no pathological changes or lesions attributable to the *Pistacia lentiscus* essential oil. Concerning histological examination, no possible histological changes in the target organs were observed in female mice. The (Figure 3) shows representative images of the kidneys, liver, stomach and spleen. Results demonstrate that only at high dose, the PLEO extract induced mild damages in livers of male mice such as liver congestion.

Biochemical parameters changes by Pistacia lentiscus essential oil

As demonstrates in (Table 3), biochemical analyses show no significant difference in all biochemical parameters in mice treated with all biological doses. Daily administration of PLEO at (50 mg/kg) produced slight variation of lever parameters such as ALAT and ASAT only in male mice group in comparison to the negative control.

Toxicological profile was evaluated using sub-acute toxicity assessment, results indicates that administration of PLEO at 28day did not provoke any lesions or abnormalities in target organs (spleen, rate and kidney) in male and females. Hepatic biochemical parameters were moderately changed only at high dose. To date in our knowledge, no study was done on sub-acute toxicity of PLEO, which suggests that our research will be the first.

Neurobehavioral effect of PLEO in inverted screen test, rotarod test and open field assay.

The neurobehavioral effects of *Pistacia lentiscus* essential oil are shown in (Table 4). The 28-day post treatment with PLEO did not modify rearing or count numbers in female or male groups submitted to the open field assay. In the inverted screen test, many sex differences were induced by the PLEO because the highest pharmacological doses (50 mg/kg) produced an important significant diminution in the grasping time in females compared to the negative control. In the other hand, assessment of animals in Rotarod test demonstrates that the 28 daily administrations with PLEO were unable to provoke alterations in motor coordination in both sexes.

Gastro-protective effect of Pistacia lentiscus essential oil

The HCl/Ethanol solution was used to induce gastric lesions, as reported in (Figure 6) the pretreatment with PLEO reduce the ulceration index significantly in comparison to the group that receive vehicle. It is also to be noted that triple administration produces a

strong protective effect of PLEO suggesting possible preventive and curative effect of this plant. The macroscopic observations as illustrate in (Figure 7) demonstrates that groups treated with PLEO represent a minor ulcer in removed stomach. Whereas, removed stomachs of the negative group present a major lesion, therefore increasing of ulceration index.

Moroccan population had always resorted to aerial parts of *Pistacia lentiscus* to treat gastrointestinal diseases, including gastric ulcers. To evaluate the possible antiulcer activity of PLEO, the HCl/Ethanol mixture was used to induce gastric lesions. As mentioned previously in results section, triple administration of PLEO significantly reduces the ulceration index in comparison to the control. The observed effect is comparable to the reference drug (Omeprazole).

DISCUSSION

The present investigation of *Pistacia lentiscus* essential oil from fruits indicates that 28 daily administrations of PLEO did not induce any significant change in body weight and relative organs weight at all doses. In addition, our results indicate for the first time that PLEO did not possess a sub-acute toxicity. Nevertheless, high dose of PLEO modify slightly some biochemical parameters such as hepatic markers (ALT) for both sexes. Furthermore, histopathological examination of target organs 28 day later shows any microscopic alterations. On the other hand, PLEO as mentioned in results section, possess an antidepressant and anxiolytic-like effects. In addition, 28 daily administrations of PLEO did not affect motor coordination activity.

In our previous study, phytochemical analysis reveals the presence of various monoterpenes compounds. Chemical composition it depends to the altitude, harvesting season and climatic conditions. The obtained outcomes are in accordance with literature described in details in our previous reports.[10]

The level of anxiety was assessed. One currently used assay for investigation into a potential anxiolytic effect of new drugs is the elevated plus maze test. Anxiolytic drugs can actually enhance the frequency of entries and duration in the open arms. To this purpose, the administration of PLEO increase significantly the period of permanence and frequency in the open arms indicating a strong anxiolytic like effect of *Pistacia lentiscus* essential oil from fruits. These findings are consistent with those reported previously by.[20] In addition, open field test was used to evaluate the locomotor and exploratory activity. Results shows that administration of PLEO extends period of immobility and reduce numbers of crossing. As demonstrated, *Pistacia lentiscus* essential oil possesses a strong anxiolytic action, this effect according to[27,28] may be related to the presence of monoterpenoids compounds, theses lasts acting by GABA transmission and Chlore-channel complex. Furthermore, the hydrophobic properties of terpenoid molecules allow them to easily pass the blood brain barrier and exert their anxiolytic effect on the central nervous system.

For the first time when the antidepressant effect of *Pistacia lentiscus* was evaluated and especially in mice. The results we found from male mice might be applied to the female mice, where depressive disorders are known to be more common. It is widely recognized that female menstrual variations throughout the estrous cycle might affect anxiolytic and antidepressant drugs effects[26]. As results, hormonal repair and ovariectomy trough progesterone and estradiol are required for the assessment of novel psychoactive drugs in females, such as *Pistacia lentiscus* essential oil. We'll use these techniques when we perform further research. The triple administration of PLEO elicited strong antidepressant effect in similar manner noradrenalin/serotonin selective reuptake inhibitors. The major depressive illnesses have linked to abnormalities in monoaminergic systems such as, serotoninergic, dopaminergic or noradrenergic systems. Recently, several investigations reported the implication of α2, D2, β and 5HT2 receptors.[14] In order to assess the implication of 5HT2, D2, α2 and β receptors in the antidepressant like effect mentioned previously, mice were pretreated respectively with cyproheptadine, yohimbine, propranolol and olanzapine as antagonists. The antidepressant like effect of PLEO was reversed when animals were pretreated with yohimbine, olanzapine and cyproheptadine suggesting that PLEO acting by adrenergic, dopaminergic and serotoninergic receptors. Our outcomes confirm several previous reports which indicate the implication of noradrenergic system in depression disorders. The dopaminergic receptor (D2) possesses a positive effect in the treatment of depressive disorders, since antagonists of this receptor inverse the antidepressant like effect of various antidepressant drugs.[14] We recommended this particular plant as a safer option for reducing depression and anxiety since *Pistacia lentiscus* essential oil did not cause any side effects after a single (or even prolonged) treatment.

This contrasts with the serious negative consequences generated by diazepam as established throughout this study, including loss of muscular tone and reduce motor coordination. When combined, the symptoms of anxiety, depression, metabolic and gastrointestinal disorders have an adverse effect on health. In certain circumstances, gastric disorders may occur before the first signs of depression.[21-23]

A thorough visual assessment of important organs like kidney, spleen and liver revealed nothing abnormal. Whereas there were certain treatment and gender related modifications in body weight, these wide modifications in physical structure of animals had no effect on the relative organ weight, supporting the idea that the impact of PLEO administration on body weight is not correlated with adverse effect on animal's general health. The outcomes of the present investigation demonstrate that female and male presented excellent increasing in weight gain over the daily treatment (28 day) with PLEO. Also, males gain less weight than females; this variation may be related to the sex hormones. The involvement of sexual hormones must be considered in such studies to establish their effect on metabolism and/or food behavior.

Sub acute exposure to pharmacological drugs may cause several side effects. To this end, mice were assessed to a battery of neurobehavioral tests after 28 day of treatment. The rota-rod and inverted screen tests were used to evaluate the locomotor activity. In inverted screen test mice those treated with PLEO possesses a similar latency for failing in comparison to the control group. However, the latency for failing decrease significantly in diazepam treated group. Failure of ambulatory movement in mice is a sign of disrupted of central nervous system functions[16], but our outcomes any dose of PLEO affected spontaneous ambulatory behavior by either depression or activating brain activity. Similarly, obtained results demonstrate the absence of impaired motor coordination or exploratory activity. These lasts were confirmed by inverted screen test, when mice showed strong grip strength through time testing.

Concerning antiulcer activity, our findings are in accordance with several previous reports.[24, -26] Nevertheless, antiulcer activity of PLEO could be attributed to the presence of monoterpenoids compounds. Several mechanisms have been proposed to explain terpenoids antiulcer action, including increased mucosal prostaglandin content, decreased histamine production from mast cells via inhibition of histidine decarboxylase and inhibiting *Helicobacter pylori* development.[30]In another study in vivo, four week following infection. Mice were given 2 g of mastic gum of *Pistacia lentiscus* twice day for 7 days as antimicrobial chemotherapy. The findings showed that mastic was unable to eliminate *Helicobacter pylori* in mice [31].

CONCLUSION

This investigation demonstrated that *Pistacia lentiscus* essential oil produced a remarkable anxiolytic like effect, and for the first time in our knowledge, we reported the antidepressant like effect. This effect was reversed when mice were pretreated with yohimbine, olanzapine and cyproheptadine, suggesting the implication of dopaminergic, adrenergic and serotoninergic pathways in modulation of depression disorders. Even after lengthy therapy, histopathological examination of selected organs revealed no deleterious alterations or morphological abnormalities induced by intraperitoneal administration of *Pistacia lentiscus* essential oil. In addition, motor coordination and exploratory behavior were not affected after 28 days of treatment. It also provided a beneficial effect on gastric ulcer. This confirms the excessive use of this plant in traditional pharmacopeia to treat several illnesses.

ACKNOWLEDGMENTS

Big thank for Mr A. Regragui for his assistance in providing drugs and animals to the authors.

Declaration of Competing Interest

Authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

Chafik Terrafe: Methodology, writing original draft preparation. **Majda Badaoui**: Conceptualization writing-review. **Fatimazahra agourram**: Essential oils analysis and compounds identification. **Ismail bouargalne**: Data analysis and acquisition. **Rachida Aboufatima**: Validation, writing review & editing. **Abderrahman Chait:** Methodology, Validation, Writing original draft preparation & editing.

Table 1. Chemical characterization of *Pistacia lentiscus* essential oil by GC-MS.

Peak N°	RRI[a]	RRI[b]	RT (min)	Compounds PLFEO	GC-MS %
1	1010	1011	3,25	Tricycline	0,35
2	1023	1008-1029	4,15	α-Thujen	7,42
3	1064	1054-1097	7,25	Camphene	0,66
4	1098	1086-1112	7,89	α-Pinene	15,89
5	1121	1097-1128	8,14	α-Myrcene	22,98
6	1193	1171-1217	8,56	α-Phellandrene	2,38
7	1231	1195-1239	10,59	Cadinene	0,43
8	1276	1207-1296	11,25	o-Cymene	0,28
9	1285	1246-1300	11,98	p-Cymen	2,25
10	1310	1271-1324	14,45	Borneol	0,26
11	1386	1307-1399	15,32	Terpinene	0,96
12	1413	1381-1434	15,86	Verbenol	0,41
13	1428	1436-1474	16,5	β-Phellandrene	0,32
14	1467	1450-1494	17,25	Limonene	0,33
15	1482	1462-1520	17,79	Transtagetone	0,14
16	1520	1502-1578	18,52	α-Humulene	0,18
17	1545	1534-1589	19,32	Terpinen-4-ol	0,38
18	1594	1563-1593	20,19	Myrtenol	1,28
19	1601	1586-1610	21,14	Terpineol	24,12
20	1614	1591-1628	21,87	Linalool	0,69
21	1649	1611-1657	22,66	Cis-β-Ocimene	0,25
22	1675	1626-1680	23,51	Bornylacetate	0,13
23	1683	1638-1698	25,14	Caryophyllene oxide	2,36
24	1708	1667-1710	26,19	Sabinene	1,99
25	1765	1688-1774	27,65	Germacrene D	2,21
26	1795	1731-1802	28,44	. τ-Muurolol	2,78
27	1804	1797-1813	30,08	ç Muurolene	1,72
28	1817	1801-1858	30,78	α-Cadinol	0,17
29	1862	1824-1881	31,31	p-Camphrene	1,19
30	1893	1876-1907	32,2	α-Caryophyllene	0,31
31	1901	1894-1911	33,01	6-Epicarotol	3,08
Total					**97,9**
Grouped compound (%)					
Monoterpene hydrocarbons			56,93		
Oxygenated monoterpenes			27,28		
Sesquiterpenes hydrocarbons			4,81		
Oxygenated sesquiterpenes			5,31		
Others			3,57		

RRI[a]: relative retention indices calculated against n-alkanes; RRI[b]: relative retention indices estimated from the literature

Table 2. Effect of PLEO on the relative organ weight: 28-day treatment.

	Vehicle	Treatment (mg/kg)		
		12,5	25	50
organ (mg)				
ROW male				
Kidney	6,9±0,4	7,1±1,2	7,9±0,8	7,2±1,7
Liver	45,3±0,7	43,5±0,9	48,1±1,3	47,3±1,2
Spleen	5,2±0,8	4,5±1,2	4,9±0,6	5,6±0,8
ROW female				
Kidney	6,4±1,1	6,1±0,3	6,8±0,3	5,7±0,5
Liver	43,6±1,7	44,1±2,9	45,3±3,1	42,1±1,3
Spleen	7,1±1,2	7,2±0,5	6,8±0,2	7,8±0.9

Table 3. Effect of *Pistacia lentiscus* essential oil on biochemical parameters of male and female mice: 28-day treatment.

		Treatment (mg/kg)		
	Vehicle	12,5	25	50
Analyte				
Male				
ALT U/L	65,1± 0,1	74,2±2,4	91,5 ± 0,9	81,2 ± 1,5
AST U/L	139± 3,2	124 ± 2,1	142 ± 4,3	142 ± 5,3
GLC (mmol/L)	3,78± 0,1	3,28 ± 0,6	4,81 ± 0,5	3,87 ± 0,1
UREA (mmol/L)	13,4± 0,5	12,4 ± 0,2	11,4 ± 0,3	10,4 ± 0,1
CHOL (mmol/L)	2,3± 0,4	2,8 ± 0,6	2,1 ± 0,7	2,5 ± 0,1
ALBUMINE	33,5± 2,1	30,5 ± 1,5	29,2 ± 1,9	34,5 ± 0,8
Female				
ALT U/L	85,1± 5,7	75,1 ± 4,8	69,1 ± 2,9	78,1 ± 5,7
AST U/L	143± 3,5	134 ± 9,2	139 ± 8,1	121 ± 3,4
GLC (mmol/L)	4,35± 0,2	3,18 ± 0,3	3,78 ± 0,6	4,50 ± 0,2
UREA (mmol/L)	13,4±2.7	10,4 ± 2,5	11,4 ± 4,6	10,4 ± 5,8
CHOL (mmol/L)	1,9± 0,7	2,2 ± 0,2	2,4 ± 0,1	2,1 ± 0,3
ALBUMINE	27,4± 5,4	29,5 ± 3,1	31,5 ± 2,6	34,5 ± 2,3

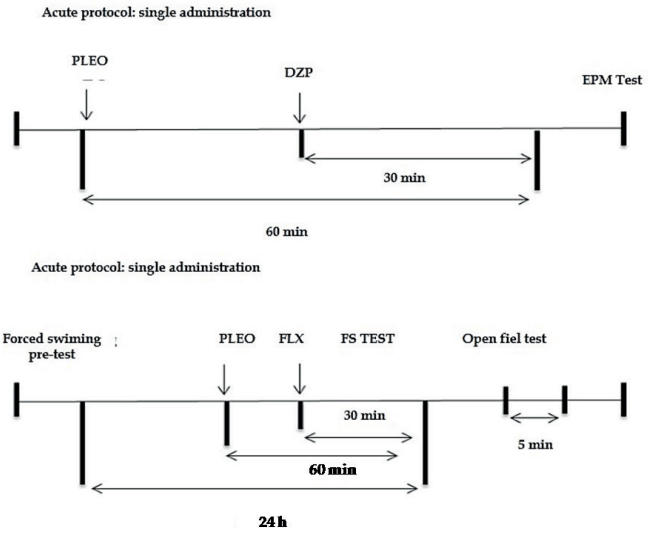


Figure 1. Schematic time line of acute protocol single administration (FS: Forced swimming; DZP: Dizepam)

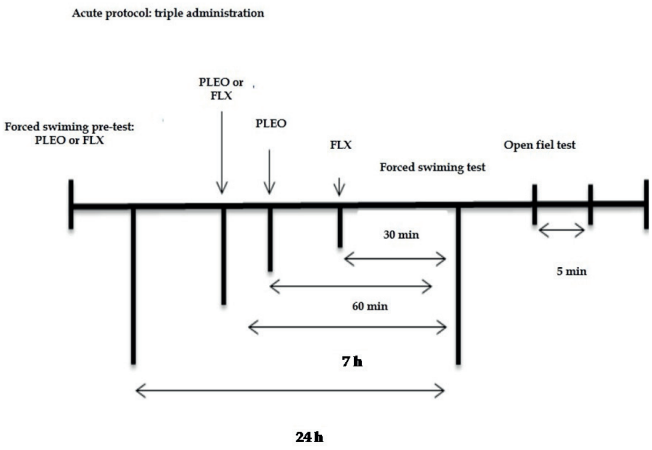


Figure 2. *Schematic timeline of acute protocol triple administration (FLX: Fluoxetine; PLEO: Pistacia lentiscus essential oil).*

Table 4. Neurobehavioral effect of *Pistacia lentiscus* essential oil and diazepam in mice.

Treatment	Dose	OFT count number /5min		IST Latency for failling (%)		Rota-rod test latency to the first fall (s)	
		Males	Females	Males	Females	Males	Females
Vehicle	-	75	104	100	100	300	300
PLEO	12,5 mg/kg	64	98	85,4	94,1	300	300
	25 mg/kg	61	85	77,3	70,4	294	287
	50 mg/kg	53	79	61,6	50,4	290	294
DZP	2 mg/kg	31	53	30,3	48,2	210	187

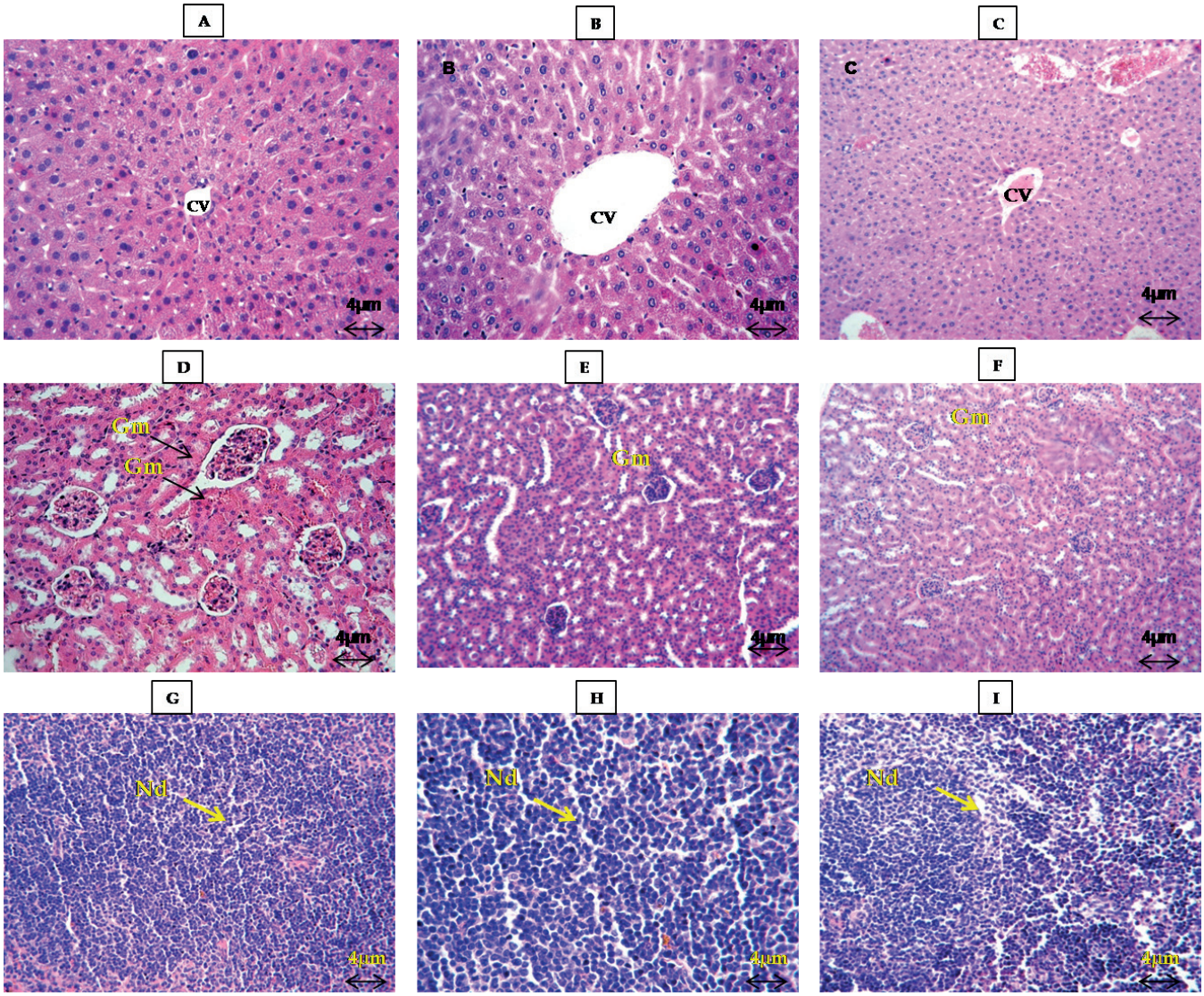


Figure 3. Histopathological examinations of liver, kidney and spleen in sub-acute toxicity 28 days later. Liver tissue: control (A), PLEO at 25 mg/kg (B) and PLEO at 50 mg/kg (C) (CV= central vein). Kidney tissue: control (D), PLEO at 25 mg/kg (E), and PLEO at 50 mg/kg (F) without any alterations (Gm=glomerulus). Spleen tissue: control (G), PLEO at 25 mg/kg (H) and PLEO at 50 mg/kg (I) (Nd=lymphatic nodes).

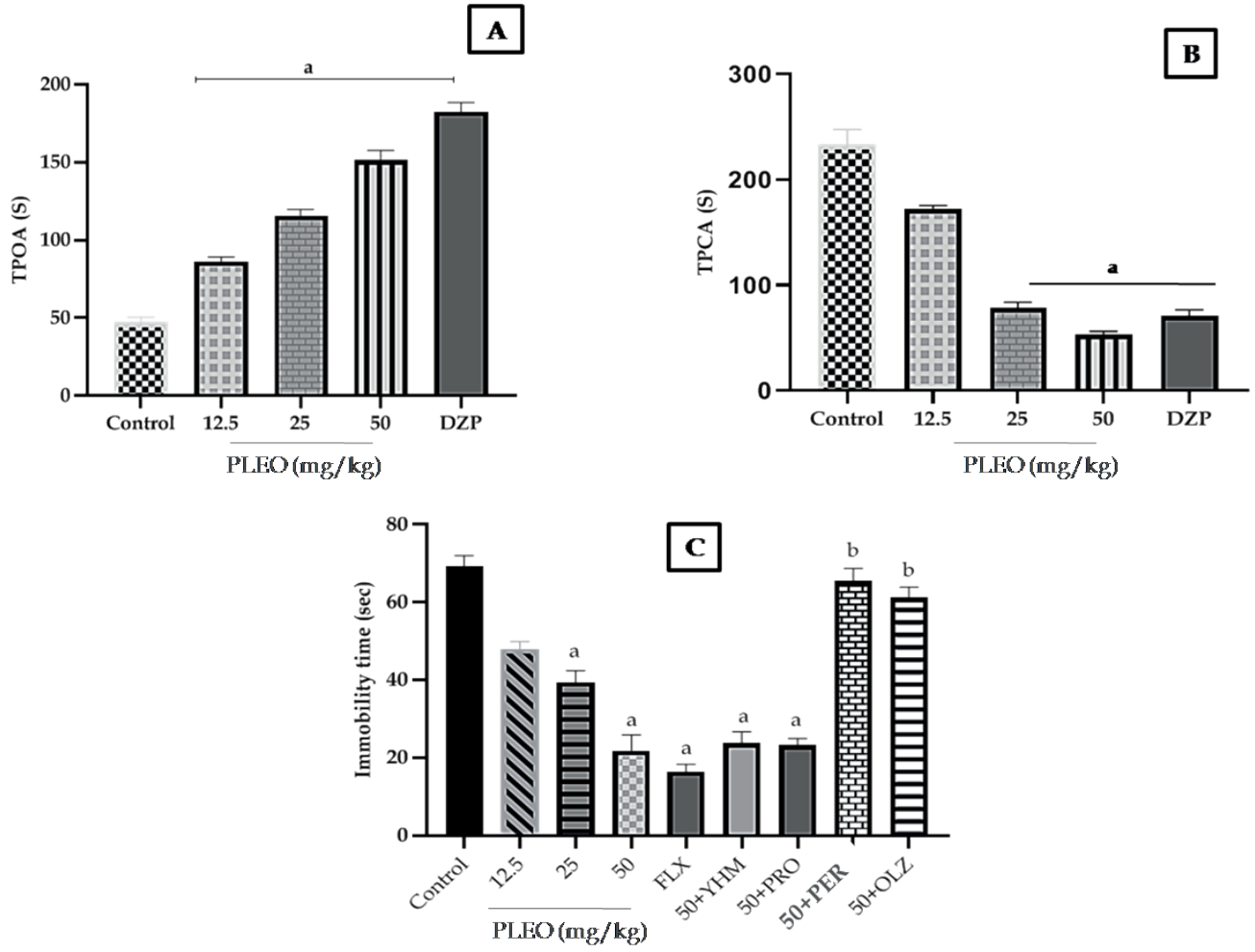


Figure 4. (Panel A & B): Effect of acute treatment (single administration) of PLEO or Diazepam in mice submitted on the elevated plus maze test (n=6 in each group). Results are presented as mean ± SEM, where a indicates significant differences between negative and treated group.

(Panel C): Effect of acute treatment (triple administration) of PLEO or FLX and pretreatment with Yohimbine (2 mg/kg), Propranolol (5 mg/kg), Cyproheptadine (3 mg/kg), Olanzapine (2 mg/kg) in male mice submitted on the forced swimming test (n=6 in each group). All data are expressed as mean ± SEM, where a indicate significant difference between treated groups and control, b indicate significant difference between antagonists and group treated with PLEO (50 mg/Kg).

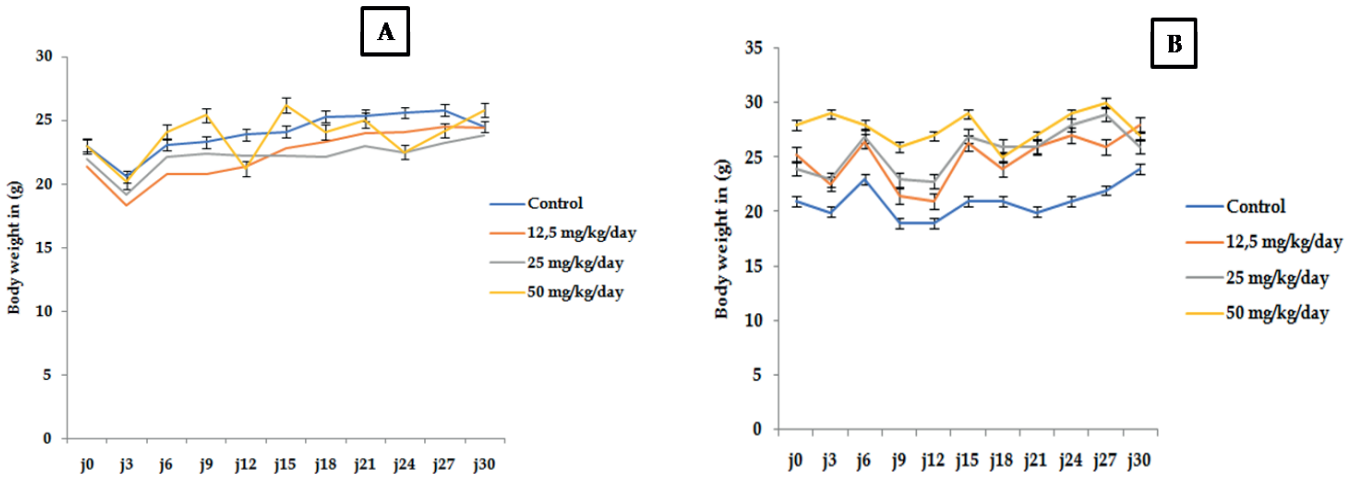


Figure 5. Body weight variation in animals produced by intraperitoneal administration of *Pistacia lentiscus* essential oil or vehicle of 28 days. Panel A: male mice, Panel B: female mice. Data are expressed as mean±SEM

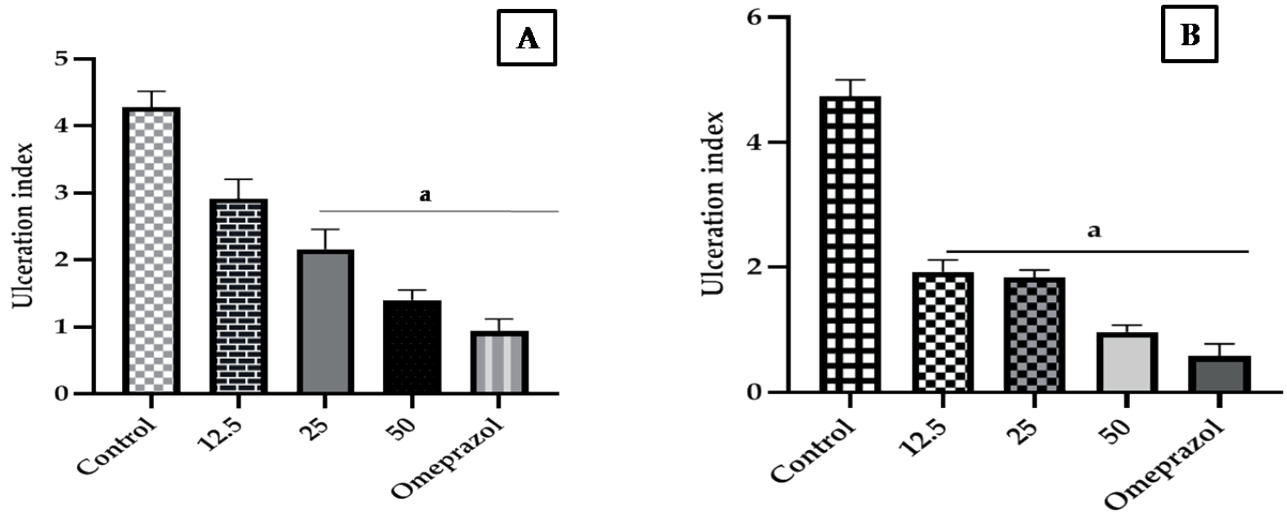


Figure 6. Gastro-protective effect of *Pistacia lentiscus* essential oil and omeprazole on Hcl/Ethanol induced gastric lesions (n=6/group). Results are expressed as mean ± SEM, where a indicates the significant difference between, positive control, treated groups and negative control. (Panel A): single administration; (Panel B): triple administration.

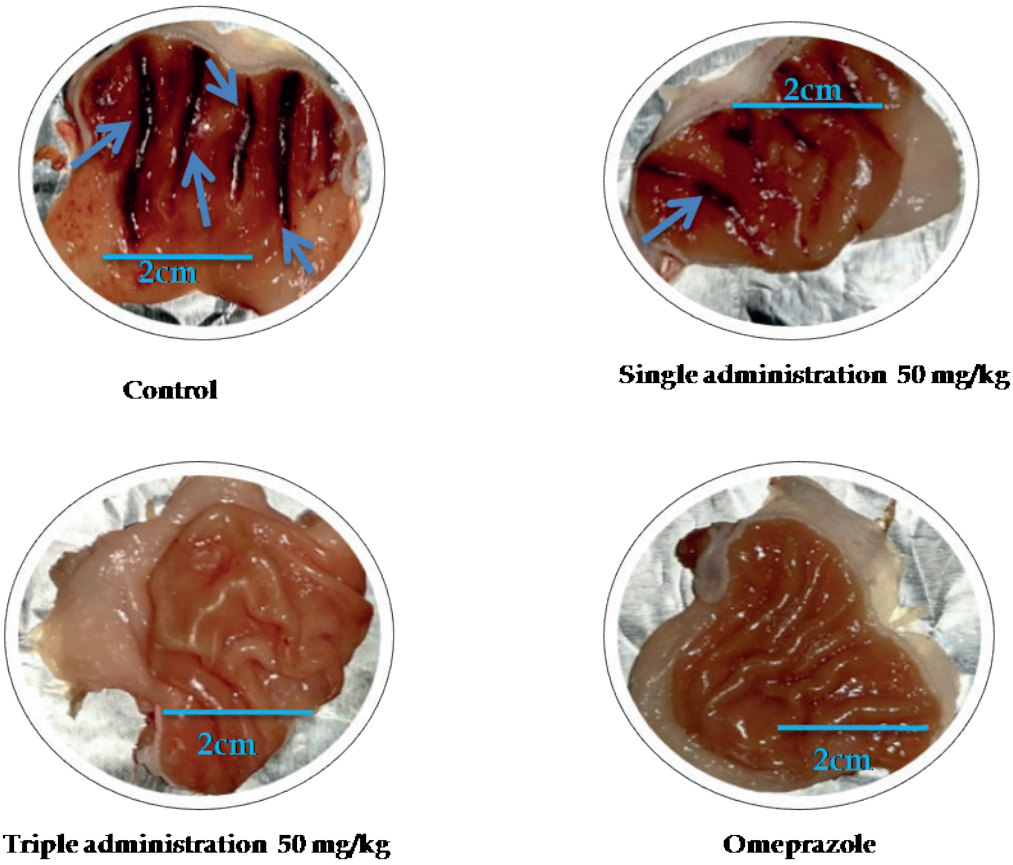


Figure 7. Antiulcer activity of *Pistacia lentiscus* essential oil in HCl/Ethanol induced ulcer gastric.

References:

1. Bouyahya, J. Abrini, A. Et-Touys, Y. Bakri, N. Dakka. Indigenous knowledge of the use of medicinal plants in the North-West of Morocco and their biological activities. European Journal of Integrative Medicine. 2017; **13:** 13-9-25.
2. Maxia, C., Sanna, M. A., Frau, A., Piras, M. S,. Karchuli, V., Kasture. (2011). Anti-inflammatory activity of *Pistacia lentiscus* essential oil: involvement of IL-6 and TNF-α. *Natural product communications*. 2011; **6:** 1934578X1100601033.
3. M. Bozorgi., Memariani, Z., Mobli, M., Salehi. Surmaghi., M. H., Shams-Ardekani., M. R,. R.Rahimi. Five Pistacia *species (P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus)*. A review of their traditional uses, phytochemistry, and pharmacology, The Scientific World Journal. 2013.
4. Giaginis, S., Theocharis. Current evidence on the anticancer potential of Chios mastic gum. *Nutrition and cancer*. 2011; **63:** 1174–1184.
5. S. Catalani., F. Palma,. S. Battistelli., S. Benedetti. Oxidative stress and apoptosis induction in human thyroid carcinoma cells exposed to the essential oil from *Pistacia lentiscus* aerial parts. *PloS one*. 2017; **12:** e0172138.
6. F. Z. Mharti., B. Lyoussi., A. Abdellaoui. Antibacterial activity of the essential oils of *Pistacia lentiscus* used in Moroccan folkloric medicine. *Natural product communications*. 2014; **1934578X1:** 1006-1024.
7. Akmar, S. L. ., Ansari, M. ., Berahim, Z. ., & Shima Shahidan, W. N. . (2022). Phytochemical compound and non-cytotoxicity effect of sting bee and stingless bee honey against normal human gingival cell lines. *Bangladesh Journal of Medical Science*, **21**(1): 158–164. https://doi.org/10.3329/bjms.v21i1.56343
8. Yan, P., Hong, T. K., & Alshagga, M. (). Prevalence of herbal products use and perceptions on drug-herb interactions among university students in Klang Valley Malaysia- a cross sectional study. *Bangladesh Journal of Medical Science*, 2021;**20**(2): 361–367. https://doi.org/10.3329/bjms.v20i2.51549

9. S. Paraschos. Phytochemical and Pharmacological Study of Chios Mastic Gum. National and Kapodistrian university of athens, *Greece*. 2010; https://doi.org/10.12681/eadd/28819
10. M.S. Sharifi. Hazell. Fractionation of mastic gum in relation to antimicrobial activity. *Pharmaceuticals*. 2009; **14:** 22–10. https://doi.org/10.3390/ph2010002.
11. C. Terrafe., R. Aboufatima., M. Badaoui., M. A, Laaradia., H. Kabdy., A. Chait. Neuropharmacological effects of *Pistacia lentiscus* and its possible mechanisms of action, ||| *Bangladesh Journal of Pharmacology*|||. 2022; **17***:* 114-123.
12. A.S.H. Nahida., A.N. Siddiqui. *Pistacia lentiscus:* a review on phytochemistry and pharmacological properties, *Int. J. Pharm. Pharmaceut. Sci*. 2012; **4:** 16–20.
13. C. Terrafe., M. Badaoui., A. L. Mehdi., S. Moubtakir., R. Aboufatima., C. Abderrahman. Toxicity, repellency and chemical composition of essential oils from aerial parts of *Pistacia lentiscus* (L) against *Tribolium casatneum* (Coleoptera: Tenebrionidae). *Journal of Essential Oil & Plant Composition*. 2023; **1:** 176-184.
14. M. Badaoui, S. Moubtakir, C. Terrafe, R. Aboufatima, & A. Chait. Antianxiety and antidepressant effects of aqueous latex extract of Euphorbia resinifera, ||| *Bangladesh Journal of Pharmacology*. 2022; 1732-41.
15. P. U. Ezurike, E. Odunola, T. A, Oke, A. G. Bakre, O. Olumide, Odetoye., & Abiodun, Ganoderma. lucidum ethanol extract promotes weight loss and improves depressive-like behaviors in male and female Swiss mice, *Physiology & Behavior*. 2023; **265:** 114-155.
16. J.Cassani, O.A. Ferreyra-Cruz, A.M. Dorantes-Barron, R.M. Vigueras Villasenor, D. Arrieta- R. BaezEstrada-Reyes. Antidepressant-like and toxicological effects of a standardized aqueous extract of Chrysactinia mexicana A. Gray (Asteraceae) in mice, *J. Ethnopharmacol*. 2015; **171:** 295–306. https://doi.org/10.1016/j.jep.2015.05. 055.
17. K. S. Dimas, P. Pantazis, R. Ramanujam. Chios mastic gum: a plant-produced resin exhibiting numerous diverse pharmaceutical and biomedical properties. in vivo. 2019; **26:** 777-785.
18. H. Y. Matsuda., M.Yoshikawa. Gastroprotection of Escins. IA, IB, IIA, and IIB on ethanol-induced gastric mucosal lesions in rats, *European Journal of Pharmacology*. 1999; **373:** 63–70.
19. M. Mohammadi-Nasab, I. Fatemi, M. Pak-Hashemi, M. Abbasi-Fard, & M. Hassanipour. The Effects of Pistacia Vera Seed Oil on Anxiety and Depressive-Like Behaviors in Rats with Polycystic Ovary Syndrome, *Pistachio and Health Journal*. 2019; **2:** 1-9.
20. S.D.Baldwin, Depression and sexual dysfunction. *Br. Med. Bull*. 2001; **57:**81–99. https://doi.org/10.1093/bmb/57.1.81.
21. Ali-Shtayeh., M.S., Yaniv, Z., Mahajna, J., Bank, W. Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants, *J. Ethnopharmacol*. 2000; **73:** 221–232.
22. E.Hanlidou., R.Karousou., V.Kleftoyanni., S.Kokkini. The herbal market of Thessaloniki (N Greece) and its relation to the ethnobotanical tradition, *J. Ethnopharmacol*. 2004; **91:**281–299. https://doi.org/10.1016/j.jep.2004.01.007.
23. A, Rachida., L. Jawad., A.L.Mehdi., B, Kenza., E. G, Zineb., O. Sara, F. Hind, E. A, Abderrazak, Z. Sokar and C. Abderrahman. Study of protective effects of pistacialentiscus leaves aqueous extract against gastric ulcer and renal stone formation in rats, *european journal of pharmaceutical and medical research.* 2019; **6:** 41-47.
24. P.De Sousa., P. D. A. S.Hocayen., L. N.Andrade., & R. A. Andreatini. Systematic review of the anxiolytic-like effects of essential oils in animal models, *Molecules*. 2015; **20:** 18620-18660.
26. S. Milanos., S. A. Elsharif., D. Janzen., A. Buettner., C. Villmann. Metabolic products of linalool and modulation of GABAA receptors, *Frontiers in chemistry*. 2017; **5:** 46.
27. A.Fernandez-Guasti., L. Martinez-Mota. E.Estrada-Camarena., C.M. Contreras., C.Lopez- Rubalcava. Chronic treatment with desipramine induces an estrous cycle dependent anxiolytic action in the burying behavior but not in the plus maze, Pharmacol. *Biochem. Behav*. 1999; **63:**13–20, https://doi.org/10.1016/S0091- 3057(98)00231-7.
28. MS. Al-Said., AM, Ageel., NS, Parmar., M, Tariq. Evaluation of mastic, a crude-drug obtained from *Pistacia lentiscus* for gastric and duodenal anti-ulcer activity, *J Ethnophar-macol*. 1986; **15:** 271-278.
29. Hussain, M. A., Alam, M. N., Ahmed, N., Said, H., Afrin, S. F., Hassan, Z., Mamun, A. A., & Mahmood, A. K. A. (). Investigation of efficacy of an herbal product (Habb-E-Munish) in the treatment of ED Using IIEF-5 scores and its effect on liver and renal functions. *Bangladesh Journal of Medical Science*, 2019; **19**(1), 176–179. https://doi.org/10.3329/bjms.v19i1.43894.
30. Anwar, R. ., Amna Gulzar, D. e, Sattar, A. A. us, & Ashiq, K. . (). Amelioration of streptozotocin-induced neurotoxicity in rats by a standardized extract of Cydonia oblonga seeds. *Bangladesh Journal of Medical Science*2022; **21**(4), 848–857. https://doi.org/10.3329/bjms.v21i4.60284
31. Dimas, K.S., Pantazis, P., Ramanujam, R. Chios mastic gum: a plant-produced resin exhibiting numerous diverse pharmaceutical and biomedical properties Review, In: *Vivo.* 2012; **26:** pp. 777–785.