Clinical and Experimental Health Sciences

Phytochemical, Pharmacological, and Toxicological Studies on *Peganum harmala* L.: An Overview of the Last Decade

İçim Gökkaya, Gülin Renda, Tuğba Subaş, Ufuk Özgen

Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, Türkiye.

Correspondence Author: İçim Gökkaya
E-mail: icim1995@hotmail.com
Received: 02.06.2022 **Accepted:** 04.04.2023

ABSTRACT

Objective: Plants have been used to treat ailments since the dawn of humanity. The use of medicinal plants for various purposes such as preventing diseases, treating diseases and supporting medical treatment is increasing day by day. On the other hand, medicinal plants are important sources of raw materials for the pharmaceutical industry. It has been demonstrated that *Peganum harmala* L. and the phytochemicals it contains have a wide variety of pharmacological activities. *P. harmala* and its active ingredients can be an important resource for the pharmaceutical industry. In this review, the phytochemistry, pharmacological effects, clinical studies, and toxicity of *P. harmala* are discussed under the current information.

Methods: Studies on *P. harmala* were searched using Pubmed, Scopus, Science Direct databases, and Google Scholar search engine. As a result of the searches, 96 articles were included in the study.

Results: The main group of secondary metabolites responsible for the biological activities of *P. harmala* is alkaloids. The plant and its isolated secondary plant compounds have been shown to have many pharmacological actions, counting antiamnestic, anticancer, antidepressant, antiinflammatory, cardiovascular, gastroprotective, hepatoprotective, nephroprotective, and vasodilator activities. Studies evaluating the plant's clinical effects have been carried out in recent years. However, it has been recorded in the literature that the use of *P. harmala* causes poisoning with symptoms such as neurosensory symptoms, visual hallucination, bradycardia, hypotension, agitation, tremor, ataxia, and vomiting.

Conclusion: Considering the pharmacological effects, the number of studies on the efficacy and safety of *P. harmala* and its secondary metabolites should be increased.

Keywords: *Peganum harmala*, alkaloid, β-carboline, antidepressant, anticancer, toxicity

1. INTRODUCTION

Peganum harmala L. (family Nitriaceae) is known in Iran as "Espand," in North Africa as "Harmel," in the United States as "African rue," "Mexican rue," or "Turkish rue," and in Türkiye as "üzerlik," "lezik," "ülerzik," and "uzarih". It is a herbaceous suffrutescent perennial that is common around the world, particularly in North Africa, the Middle East, Türkiye, Pakistan, India, Iran, Kazakhstan, Mexico, and South America (1). The stem is erect, glabrous, and can grow up to 70 cm. Leaves are 3-5 cm long, alternate, with small and lower stipules, that are multiply split into linear, lanceolate, or narrowly elliptic segments usually with three parts at the base. The flowers are sessile and often located opposite the leaves. Sepals are linear, green, similar to leaf segments, usually irregularly segmented, and have a very small epicalyx. Petals are white, elliptical, 10-13(-19) mm long, and usually slightly exceeding the sepals. The flowering period in Türkiye is May-July. The fruit is a short-stalked, broadly obovoid or spherical, 8x8 mm, locicid capsule (2).

For generations, the plant has been utilized in folk medicine for therapeutic purposes. The seeds are used in Pakistan in powder form and internally to treat asthma, colic, and jaundice, to reduce malaria fever, and as an antihelminthic. The decoction prepared from the seeds is used to increase breast milk, for abortion, as a stimulant, and to treat laryngitis (3). In India, seeds cooked in sesame oil are dripped into the ear and used to relieve earache (4). In Türkiye, the seeds are used as an anthelmintic, menstrual remedy, to treat hemorrhoids, stimulate the nervous system, and relieve abdominal pain. The powdered seeds are used externally to treat eczema and hemorrhoids (5,6,7). It is known that the aerial parts and the seeds of the plant are used as an infusion, decoction, and powder for the treatment of diabetes and hypertension in northern Algeria (8). In Kyrgyzstan, the decoction prepared from the roots is used in washing and

DOI: 10.33808/clinexphealthsci.1125345

bathing to cleanse infected skin areas (9). In Iran, fruits in the form of powder and decoction are used to treat toothache, gynecological infections, and menstrual cramps (10). In Pakistan, incense made by mixing and burning the leaves of *Skimmea laureola* is used to drive away evil and against the evil eye (11). The seeds of *P. harmala* are used instead of *Banisteriopsis caapi* in the preparation of Ayahuasca, which is consumed as a hallucinogenic beverage by tribes in the Amazon. For this reason, the plant is known as an analogue of Ayahuasca Tea (12).

P. harmala contains mainly alkaloids, fatty acids, triterpenoids, anthraquinones, flavonoids, phenolic acids, and essential oils. Studies have shown that ethanolic, methanolic, and aqueous extracts prepared from seeds, and the alkaloid fraction obtained from these extract have a wide range of pharmacological effects, including antiinflammatory, antimicrobial, antidepressant, antitumoral, analgesic, antihypertensive, hypoglycemic, gastroprotective, and neuroprotective effects (1,13,14).

Göbel isolated harmaline, a *β*-carboline alkaloid, from the seeds and roots of *P. harmala* in 1841. The evaluation of the efficacy of harmaline and other *β*-carboline alkaloids and their synthetic derivatives in chronic diseases such as cancer, Alzheimer's disease, Parkinson's disease, diabetes, and hypertension has been the subject of many studies (13). Studies on the isolation of alkaloids from *P. harmala*, elucidation of the structures of isolated compounds, and determination of their pharmacological activities have continued since 1841 until today. In recent years, the number of clinical studies investigating the efficacy of *P. harmala* in humans has increased. However, case reports have indicated that toxic effects occur when the plant is used concomitantly with conventional drugs or taken in high doses.

In the literature, there are comprehensive review studies on the phytochemistry, biological effects, and toxicity of the genus *Peganum*. These studies did not focus directly on the *P. harmala* species, but also covered other *Peganum* species (1,13). When the previous studies on *P. harmala* were examined, it was seen that the number of comprehensive studies was limited and current data as well as the results of clinical investigations were lacking. In this review, the phytochemistry, pharmacological effects, clinical studies, interactions with herbal products/conventional drugs, and toxicity of *P. harmala* are discussed in accordance with current information. Pubmed, Scopus, Science Direct, and Google Scholar databases were used to search for studies concerning *P. harmala*. The following keyword combinations were used for the search: "phytochemistry" or "chemical compound" or "pharmacological effects" or "biological effects" or "clinical trials" or "drug interaction" or "interaction with herbal products" or "toxicity" or "case report" and "*Peganum harmala*". The current data between the years 2010-2021 are discussed in the review. Since the number of available studies evaluating the toxic effects of the plant is limited, the literature was searched without specifying the time interval only for this part of the manuscript. The references listed in the chosen publications were searched for additional reports which were not included in the databases. For studies with similar results, the most recent was examined. A total of 96 accessible articles whose language was English were included in the study.

2. PHYTOCHEMISTRY

The most important group of secondary metabolites responsible for the biological effects of *P. harmala* is the alkaloids. In the studies, the seeds of the plant were found to have high alkaloid content (2-7.7%). Various alkaloid groups with *β*-carboline, quinazoline, and indole structures were identified in the seeds. In addition to alkaloids, *P. harmala* also contains fatty acids, triterpenoids, anthraquinones, flavonoids, phenolic acids, and other phytochemicals (13). The secondary metabolite groups and chemical compounds contained in the plant, the plant parts from which these compounds are extracted, and the solvent/solvent systems used in the extraction are listed in Table 1.

Different parts of *P. harmala* were found to contain essential oil. The chemical composition of the essential oils may vary due to differences in the geographical region, harvesting time, stage, drying method, and essential oil extraction procedures. The results of the studies in which the essential oil composition of *P. harmala* was investigated are shown in Table 2.

3. PHARMACOLOGICAL EFFECTS

3.1. Antiamnesic Activity and Effect on Alzheimer's Disease

Liu *et al*. investigated the *in vivo* acetylcholinesterase (AChE) inhibitory activity of ethanol extract (EXT_E), alkaloid fraction (ALK) and flavonoid fraction (FLA) prepared from the aerial parts of *P. harmala*. Mice were treated with the EXT_E at doses of 183, 550, and 1650 mg/kg with the ALK and FLA at doses of 10, 30, and 90 mg/kg. All treatments were administered orally in a single dose. As a result, AChE activity was significantly decreased and acetylcholine content was significantly increased in the cortex and hippocampus of mice in the EXT_E and ALK groups (at all doses, $p<0.05$). There was no significant change in the FLA group. In the continuation of the study, the effect of EXT_E and ALK on scopolamine-induced memory deficits was investigated using the Morris water maze (MWM) tasks. In this phase of the study, EXT_E (at doses of 183, 550, and 1650 mg/kg) and ALK (at doses of 10, 30, and 90 mg/kg) were administered by oral gavage for 1 week. The scopolamine-induced reduction in swimming time within the target zone and reduction in the number of crossings in the platform were significantly reversed by EXT_E and ALK ($p<0.05$ for EXT_E at 550, 1650 mg/kg and ALK at 30, 90 mg/kg). AChE activity and protein expression were significantly reduced and acetylcholine content was significantly increased (at all doses, $p<0.05$). There was no significant change in choline acetyltransferase (ChAT) activity, ChAT protein expression, and choline content (41). The results of another *in vivo* study showed that administration of deoxyvasicine (DVAS; at doses of 5, 15, and 45 mg/kg, 7 days, orally) improved learning and memory deficits in the MWM test in male C57BL/6J mice. DVAS

reduced AChE levels (at all doses, $p<0.05$) in the hippocampus and cortex. Treatment with DVAS significantly increased ChAT levels in the hippocampus at three doses administered (at all doses, $p<0.01$). However, only the 45 mg/kg dose of DVAS was significantly effective in increasing ChAT levels in the cortex ($p<0.05$). It ameliorated scopolamine-induced neuronal damage by increasing hippocampal brain-derived neurotrophic factor levels (at all doses, $p<0.05$). It decreased neuroinflammation by suppressing tumor necrosis factor-α (TNF-α) levels (at all doses, $p<0.01$) and oxidative stress by increasing glutathione peroxidase (GSH-px) levels and activity (at all doses $p<0.01$). DVAS also affected neurotransmitter levels by increasing levels of acetylcholine, 5-hydroxytryptamine, and γ-aminobutyric acid, and decreasing levels of 5-hydroxy indole-3-acetic acid and glutamic acid (42).

The effect of a methanol extract prepared from the seeds of *P. harmala* (approximately 14% and 21% (w/w) harmine and harmaline content, respectively) on learning and memory problems in a rat model of Alzheimer's-like pathology induced by aluminum chloride ($AlCl_3$) was evaluated. The extract was administered orally at a dose of 187.5 mg/kg for 4 weeks, beginning 2 weeks after exposure to $AlCl_3$. It increased hippocampal levels of insulin, glucagon-like peptide-1 (GLP-1), phosphorylated Akt at serine 473 (pS473-Akt), and glucose transporter type 4 (GLUT 4). It reduced the level of insulin receptor substrate-1 phosphorylation at serine 307 (pS307-IRS-1), beta-amyloid(Aβ)42, augmented pS9-glycogen synthase kinase-3β (pS9-GSK-3β) and phosphorylated tau (*p*-tau) in the hippocampus. The increased nuclear factor erythroid 2-related factor 2 (Nrf 2) and improved hippocampal oxidative stress markers. As a result, it was found that the extract attenuated $AlCl_3$-induced cognitive impairment by affecting the indicated biochemical parameters and signaling pathways (43).

3.2. Antibacterial Activity

The antibacterial effect of the harmala alkaloid-rich fraction contained in chitosan-coated poly(lactic-co-glycolic acid) nanoparticles (H/CS/PLGA NP) on *Staphylococcus aureus* and *Escherichia coli* was studied by the broth macro dilution method. H/CS/PLGA NP showed high antibacterial activity against *S. aureus* and *E. coli* with minimum inhibitory concentration (MIC) values of 0.125 and 0.06 mg/mL, respectively (MIC value of 0.5 mg/mL for the harmala alkaloid-rich fraction only; 0.18 mg/mL MIC value for the chitosan/PLGA-coated blank) (44).

3.3. Antidepressant Activity

Herraiz *et al*. investigated the effects of acidic methanolic extracts from the leaves, stems, seeds, and roots of *P. harmala* on monoamine oxidase-A (MAO-A). Seed and root extracts strongly inhibited MAO-A with IC_{50} values of 27.6±1.3 and 159.3±17.5 µg/L, respectively. Stem and leaf extracts inhibited MAO-A by 50% and 40%, respectively, at a concentration of 2.5 mg/mL. Although the stem and leaf extracts are considered MAO-A inhibitors, their effect was shown to be lower than that of the seed and root extracts. As a result of the quantitative studies, it was found that MAO-A inhibition of the seed extract was dependent on harmaline and harmine content, while the root extract was dependent only on harmine. Stem and leaf extracts showed lower inhibitory activity at lower harmine content. In the continuation of the study, kinetic studies were performed with the seed extract. The seed extract competitively and reversibly inhibited MAO-A. It showed no significant inhibitory effect on MAO-B with an IC_{50} value of 416 µg/L. As a result, the extract inhibited MAO-A more selectively than MAO-B (12). In another study, peganin, deoxypeganin, and peganin glucoside isolated from seeds were found to weakly and insignificantly inhibit the isoenzyme MAO-A, but not the isoenzyme MAO-B. As a result of the study, quinazoline alkaloids were not accepted as MAO inhibitors. MAO-A inhibition was associated only with the content of β-carboline alkaloids (23).

Sassoui *et al*. reported that an ethanol extract of *P. harmala* seeds (at doses of 100 and 300 mg/kg, single dose, orally) significantly reduced immobility time in the forced swimming test in Wistar rats. In addition, the extract decreased the level of serum adrenocorticotropic hormone and the defecation rate. According to these results, the extract was found to have an antidepressant effect, and the highest effect was observed in rats treated with a dose of 300 mg/kg (45).

3.4. Antiinflammatory Activity

Bensalem *et al*. tested the inhibitory effect of ethanol extracts from different parts of *P. harmala* and the β-carboline alkaloids on the enzyme myeloperoxidase (MPO), which plays a role in inflammation, using the taurine-chloramine assay. The extract prepared from the seed and aerial parts strongly inhibited MPO activity at a concentration of 20 µg/mL, by 97±5% and 43±4%, respectively. The root extract showed a low inhibitory effect of 15±6%. Harmine, harmaline, and harmane showed significant inhibition of MPO with IC_{50} values of 0.26, 0.08, and 0.72 µM, respectively. Harmaline was found to have the highest inhibitory activity and this effect was higher than that of 5-fluorotryptamine (IC_{50}:200 nM). In molecular docking studies, the alkaloids were found to have a high affinity for the active site of MPO. The ΔG values for harmine, harmaline, and harmane were - 4.4, - 6.1, and - 3.1 kcal/mol, respectively (46).

Methanol extract of *P. harmala* whole plant (at a dose of 200 mg/kg, *i.p*.) inhibited carrageenan-induced paw edema in Wistar albino mice by 75.14% ($p<0.001$) in 3 hours (100 mg/kg body weight indomethacin, 86.1%) (47). In another study, it was found that the cream formulation prepared with 20% *P. harmala* seed oil significantly inhibited the inflammation induced by carrageenan by 60.4% (1% diclofenac: 45.65%) (30). The effect of a methanol extract from the leaves of *P. harmala* was studied in the polyarthritic rat model induced by Complete Freund's Adjuvant (CFA). The extract was administered orally to the rats at doses of 200, 400, and

600 mg/kg/day for 21 days. CFA-induced paw edema was suppressed by 19.3±1.27% by the extract at a dose of 600 mg/kg (for 200, 400 mg/kg extracts, 5 mg/kg diclofenac sodium 14.56%±1.99%, 16.82%±3.21%, 16.80±2.73%, respectively). Treatment with the extract decreased the levels of C-reactive protein (CRP), rheumatoid factor, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). The extract ameliorated oxidative damage in polyarthritic rats by increasing the levels of superoxide dismutase (SOD), reducing glutathione (GSH), and catalase (CAT). It suppressed the levels of malondialdehyde (MDA), prostaglandin-E_2 (PGE_2), and TNF-α. In addition, the study evaluated the effect of the extract on hematological parameters. While the extract increased the number of red blood cells (RBCs), it decreased the number of white blood cells (WBCs) and platelets. It had no effect on hemoglobin levels. All effects were dose-dependent, with the highest effect observed in rats given 600 mg/kg of the extract (48). Wang *et al*. investigated the osteoclastogenic effects of harmine in the titanium-induced periprosthetic osteolysis model. Male C57BL/J6 mice were administered low (5 mg/kg/day) or high (10 mg/kg/day) doses of harmin emulsion intragastrically for 2 weeks. Both doses significantly increased bone mineral density (BMD) and bone volume/tissue volume (BV/TV), while reducing porosity area and the number of pores. It decreased interleukin (IL)-1β, TNF-α, and IL-6 levels at both doses. It suppressed inflammation by shifting macrophage polarization from M1 (pro-inflammatory phenotype ($(F4/80^+/iNOS^+)$) to M2 (anti-inflammatory phenotype ($(F4/80^+/Arg\text{-}1^+)$). Harmine was shown to reduce inflammation in periprosthetic osteolysis by suppressing c-Jun N-terminal kinase activation (49).

3.5. Antitussive, Expectorant, and Bronchodilator Activities

Liu *et al*. investigated the antitussive effects of methanol extracts (EXT_M), ALK, and FLA prepared from the aerial parts of *P. harmala* in ammonia liquor-, capsaicin-, and citric acid-induced cough models in mice and guinea pigs. Mice were treated with EXT_M at doses of 183, 550, and 1650 mg/kg and with ALK and FLA at doses of 10, 30, and 90 mg/kg (single oral administration). In all three models, EXT_M and ALK suppressed cough frequency and prolonged cough delay. FLA had no effect on cough frequency and latent period. The results showed that high-dose EXTM (1650 mg/kg) and ALK (90 mg/kg) had as good a pharmacological effect as codeine phosphate (30 mg/kg). To determine the mucolytic effect of EXT_M, ALK, and FLA, the phenol red secretion assay was performed in mice. EXT_M increased phenol red secretion by 0.64-, 1.08-, and 1.29-fold (p<0.05) at doses of 183, 550, and 1650 mg/kg, respectively. ALK increased phenol red secretion by 0.63-, 0.96-, and 1.06-fold (p<0.05) at doses of 10, 30, and 90 mg/kg. FLA had no effect on the amount of phenol red secretion. High-dose EXT_M and ALK were shown to have a higher expectorant effect than the standard drug ammonium chloride (1500 mg/kg dose). The bronchodilator effect was evaluated by acetylcholine chloride and histamine-induced bronchoconstriction test. EXT_M showed a bronchodilator effect by prolonging the pre-convulsive time by 67.34%, 101.96%, and 138.00% at doses of 183, 550, and 1650 mg/kg, respectively. ALK prolonged the pre-convulsive time by 55.47%, 97.74%, and 126.77% at doses of 10, 30, and 90 mg/kg, respectively. For FLA administered at doses of 10, 30, and 90 mg/kg, the values were 84.69%, 95.94%, and 154.52%, respectively. The standard drug aminophylline at a dose of 50 mg/kg prolonged the pre-convulsive time by 162.28% (50).

3.6. Antiviral Activity

Benzekri *et al*. tested the anti-HSV2 activity of sixteen extracts of seeds, stems, leaves, and flowers of *P. harmala* using hexane, dichloromethane, ethyl acetate, and methanol in the plaque reduction assay. As a result, the methanolic extract of seeds was the only extract that showed anti-HSV2 activity (selectivity index (SI): 13.19, IC_{50}: 161 µg/mL). The extract showed antiviral activity by exhibiting virucidal effects on both virus entry and release of the newly formed virions. Continuing the study, the active component of the extract was isolated and defined as harmine. It showed a synergistic effect with a combination index of 0.5 when co-administered with aciclovir (51). In another study, the antiviral activity of ethanolic extract and total alkaloid fraction from *P. harmala* seeds against influenza A/Puerto Rico/8/34 (H1N1; PR8) virus was investigated using Madin Darby canine kidney epithelial cell line. The total alkaloid fraction (IC_{50}: 5.8 µg/mL, SI: 23.1) had higher antiviral activity than the crude extract (IC_{50}: 9.87 µg/mL, SI: 12.45). The extract inhibited viral RNA replication as well as polymerase activity. However, the extract did not cause hemagglutination inhibition and did not exhibit virucidal activity (52).

3.7. Cardiovascular Activity

Keihanian *et al*. studied the effect of an ethanol extract from the seeds of *P. harmala* in the treatment of chronic heart failure in an isoproterenol-induced rat model. The extract was administered intraperitoneally to Wistar rats at a dose of 100 mg/kg/day for 30 days. The extract improved ejection fraction by 26.08% (p<0.01). Left ventricular end-diastolic diameter was significantly decreased in the *P. harmala* treated group. The extract decreased the levels of N-terminal proatrial natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (hs-CRP), creatine kinase myocardial band (CK-MB), and angiotensin-converting enzyme (ACE) in serum (53). Huang *et al*. studied the therapeutic effect of harmine on cardiac hypertrophy in a spontaneous hypertension rat model. Wistar-Kyoto rats were treated with 0.05% harmine (at a dosage of approximately 50 mg/kg/day) added to the diet for 12 weeks. Harmine was shown to ameliorate cardiac hypertrophy caused by pressure overload. On the other hand, harmine had no effect on blood pressure. Harmine significantly increased left ventricular systolic inner diameter (LVID) and significantly decreased interventricular septal thickness in diastole (IVSd), left ventricular posterior wall end-diastolic thickness (LVPWd), left ventricular mass index (LVMI), ejection fraction (EF), and fractional shortening (FS). Harmine upregulated the fetal genes β-myosin heavy chain

(*β*-MHC), B-type natriuretic peptide (BNP), NT-proBNP, and atrial natriuretic factor (ANF). Harmine decreased the expression of structure - and function-related proteins such as NFAT-activated mediators, myocyte-specific enhancer factor 2C (Mef2C), and sarcoplasmic/reticular Ca^{2+}-ATPase 2A (SERCA2A). In the continuation of the study, the mechanisms underlying the attenuating effect of harmine on cardiac hypertrophy were investigated. Harmine decreased the mRNA levels of inflammatory mediators such as IL-1*β*, IL-6, IL-10, and TNF-*α*. It suppressed the mRNA levels of macrophage maker (Emr1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in cardiac tissue. It decreased the mRNA levels of chemokines (CCL2, CXCL1) and chemokine receptor-2, a chemoattractant for inflammatory cells. In conclusion, harmine was found to inhibit the activation of the nuclear factor kappa B (NF-κB) signaling pathway and suppress the expression of proinflammatory cytokines in the heart (54).

3.8. Effect on Cancer

The cytotoxic, antiproliferative, and antiangiogenic effects of the hydroalcoholic extract prepared from *P. harmala* seed on human umbilical vein endothelial cells (HUVEC) at different concentrations were studied *in vitro*. The cytotoxic concentration of the extract was determined to be 150 µg/mL. The extract strongly inhibited the proliferation of HUVEC (IC_{50}: approximately 85 µg/mL) such a dose-dependent manner. It showed a partial antiangiogenic effect at concentrations of 40 and 80 µg/mL. At higher concentrations (100 and 120 µg/mL), it inhibited the production of endothelial tubular formations and suppressed endothelial cell branching. In addition, the extract reduced vascular endothelial growth factor secretion in HUVEC (55).

The anticancer activities of many compounds, mainly alkaloids, isolated from *P. harmala* are being studied and attempts are being made to elucidate their mechanisms of action. The effect of harmine 24, 36, and 48 h incubation at concentrations of 2, 4, 8, 16, 32, and 64 *µ*g/mL on the TPC-1 line was investigated by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay. The IC_{50} values of harmine against TPC-1 cells at 24, 36, and 48 hours were 16.57 ± 1.4, 9.48 ± 1.1, and 5.51 ± 0.7 µg/mL, respectively. Harmine suppressed TPC-1 cell proliferation significantly in a concentration- and time-dependent manner. Harmine treatment resulted in a dose-dependent increase in Bax expression and a decrease in Bcl-2 expression. Consequently, it was found to exhibit apoptotic effects by significantly reducing the Bcl-2/Bax ratio. It also induced caspase-3 activity, which plays a role in apoptosis. In addition, it suppressed TPC-1 cell invasion and migration in a dose-dependent manner (56). Hamsa *et al*. studied the effect of harmine on metastatic lung tumors using three different methods, including concurrent, prophylactic, and after-tumor development administrations. Harmine inhibited tumor nodules at rates of 83.6%, 68.4%, and 51.6% in the concurrent, prophylactic, and post-tumor administration models, respectively. The survival rate of metastatic rats in these three models improved significantly after harmine administration by 172.54%, 147.18%, and 120.76%, respectively. Harmine administration decreased the concentration of biochemical parameters in the lungs, such as collagen, hydroxyproline, hexosamine, and uronic acid, which were elevated in metastatic tumors. It suppressed the levels of proinflammatory cytokines such as IL-1*β*, IL-6, TNF-*α*, and granulocyte-macrophage colony-stimulating factor (GM-CSF). It downregulated the expression of several pro-metastatic genes such as extracellular signal[en] regulated kinase (ERK)-1, ERK-2, VEGF, metalloproteinase (MMP)-2, and MMP-9 while upregulating the expression of anti-metastatic genes such as nm23, tissue inhibitor of metalloproteinase (TIMP)-1, and TIMP-2 (57). Peganumine A was reported to be moderately cytotoxic in MCF-7 (IC_{50}: 38.5 µM), PC-3 (IC_{50}: 40.2 µM), and HepG2 (IC_{50}: 55.4 µM) cell lines. This compound showed selective effects in HL-60 cells with an IC_{50} value of 5.58 µM. In another study, pegaharmaline A (IC_{50}: 9.4 µM) and pegaharmaline B (IC_{50}: 13.6 µM) were found to have significant cytotoxic effects in HL-60 cells. In HL-60 cells, peganumaline B (IC_{50}: 21.54 µM), peganumaline F (IC_{50}: 24.55 µM), and pegaharmine E (IC_{50}: 25.07 µM) showed moderate cytotoxic effects (17,18,28). Wang *et al*. found that harmalacidine had potent cytotoxic activity on U937 with an IC_{50} value of 3.1±0.2 µmol/L. Harmalacidine was found to act on mitochondrial and protein tyrosine kinase signaling pathways (PTKs-Ras/Raf/ERK) (27).

3.9. Effect on Parkinson's Disease

Yalçın *et al*. reported that *P. harmala* seed methanol extract exhibited an inhibitory effect (IC_{50}: 1.09±0.33 µg/mL) on catechol-*O*-methyltransferase (COMT). Analysis of the extract revealed that the main alkaloid contained therein is harmaline. Later in the investigation, the alkaloid responsible for the inhibitory effect was determined using harmaline and harmalol standards. Harmaline (IC_{50}: 0.98±0.12 µg/mL) strongly inhibited COMT. This effect was higher than that of harmalol (IC_{50}: 3.59±0.37 µg/mL) (58).

The effect of *P. harmala* seed aqueous extract (10 mg/kg, *i.p*.) on 6-hydroxydopamine-induced Parkinson's disease symptoms and markers of oxidative stress was studied in Wistar rats. The extract improved muscle stiffness in Murprogo's test and apomorphine-induced one-direction rotation behavior in the rotation test. Markers of oxidative stress, such as lipid peroxidation and protein oxidation, were found to be significantly lower in the brains of rats treated with the extract. In addition, the extract inhibited ACE activity in the brain. The histological study, it prevented the degeneration of dopaminergic neurons. These results show that the extract can effectively reduce the symptoms of Parkinson's disease and the markers of oxidative stress (59).

Table 1. *Chemical compounds contained in P. harmala*

	Compound/Compounds	Plant Parts	Extract Type	References
	Alkaloids			
β-carboline alkaloids	Harmaline	S	$HClO_4$:MeOH (1:1)	(12)
		AP	MeOH:H_20 (7:3)	(15)
	Harmine	S, R, St, L	$HClO_4$:MeOH (1:1)	(12)
		S	85% EtOH	(16)
		AP	MeOH:H_20 (7:3)	(15)
	Harmalol	S, R	$HClO_4$:MeOH (1:1)	(12)
		S	85% EtOH	(16)
	Harmol	S	85% EtOH	(16)
		R, St	$HClO_4$:MeOH (1:1)	(12)
	Tetrahydroharmine	S	$HClO_4$:MeOH (1:1); 85% EtOH	(12, 16)
	Harmane, 3-hydroxylated harmine, 1-hydroxy-7-methoxy-*β*-carboline, acetylnorharmine, harmic acid methy ester, harmaline, harmalanine, harmine *N*-oxide, 2-aldehyde-tetrahydroharmine	S	85% EtOH	(16)
	Pegaharmalines A and B	S	-	(17)
	Peganumine A	S	95% EtOH	(18)
	Peganumines B, C, F, G, H	S	85% EtOH	(19)
	Pegaharmines A–D	S	95% EtOH	(20)
	Pegaharmines F–K	S	95% EtOH and 75% EtOH	(21)
	Pegaharines A–G	S	95% EtOH	(22)
Quinazoline alkaloids	Peganine (vasicine)	S	$HClO_4$:MeOH (1:1); 85% EtOH; 95% EtOH; 70% aqueous MeOH	(16,23-25)
		Fr, Fl, St R	$HClO_4$:MeOH (1:1)	(23)
		AP	MeOH:H_20 (7:3)	(15)
	Deoxypeganine (deoxyvasicine)	S	$HClO_4$:MeOH (1:1); 85% EtOH	(16, 23)
		Fr, Fl, St	$HClO_4$:MeOH (1:1)	(23)
	Peganine *β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (peganine glucoside)	S	$HClO_4$:MeOH (1:1)	(23)
	Vasicinone	S	85% EtOH; 95% EtOH; 70% aqueous MeOH	(16, 24, 25)
	Deoxyvasicinone	S	85% EtOH; 95% EtOH; 70% aqueous MeOH	(16, 24, 25)
	(*S*) vasicinone-1-*O*-*β*-D-glucopyranoside	S	70% aqueous MeOH; 95% EtOH	(24,25)
	(*R*) vasicinone-1-*O*-*β*-D-glucopyranoside	S	70% aqueous MeOH	(25)
	(*S*) and (*R*)-vasicinone *β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, quinanolin-4(3H)-one, methyl 3-(4-oxoquinazolin-3(4H)-yl) propanoate, vasicinolone, 1-(2-aminobenzyl)-3-hydroxypyrrolidine-2-one, (*S*) – 1-(2-aminobenzyl)-3-hydroxypyrrolidin-2-one *β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside, (*R*)-1-(2-aminobenzyl)-3-hydroxypyrrolidin-2-one *β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside	S	95% EtOH	(24)
	2-carboxyl-3,4-dihydroquinazoline	S	85% EtOH	(16)
	Pegamine	S	85% EtOH; 95% EtOH	(16,24)
	Peganumines D and E	S	85% EtOH	(19)
β-Carboline-quinazoline dimers	Pegaharmols A and B	R	95% EtOH	(26)
Indole alkaloids	2-(indol-3-yl)ethyl-*β*-D-glucopyranoside, 2-(indol-3-yl)ethyl-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, (*S*)-3-hydroxy-3-(*N*-acetyl-2-aminoethyl)-6-methoxyindol-2-one	S	70% aqueous MeOH	(27)
	6-methoxyindoline	S	85% EtOH	(16)
	(±)-peganines A–B; (±)-peganumalines A-E, peganumaline F	S	95% EtOH and 75% EtOH	(21,28)

Thiazole derivatives alkaloids	Peganumals A–B	S	95% EtOH and 75% EtOH	(21)
Fatty acids				
Saturated fatty acids	Tetradecanoic, pentadecanoic, tridecanoic, hexadecanoic, heptadecanoic and octadecanoic acids	WP	*n*-hexane	(29)
	Palmitic, stearic, arachidic, behenic and tetracosanoic (lignoceric) acids	S(oil)	*n*-hexane	(30,31)
Saturated fatty acid derivatives	12-methyl tetradecanoic, 5,9,13-trimethyl tetradecanoic, 2-methyl octadecanoic acids	WP	*n*-hexane	(29)
Unsaturated fatty acids	(*E*)-9-dodecenoic, (*Z*)-9-hexadecenoic, (*Z,Z*)-9,12-octadecadienoic and (*Z,Z,Z*)-9,12,15-octadecatrienoic acids	WP	*n*-hexane	(29)
	Linoleic, oleic, palmitoleic, linolenic, gadoleic, erucic, and vaccenic acids	S (oil)	*n*-hexane	(30, 31)
Triterpenoids				
Lupane type triterpenoids	3α-acetoxy-14α-hydroxy-lup-20(29)-en-11-oxo-28-oic acid methyl ester, 3*β*-acetoxy-27-(4-hydroxy-3-methoxy-*E*-cinnamoyloxy) lup-en-28-oic acid methyl ester and 3*β*-acetoxy-27-hydroxy-lup-20(29) – en-28-oic acid	S	70% EtOH	(32)
Oleanane type triterpenoids	3α-acetoxy-27-hydroxy-olean-12-en-11-oxo-28-oic acid methyl ester, 3α-hydroxy-olean-27-(4-hydroxy-3-methoxy-*E*-cinnamoyloxy)-12-en-28-oic acid methyl ester, 3α-acetoxy-27-hydroxyolean-12-en-28-oic acid	S	70% EtOH	(32)
Pentacyclic triterpenoids	3α-acetoxy oleanolic acid, urs-12-ene-28-carboxy-3α-tetradecanoate, methyl-lup-20 (29)-en-3-on-28-oate, betulonic acid, lup-20 (29)-en-3-on-28-oic acid, 3-oxo-27-hydroxylup-20 (29) – en-28-acid methyl ester, 3-oxo-27-hydroxylup-20(29)-en-28-oic acid, 3α-acetoxy-27-hydroxylup-20 (29)-en-28-oic acid methyl ester, 3*β*-acetoxy-27-hydroxylup-20(29)-en-28-oic acid methyl ester, 3α,27-dihydroxylup-20(29)-en-28-oic acid methyl ester	S	70% EtOH	(32)
Anthraquinone				
Anthraquinones	Peganone 1 (3,6-dihydroxy-8-methoxy-2-methyl anthraquinone), Peganone 2 (8-hydroxy-7-methoxy-2-methyl anthraquinone)	S	EtOH	(13)
Flavonoids				
Flavone glycosides	Peganetin, deacetylpeganetin	AP	MeOH:$H_2$0 (7:3)	(15)
	Acacetin-7-*O*-rhamnoside	AP	MeOH	(13)
	Acacetin-7-*O*-[6''-*O*-glucosyl-2''-*O*-(3'''-acetylramnosyl) glycoside	AP	MeOH	(13)
	Acacetin-7-*O*-(2''''-*O*-rhamnosyl-2''-*O* glucosylglucoside)	AP	MeOH	(13)
	Glycoflavone 2''-*O*-rhamnosyl-2''-*O*-glucosylcytoside	AP	MeOH	(13)
	Cynaroside (Luteolin 7-glucoside)	L	MeOH	(33)
Flavonol glycosides	Rutin	S	MeOH	(34)
Flavanols	Catechin	S	MeOH	(34)
Flavanones	Hesperetin	S	MeOH	(34)
Phenolic acids				
Hydroxycinnamic acid derivative phenolic acids	*p*-coumaric acid, chlorogenic acid	S	MeOH	(34)
	Hydrocaffeic, caffeic, rosmarinic acids	L	MeOH	(33)
Hydroxybenzoic acid derivative phenolic acids	Protocatechuic acid	L	MeOH	(33)

S: Seed, AP: Aerial part, R: Root, St: Stem, L: Leaf, Fr: Fruit, Fl: Flower, WP: Whole plant, HClO4: perchloric acid, EtOH: ethanol, MeOH: metanol

Table 2. *Composition of essential oil samples obtained from P. harmala*

Location	Part of the plant used	Essential oil extraction method	Major Compounds	References
Egypt	Aerial part	Hydrodistillation	1-hexyl-2-nitrocyclohexane (9.07%), *Z*-2-octadecen-1-ol (8.13%), 3,5,24-trimethyltetracontane (7.84%)	(35)
Pakistan	Aerial part	-	Camphor (28,244%), capillin (13,176%)	(36)
China	Aerial part	Hydrodistillation	Limonene (14.5%), thymol (%11.5)	(37)
Persia	Fruit	Hydrodistillation	α-pinene (72.6%), trans-verbenol (3.9%), sabinene (2.6%)	(38)
Algeria	Seed	Hydrodistillation	Eugenol (17.5%), thymol (7%), α-isomethyl-(*E*)-ionol (7%), dihydro carveol acetate (6.2%)	(39)
Egypt	Seed	Hydrodistillation	Eugenol (17.2%), *n*-tetradecanol (12.3%), dodecanoic acid (5.9%)	(39)
Libya	Seed	Hydrodistillation	Eugenol (17.8%), *n*-tetradecanol (11.3%), β-acorenolo (% 7.4)	(39)
Morocco	Seed	Hydrodistillation	Eugenol (13.2%), *n*-tetradecanol (11.1%), bakerol (7.5%)	(39)
Tunisia	Seed	Hydrodistillation	Eugenol (69.2%), (*E*)-anethole (6.9%)	(39)
Iran	Seed	HS-SPME	2,3-dimethyl benzofuran (28.32%), cis-linalool oxide (7.46%), [2E] – decenal (6.57%)	(40)

Table 3. *Cases of poisoning due to P. harmala*

Patient's History	Part of the Plant Used, How to Use and Amount of Usage	Reason for Use	Clinical Findings	Reference
76 F, Parkinson's disease. History of use of levodopa/benserazide, rasagiline, and long-acting carbidopa levodopa	100 g of seed infusion, orally	Unspecified	Pulse ↑(120 beats/min), tachycardia, BP ↑ (165/90 mmHg), GCS 15/15; serotonergic syndrome	(88)
20 F, pregnancy history	A handful of seeds, orally	To induce abortion	Bradycardia at 45 pulse/minute then a tachycardia at 132 pulse/minute, LFTs ↑, kidney function tests ↑, troponine and creatine phosphokinaseMB ↑	(89)
31 F, pregnancy history	1 cup (~ 50g) of powdered seeds drunk with water	To induce abortion	Leukocytosis	(90)
58 F, 15 years of Type 2 DM patient, history of metformin and glibenclamide use	Boiled herb seeds, orally, 250 mL (one glass)	Regulation of blood sugar	Pulse ↑(120 beats/min), tachycardia, BG (250 mg/dl) ↑, HbA1c ↑ (>8), LFTs ↑, LDH↑, WBC ↑, PT and aPTT ↑, GCS 14/15	(91)
54 F, history of HT and atenolol use	Mixture of water, sugar and seeds, oral, 50 g	Treatment of chronic constipation	Pulse ↑(105 beats/min), tachycardia, BP ↑ (160/90 mmHg)	(92)
24 F, 22 weeks of pregnancy history	Seed. The use and amount of the seeds are not specified.	To induce abortion	Respiratory rate (30/min) ↑, LFTs ↑, Troponin ↑, Creatine phosphokinase –MB ↑, GCS 12/15 (Kidney failure, liver damage, fetal death)	(93)
45 F	Seeds mixed with a spoonful of honey, orally, 50 g	Treatment of hypermenorrhea	BP↓ (90/60 mmHg)	(94)
41 F	100 g of seed infusion, orally	To reduce anxiety	BP↑ (138/103 mmHg), Pulse ↑ (110 beats/min), Respiratory rate ↑ (30/min), GCS 8 BG↑ (225 mg/dL)	(95)
35 M	150 g of seed, orally	Unspecified	BP↓ (80/40 mmHg), Pulse ↑ (100 beats/min), mild anemia (Hb of 12.9 g/dl)	(96)

F: female, BP: Blood pressure, GCS: Glasgow coma scale, LFTs: Liver function tests, DM: diabetes mellitus, BG: Blood Glucose, HbA1c: glycosylated hemoglobin, LDH: lactate dehydrogenase, PT: prothrombin time, aPTT: Activated Partial Thromboplastin Time, HT: Hypertension, M: male

3.10. Gastroprotective Activity

Singh *et al*. investigated the gastroprotective effect of peganine hydrochloride which was purified from *P. harmala* seed ethanolic extract, in Sprague-Dawley rats using models of cold-induced stress (CRU), aspirin (AS), alcohol (AL), and pyloric ligation (PL) that caused the gastric ulcer. Peganine (20 mg/kg, single dose, oral) provided 50.0% (p<0.05), 89.41% (p<0.001), 58.50%, and 62.50% (p<0.01) protection in CRU, AL, AS and PL models, respectively (omeprazole at a dose of 10 mg/kg: 77.4%, 49.97% and 69.42% in CRU, AS, PL models. 500 mg/kg sucralfate: 62.5% in AL model). It reduced free acidity by 33.38% (p<0.01), total acidity by 38.09% (p<0.01), and increased mucin secretion by 67.91% (p<0.01) in the PL model. *In vitro* studies showed that the antisecretory mechanism of action of peganine (1-100 µg/mL at different concentrations) was related to its inhibition of H^+K^+ ATPase activity. Peganine inhibited the proton pump (IC_{50}: 73.47 µg/mL). The results support that peganin has remarkable gastroprotective activity in rats (60).

3.11. Nephroprotective Activity

Niu *et al*. studied the effect of harmine on lipopolysaccharide-induced acute kidney injury in male Kunming mice. Harmine was administered intragastrically at a dose of 25 or 50 mg/kg for five days. Harmine decreased blood urea nitrogen (BUN) and creatinine levels in a dose-dependent manner. At the 50 mg/kg dose, it significantly suppressed the increase in serum cystatin C levels. It decreased the dose-dependent formation of MDA and MPO and increased the activities of SOD and GSH. Harmine suppressed Toll-like receptor 4 (TLR4) expression and phosphorylation of NF-κB p65 and inhibitor of κBα. In addition, harmine inhibited the expression of NLRP3, caspase-1, and IL-1*β*. Harmine ameliorates acute kidney injury by blocking TLR4-NF-κB and NLRP3 signaling pathways (61).

3.12. Others

Results of other studies revealed that *P. harmala* extracts and isolated secondary metabolites exhibited antidiabetic, diuretic, vasorelaxant, antiparasitic, acaricidal, hepatoprotective, and wound healing activities (62-69).

4. CLINICAL STUDIES

In a double-blind, controlled, randomized clinical trial, the efficacy of *P. harmala* seed oil (standardized with 0.0025% harmaline and 0.057% harmine) was evaluated in patients with knee osteoarthritis. Four drops of the *Peganum* oil or the control group (olive oil) were applied to the knees three times a day for four weeks. The Visual Analog Scale (VAS) was used at weeks 0 and 4 to determine the patients' pain relief. The Western Ontario and McMaster Universities Arthritis Index (WOMAC) was used to determine improvement in pain, stiffness, and functional symptoms. When scores were compared from VAS, pain decreased significantly in both groups (p<0.05). In the group of patients receiving *Peganum* oil, a decrease of 52.56% was observed (Control group: 17%). Except for stiffness, the WOMAC variables were significantly decreased (p<0.001). Based on the WOMAC results, it was found that pain decreased by 37.89% in the *Peganum* oil group. (Control group: 16.41) Pain and functional limitations decreased significantly in the *Peganum* group compared to the control group after 4 weeks (p<0.001). On physical examination, knee tenderness was found at baseline in both groups, but tenderness decreased significantly after 4 weeks of treatment (p<0.001) (70).

Hafshejani *et al*. conducted a randomized controlled clinical trial to evaluate the efficacy of *P. harmala* in female patients (n=100) diagnosed with osteoporosis. During the study, female patients received medical treatments, including calcium D (500 mg) twice daily and Osteofos® (70 mg, alendronic acid) twice weekly. In addition to medical treatment, patients in the treatment group received capsules of *P. harmala* seed (500 mg) twice daily for three months. It was reported that the mean BMD of the femur and spine significantly improved in the treatment group (p<0.001). BMD changes in the femur (-0.37) and spinal cord (-0.44) were higher in the treatment group than in the control group (BMD changes in the control group for the femur and spinal cord were - 0.24 and - 0.22 p<0.001, respectively) (71).

A clinical investigation was conducted on 90 patients who were diagnosed with benign prostatic hyperplasia (BPH) using the International Prostate Symptom Score (IPSS). The patients were separated into three groups. The first group received 1 g of *P. harmala* seed oral capsules, the second group received tamsulosin with 1 g of *P. harmala* seed oral capsules, and the third group received tamsulosin alone. The established treatment regimen was applied for four weeks. As a result, the difference in IPSS mean scores before and after treatment was determined to be significant (p=0.001). The lowest IPSS score was calculated in the group of receiving capsules with *P. harmala* and tamsulosin simultaneously (12.0±4.4 points). The highest score was calculated in the group receiving only tamsulosin (16.5±3.7 points). A significant reduction in urine frequency in urinary frequency (p=0.002), nocturia (p=0.001), and intermittent voiding (p=0.002) were observed in all three groups before and after the intervention. Finally, it was discovered that oral administration of *P. harmala* seeds may be effective in lowering urinary symptoms in BPH patients and that the combination with tamsulosin may be more effective in relieving urinary symptoms compared to the other groups (72).

In a randomized clinical trial, 80 patients with renal and ureteral stones of 4 to 10 mm were given capsules of tamsulosin (0.4 mg/day) or *P. harmala* (50 mg/kg/day) for two weeks. Stone size was significantly reduced after treatment in both groups. The mean stone size decreased from 13.31±7.16 mm to 4.07±3.66 mm in the group receiving *P. harmala* (tamsulosin: decrease from 10.79±4.82 mm to 5.15±3.63 mm). In terms of stone size, there has been no statistical variance (p=0.21). The number of stones

decreased, with no significant difference between groups. A more significant decrease in pain scores was observed in the group receiving *P. harmala* (p=0.002). The efficacy of the treatment was more than 75% in both groups. The results of the study showed that *P. harmala* was as effective as tamsulosin. In addition, *P. harmala* was reported to be more effective in relieving pain than tamsulosin (73).

Within a random, double-blind, placebo-controlled clinical study of 61 patients with gastroesophageal malignancy, groups were separated to control and treatment. All groups continued to receive the chemotherapeutic agents 5-fluorouracil, uracil, and cisplatin throughout the study. The treatment group, in addition to their own medications, received the drug Spinal-Z (containing a mixture of *P. harmala* seed methanolic extracts and *Dracocephalum kotschyi* Boiss leaves) at a dose of 600 mg/m^2/day for 6 months. Complaints like abdominal pain (p=0.004), anorexia (p<0.001), and constipation (p=0.01) decreased in the group receiving Spinal Z compared to pretreatment. Compared to the control group, complaints of abdominal pain, heartburn, constipation, and vomiting decreased significantly in the treatment group (74).

4. TOXICITY

In vitro and *in vivo* studies were conducted to evaluate the toxicity of *P. harmala* and its secondary metabolites, mainly alkaloids. Apart from cytotoxicity studies, studies on healthy cells are given in this section.

The cytotoxic effect of *P. harmala* hydroalcoholic seed extract (10%) on L929 fibroblast cells was tested in the MTT method. Cell viability decreased as the incubation period increased. After 1, 24, and 72 hours of incubation, the percentages of cell viability were confirmed to be 94%, 69%, and 51%, respectively. In the study, it was revealed that the cytotoxic effect was lower when compared to 5.25% sodium hypochlorite (75). The toxic selectivity of indole alkaloids isolated from the plant against human embryonic kidney cells (HEK-293) was evaluated. Indole alkaloids showed a very low toxic effect in HEK-293 cells (IC_{50} values above 200 µmol/L) when compared to human leukemia cell lines (27).

Studies in the literature have shown that *β*-carboline alkaloids have genotoxic, mutagenic, and cytotoxic properties. Genotoxic effects of harman and harmine (20, 40, 80 µg/mL) on V79 Chinese hamster lung fibroblast cells were investigated by single-cell gel electrophoresis test (Comet assay). In comparison to the control groups, both alkaloids increased the frequency of abnormal cells and induced DNA damage at all concentrations. It has been stated that harman and harmine have genotoxic effects on V79 cells and this effect is associated with the induction of DNA strand breaks (76). In another study, yeast *Saccharomyces cerevisiae* was incubated with harman and harmine at concentrations of 100, 200, 300, and 400 µg/L. Both alkaloids caused DNA damage by causing single and/or double-strand breaks in DNA. Harmine was found to be more cytotoxic than harman in haploid and diploid cells (77).

Acute Toxicity: Benbott *et al*. reported that *intraperitoneal* (*i.p.*) administration of an alkaloid extract prepared from the seeds was moderately toxic to Wistar albino rats with an LD_{50} of 350 mg/kg body weight. Clinical changes such as convulsions, agitation, tachycardia, dyspnea, somnolence, decreased locomotor activity, and anorexia were observed in the animals (78). The *intramuscular (i.m.)* administration of an aqueous extract of *P. harmala* to rats resulted in an LD_{50} value of 420 mg/kg (79). In another study, when the aqueous extract was given orally in an acute dose, the LD_{50} value was discovered to be 2.70±0.05 g/kg (80).

Subacute Toxicity: The total alkaloid extract from the seeds of *P. harmala* was administered orally to rats for 28 days at doses of 15, 45, and 150 mg/kg/day. The extract was found to be safe at doses of 15 and 45 mg/kg/day. In the first three days of the study, tremors occurred 15 minutes after administration in rats receiving the extract at a dose of 150 mg/kg and continued for 4 hours. The extract's no observed adverse effect level (NOAEL) was revealed to be 45 mg/kg/day (81).

Chronic Toxicity: Oral administration of *P. harmala* aqueous extract to rats at doses of 1, 1.35, and 2 g/kg 6 times a week for 3 months raised transaminase levels. In the histological examination, rats administered the extract at a dose of 2 g/kg showed signs of liver degeneration and spongiform changes in the central nervous system (80).

Genotoxicity: Abderrahman *et al*. investigated the cytological effect of the alkaloid extract from the seeds of *P. harmala* (12.5, 25, 50, and 100 mg/kg body weight, *i.p*., single dose) by calculating the mitotic index values in bone marrow cells of mice. While the mitotic index significantly decreased from 4.45 to 3.31 in the extract-treated group, the mitodepression index increased. Cytogenetic studies showed that the extract caused a significant increase in the percentage of chromosomal aberrations and induced sister chromatid exchange. These results indicate that the extract may have genotoxic effects (82).

Reproductive Toxicity: It was demonstrated that methanol extracts of *P. harmala* prepared at different doses (2, 2.5, and 3 g/kg/day) significantly prolonged the duration of the estrus phase and estrus cycle in female rats. The number of live offspring was reduced and the number of resorbed fetuses was raised when methanol extracts were administered at doses of 2.5 and 3.5 g/kg/day. It did not affect the reproductive rate, number of live offspring, number of resorbed fetuses, and implantation sites when administered at a dose of 2.0 g/kg/day (83). The effect of hydroalcoholic extract prepared from *P. harmala* seeds on the spontaneous rhythmic contraction of the isolated rat uterus was tested. It has been determined that the extract at concentrations of 12.5 and 50 µg/mL produces uterotonic effects in the presence of KCl in a calcium-free solution (84).

P. harmala aqueous extract has been shown to significantly reduce the weight of reproductive organs like the testis, epididymis, seminal vesicle, ventral prostate, and vas

deferens when given to adult male albino rats for 60 days at a dose of 300 mg/kg. The extract decreased sperm motility and density in the cauda epididymis and testicular ducts. In seminiferous tubules, it diminished spermatogenesis activity. In the continuation of the study, the serum hormonal levels of the rats were measured at 300 mg/kg dose. It has been found that the aqueous extract causes levels of testosterone and follicle-stimulating hormone to decline. It was observed that the extract reduced the number of female rats fertilized with male rats, the implantation site, and the number of viable fetuses (85).

Human Toxicity

In the literature, there are case reports of adverse effects resulting from the concomitant use of drugs or other herbal products with *P. harmala*. A 42-year-old male patient who has a mood disorder and has been taking *P. harmala* (dose unknown) for the treatment of hemorrhoids was the subject of one such case report. The patient had a history of taking quetiapine 1000 mg/day and fluoxetine 40 mg/day. The patient, who presented to the emergency department with complaints of nausea, vomiting, sweating, and tremors, was noted to have visual hallucinations, confusion, agitation, and hyperactive delirium. The patient was diagnosed with serotonin syndrome associated with the concomitant use of fluoxetine and *P. harmala* (86). Liu *et al*. reported dimethyltryptamine (DMT) poisoning in two individuals who ingested an herbal stew of *P. harmala* seeds with MAO inhibitor effect and *Acacia* tree bark containing DMT. Biochemical and toxicological analyzes of a 22-year-old male patient who reported to the emergency department with complaints of restlessness and confusion revealed a serious increase in myoglobin levels (5252 ng/mL). The patient's serum and urine showed 25 and 1206 ng/mL DMT and 3.3 and 1564 ng/mL harmaline, respectively. Another male patient, 24, was admitted to the hospital with symptoms of impulsive mood changes and violent behavior. On analysis, myoglobin level was found to be elevated similar to the other case (381 ng/mL). While 478 ng/mL DMT and 1230 ng/mL harmaline were detected in the urine, the presence of the two compounds in the serum could not be determined (87).

There are many cases of poisoning associated with uncontrolled and unconscious consumption of *P. harmala*. Neurological, gastrointestinal, and cardiovascular symptoms accounted for 34.4%, 31.9%, and 15.8% of the cases of poisoning, respectively. Following the consumption of *P. harmala* seeds, patients apply to the clinic with complaints such as visual and auditory hallucinations, locomotor ataxia, nausea, vomiting, confusion, and agitation (88-96). Some cases of poisoning by *P. harmala* are listed in Table 3.

5. CONCLUSIONS

The use of plants for various purposes such as curing diseases, preventing diseases, and improving general health is as old as the history of mankind. Today, research on medicinal plants continues and activity studies are being conducted by isolating compounds that can guide the development of new conventional medicines from medicinal plants. In this review, botanical characteristics, traditional uses, chemical composition, pharmacological effects, toxicity, interactions with drugs and other herbal products, and cases of human toxicity of *P. harmala* were studied based on scientific data.

P. harmala contains alkaloids (*β*-carboline, quinazoline, *β*-carboline-quinazoline dimers, indole, and thiazole), anthraquinones, phenolic acids (hydroxycinnamic acid and hydroxybenzoic acid derivatives), flavonoids (flavone glycoside, flavonol, flavonol glycoside, and flavanones), triterpenoids (lupane, oleanane, and pentacyclic) and fatty acids. It has been shown that the essential oils obtained by hydrodistillation from different parts of the plant contain compounds such as camphor, eugenol, *n*-tetradecanol, *α*-pinene, thymol, and limonene. The main secondary metabolite group responsible for the pharmacological effects of the herb are alkaloids in the structure of *β*-carboline and quinazoline. The plant and its alkaloids have shown anti amnestic, anticancer, antidepressant, antiinflammatory, cardiovascular, gastroprotective, hepatoprotective, nephroprotective, and vasodilator activities in preclinical studies. These effects reveal that the plant *P. harmala* can be an important resource in the pharmaceutical industry for the development of new drugs for the treatment of various diseases. In recent years, investigations examining the efficacy of the herb on humans have also been published. As a result of clinical studies, positive results have been obtained by using *P. harmala* in addition to medical treatment for diseases such as osteoporosis and BPH. In addition, it is noteworthy that it reduces the side effects caused by drugs used in the treatment of gastroesophageal malignancy. For these reasons, clinical studies are needed especially on the metabolites of *P. harmala* in areas where preclinical studies are concentrated and positive results are obtained. The therapeutic mechanisms of action of *P. harmala* and its secondary metabolites should be clarified and the pharmacological effects determined by preclinical studies should be supported by clinical studies.

In vitro and *in vivo* studies have revealed that various extracts of *P. harmala* and *β*-carboline alkaloids have cytotoxic, genotoxic, mutagenic, cytotoxic, and reproductive toxicity. However, cases of poisoning, manifested by symptoms such as neurosensory symptoms, visual hallucination, bradycardia, hypotension, agitation, tremor, ataxia, and vomiting, associated with the use of *P. harmala* in humans have been recorded. Considering these, preclinical and clinical data on the safety of the plant and its isolated compounds need to be increased.

Conflicts of interest: *The authors declare that they have no conflict of interest.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: GR, UÖ
Design of the study: GR, UÖ

Acquisition of data for the study: İG, TS
Analysis of data for the study: İG
Interpretation of data for the study: İG
Drafting the manuscript: GR, UÖ, İG, TS
Revising it critically for important intellectual content: GR, UÖ
Final approval of the version to be published: İG, GR

REFERENCES

[1] Sharifi-Rad J, Quispe C, Herrera-Bravo J, Semwal P, Painuli S, Özçelik B, Hacıhasanoğlu FE, Shaheen S, Sen S, Acharya K, Amirian M, Castillo CMS, López MD, Schoebitz M, Martorell M, Goloshvili T, Al-Harrasi A, Al-Rawahi A, Kumar M, Suleria HAR, Cho WC. *Peganum* spp.: A comprehensive review on bioactivities and health-enhancing effects and their potential for the formulation of functional foods and pharmaceutical drugs. Oxid Med Cell Longev. 2021;2021:5900422. DOI: 10.1155/2021/5900422

[2] Hürkul M, Köroğlu A. Investigation of *Peganum harmala* L. (üzerlik) fruits sold in the market from the view of pharmaceutical botany. Biodivers Conserv. 2019;12(2):65-77. DOI: 10.5505/biodicon.2019.52724

[3] Ullah M, Khan MU, Mahmood A, Malik RN, Hussain M, Wazir SM, Daud M, Shinwari ZK. An ethnobotanical survey of indigenous medicinal plants in Wana district south Waziristan agency, Pakistan. J Ethnopharmacol. 2013;150(3):918-924. DOI: 10.1016/j.jep.2013.09.032

[4] Upadhyay B, Parveen, Dhaker AK, Kumar A. Ethnomedicinal and ethnopharmaco-statistical studies of Eastern Rajasthan, India. J Ethnopharmacol. 2010;129(1):64-86. DOI: 10.1016/j.jep.2010.02.026

[5] Özdemir E, Alpınar E. An ethnobotanical survey of medicinal plants in western part of central Taurus Mountains: Aladaglar (Nigde-Turkey). J Ethnopharmacol. 2015;166:53-65. DOI: 10.1016/j.jep.2015.02.052

[6] Korkmaz M, Karakuş S, Özçelik H, Selvi S. An ethnobotanical study on medicinal plants in Erzincan, Turkey. Indian J Tradit Knowl. 2016;15(2):192-202.

[7] Güler B, Manav E, Uğurlu E. Medicinal plants used by traditional healers in Bozüyük (Bilecik-Turkey). J Ethnopharmacol. 2015;173:39-47. DOI: 10.1016/j.jep.2015.07.007

[8] Boudjelal A, Henchiri C, Sari M, Sarri D, Hendel N, Benkhaled A, Ruberto G. Herbalists and wild medicinal plants in M'Sila (NorthAlgeria): an ethnopharmacology survey. J Ethnopharmacol. 2013; 148(2):395-402. DOI: 10.1016/j.jep.2013.03.082

[9] Pawera L, Verner V, Termote C, Sodombekov I, Kandakov A, Karabaev N, Skalicky M, Polesny Z. Medical ethnobotany of herbal practitioners in the Turkestan Range, southwestern Kyrgyzstan. Acta Soc Bot Pol. 2016;85(1):3483. DOI: 10.5586/asbp.3483

[10] Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaeili S. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. J Ethnopharmacol. 2012;141(1): 80-95. DOI: 10.1016/j.jep.2012.02.004

[11] Sher H, Bussmann RW, Hart R, de Boer HJ. Traditional use of medicinal plants among Kalasha, Ismaeli and Sunni groups in Chitral District, Khyber Pakhtunkhwa province, Pakistan. J Ethnopharmacol. 2016;188:57-69. DOI: 10.1016/j.jep.2016.04.059

[12] Herraiz T, González D, Ancín-Azpilicueta C, Arán VJ, Guillén H. beta-Carboline alkaloids in *Peganum harmala* and inhibition of human monoamine oxidase (MAO). Food Chem Toxicol. 2010;48(3):839-845. DOI: 10.1016/j.fct.2009.12.019

[13] Li S, Cheng X, Wang C. A review on traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the genus *Peganum*. J Ethnopharmacol. 2017;203:127-162. DOI: 10.1016/j.jep.2017.03.049

[14] Mina CN, Farzaei MH, Gholamreza A. Medicinal properties of *Peganum harmala* L. in traditional Iranian medicine and modern phytotherapy: a review. J Tradit Chin Med. 2015;35(1):104-109. DOI: 10.1016/s0254-6272(15)30016-9

[15] Wen F, Cheng X, Liu W, Xuan M, Zhang L, Zhao X, Shan M, Li Y, Teng L, Wang Z, Wang C. Chemical fingerprint and simultaneous determination of alkaloids and flavonoids in aerial parts of genus *Peganum* indigenous to China based on HPLC-UV: application of analysis on secondary metabolites accumulation. Biomed Chromatogr. 2014;28(12):1763-1773. DOI: 10.1002/bmc.3218

[16] Yang Y, Cheng X, Liu W, Chou G, Wang Z, Wang C. Potent AChE and BChE inhibitors isolated from seeds of *Peganum harmala* Linn by a bioassay-guided fractionation. J Ethnopharmacol. 2015;168:279-286. DOI: 10.1016/j.jep.2015.03.070

[17] Wang KB, Yuan CM, Xue CM, Li DH, Jing YK, He HP, Hao XJ, Di YT, Li ZL, Hua HM. Pegaharmalines A and B, two novel *β*-carboline alkaloids with unprecedented carbon skeletons from *Peganum harmala*. RSC Adv. 2014;4:53725-53729.

[18] Wang KB, Di YT, Bao Y, Yuan CM, Chen G, Li DH, Bai J, He HP, Hao XJ, Pei YH, Jing YK, Li ZL, Hua HM. Peganumine A, a *β*-carboline dimer with a new octacyclic scaffold from *Peganum harmala*. Org Lett. 2014;16(15):4028-4031. DOI: 10.1021/ol501856v

[19] Yang YD, Cheng XM, Liu W, Han ZZ, Chou GX, Wang Y, Sun DX, Wang ZT, Wang CH. Peganumine BI and two enantiomers: new alkaloids from the seeds of *Peganum harmala* Linn. and its their potential cytotoxicity and cholinesterase inhibitory activities. RSC Adv. 2016;6:15976-15987. DOI: 10.1039/C6RA00086J

[20] Wang KB, Li DH, Hu P, Wang WJ, Lin C, Wang J, Lin B, Bai J, Pei YH, Jing YK, Li ZL, Yang D, Hua HM. A series of *β*-carboline alkaloids from the seeds of *Peganum harmala* show G-quadruplex interactions. Org Lett. 2016;18(14):3398-3401. DOI: 10.1021/acs.orglett.6b01560

[21] Wang KB, Li DH, Bao Y, Cao F, Wang WJ, Lin C, Bin W, Bai J, Pei YH, Jing YK, Yang D, Li ZL, Hua HM. Structurally diverse alkaloids from the seeds of *Peganum harmala*. J Nat Prod. 2017;80(2):551-559. DOI: 10.1021/acs.jnatprod.6b01146

[22] Wu ZN, Chen NH, Tang Q, Chen S, Zhan ZC, Zhang YB, Wang GC, Li YL, Ye WC. *β*-Carboline alkaloids from the seeds of *Peganum harmala* and their anti-HSV-2 virus activities. Org Lett. 2020; 22(18):7310-7314. DOI: 10.1021/acs.orglett.0c02650

[23] Herraiz T, Guillén H, Arán VJ, Salgado A. Identification, occurrence and activity of quinazoline alkaloids in *Peganum harmala*. Food Chem Toxicol. 2017;103:261-269. DOI: 10.1016/j.fct.2017.03.010

[24] Li SG, Wang KB, Gong C, Bao Y, Qin NB, Li DH, Li ZL, Bai J, Hua HM. Cytotoxic quinazoline alkaloids from the seeds of *Peganum harmala*. Bioorganic Med Chem Lett. 2018;28:103-106. DOI: 10.1016/j.bmcl.2017.12.003

[25] Wang CH, Zeng H, Wang YH, Li C, Cheng J, Ye ZJ, He XJ. Antitumor quinazoline alkaloids from the seeds of *Peganum*

harmala. J Asian Nat Prod Res. 2015;17(5):595-600. DOI: 10.1080/10286.020.2015.1042373

[26] Li SG, Wang YT, Zhang Q, Wang KB, Xue JJ, Li DH, Jing YK, Lin B, Hua HM. Pegaharmols A-B, Axially chiral *β*-carboline-quinazoline dimers from the roots of *Peganum harmala*. Org Lett. 2020;22(19):7522-7525. DOI: 10.1021/acs.orglett.0c02709

[27] Wang C, Zhang Z, Wang Y, He X. Cytotoxic indole alkaloids against human leukemia cell lines from the toxic plant *Peganum harmala*. Toxins. 2015;7(11):4507-4518. DOI: 10.3390/toxins7114507

[28] Wang KB, Hu X, Li SG, Li XY, Li DH, Bai J, Pei YH, Li ZL, Hua HM. Racemic indole alkaloids from the seeds of *Peganum harmala*. Fitoterapia. 2018;125:155-160. DOI: 10.1016/j.fitote.2018.01.008

[29] Moussa TAA, Almaghrabi OA. Fatty acid constituents of *Peganum harmala* plant using Gas Chromatography Mass Spectroscopy. Saudi J Biol Sci. 2016;23(3):397-403. DOI: 10.1016/j.sjbs.2015.04.013

[30] Khadhr M, Bousta D, Hanane EH, El Mansouri L, Boukhira S, Lachkar M, Jamoussi B, Boukhchina S. HPLC and GC–MS analysis of Tunisian *Peganum harmala* seeds oil and evaluation of some biological activities. Am J Ther. 2017;24(6):706–712. DOI: 10.1097/MJT.000.000.0000000402

[31] Hajji A, Bnejdi F, Saadoun M, Ben Salem I, Nehdi I, Sbihi H, Alharthi FA, El Bok S, Boughalleb-M'Hamdi N. High reserve in *δ*-tocopherol of *Peganum harmala* seeds oil and antifungal activity of oil against ten plant pathogenic fungi. Molecules. 2020;25(19):4569. DOI: 10.3390/molekül25194569

[32] Li H, Wang Z, Wang Y, Xu J, He X. Triterpenoids with anti-proliferative effects from the seeds of *Peganum harmala* L. Phytochemistry. 2020;174: 112342. DOI: 10.1016/j.phytochem.2020.112342

[33] Elansary HO, Szopa A, Kubica P, Ekiert H, Al-Mana FA, El-Shafei AA. Polyphenols of *Frangula alnus* and *Peganum harmala* leaves and associated biological activities. Plants (Basel). 2020;9(9):1086. DOI: 10.3390/plants9091086

[34] Moazeni M, Saadaty Ardakani ZS, Saharkhiz MJ, Jalaei J, Khademolhoseini AA, Shams Esfand Abad S, Mootabi Alavi A. In vitro ovicidal activity of *Peganum harmala* seeds extract on the eggs of *Fasciola hepatica*. J Parasit Dis. 2017;41(2):467–472. DOI: 10.1007/s12639.016.0830-1

[35] Selim SA, Aziz MHA, Mashait MS, Warrad MF. Antibacterial activities, chemical constitutes and acute toxicity of Egyptian *Origanum majorana* L., *Peganum harmala* L. and *Salvia officinalis* L. essential oils. Afr J Pharm Pharmacol. 2013;7(13):725-735. DOI: 10.5897/AJPP2013.3518

[36] Dastagır G, Hussaın F, Rehman IU. Essential oil composition of some plants of family Zygophyllaceae and Euphorbiaceae. Pak J Bot. 2014;46(6):2043-2049.

[37] Yang S, Bai M, Yang J, Yuan Y, Zhang Y, Qin J, Kuang Y, Sampietro DA. Chemical composition and larvicidal activity of essential oils from *Peganum harmala*, *Nepeta cataria* and *Phellodendron amurense* against Aedes aegypti (Diptera: Culicidae). Saudi Pharm J. 2020;28(5):560-564. DOI: 10.1016/j.jsps.2020.03.007

[38] Faridi P, Ghasemi Y, Mohagheghzadeh A. Chemical composition of *Peganum harmala* smoke and volatile oil. J Essent Oil-Bear Plants. 2013;16(4):850-854. DOI: 10.1080/0972060X.2013.861993

[39] Apostolico I, Aliberti L, Caputo L, De Feo V, Fratianni F, Nazzaro F, Souza LF, Khadhr M. Chemical composition, antibacterial and phytotoxic activities of *Peganum harmala* seed essential oils from five different localities in Northern Africa. Molecules. 2016;21(9):1235. DOI: 10.3390/molecules21091235

[40] Asadzadeh R, Abbasi N, Bahmani M. Extraction and identification of chemical compounds of *Peganum harmala* L. seed essential oil by HS-SPME and GC-MS methods. Trad Integr Med. 2021;6(3):229-235. DOI: 10.18502/tim.v6i3.7310

[41] Liu W, Zhu Y, Wang Y, Qi S, Wang Y, Ma C, Li S, Jiang B, Cheng X, Wang Z, Xuan Z, Wang C. Anti-amnesic effect of extract and alkaloid fraction from aerial parts of *Peganum harmala* on scopolamine-induced memory deficits in mice. J Ethnopharmacol. 2017;204:95-106. DOI: 10.1016/j.jep.2017.04.019

[42] Deng G, Wu C, Rong X, Li S, Ju Z, Wang Y, Ma C, Ding W, Guan H, Cheng X, Liu W, Wang C. Ameliorative effect of deoxyvasicine on scopolamine-induced cognitive dysfunction by restoration of cholinergic function in mice. Phytomedicine. 2019;63:153007. DOI: 10.1016/j.phymed.2019.153007

[43] Saleh RA, Eissa TF, Abdallah DM, Saad MA, El-Abhar HS. *Peganum harmala* enhanced GLP-1 and restored insulin signaling to alleviate AlCl3-induced alzheimer-like pathology model. Sci Rep. 2021;11(1):12040. DOI: 10.1038/s41598.021.90545-4

[44] Azzazy H, Fahmy SA, Mahdy NK, Meselhy MR, Bakowsky U. Chitosan-Coated PLGA nanoparticles loaded with *Peganum harmala* alkaloids with promising antibacterial and wound healing activities. Nanomaterials (Basel). 2021;11(9):2438. DOI: 10.3390/nano11092438

[45] Sassoui D, Seridi R, Azin K, Usai M. Evaluation of phytochemical constituents by GC-MS and antidepressant activity of *Peganum harmala* L. seeds extract. Asian Pac J Trop Dis. 2015;5(12):971-974. DOI: 10.1016/S2222-1808(15)60967-7

[46] Bensalem S, Soubhye J, Aldib I, Bournine L, Nguyen AT, Vanhaeverbeek M, Rousseau A, Boudjeltia KZ, Sarakbi A, Kauffmann JM, Nève J, Prévost M, Stévigny C, Maiza-Benabdesselam F, Bedjou F, Van Antwerpen P, Duez P. Inhibition of myeloperoxidase activity by the alkaloids of *Peganum harmala* L. (Zygophyllaceae). J Ethnopharmacol. 2014;154(2):361-369. DOI: 10.1016/j.jep.2014.03.070

[47] Abbas MW, Hussain M, Qamar M, Ali S, Shafiq Z, Wilairatana P, Mubarak MS. Antioxidant and anti-inflammatory effects of *Peganum harmala* extracts: an *in vitro* and *in vivo* study. Molecules. 2021;26(19):6084. DOI: 10.3390/molecules26196084

[48] Akhtar MF, Raza SA, Saleem A, Hamid I, Ashraf Baig MMF, Sharif A, Sohail K, Javaid Z, Saleem U, Rasul A. Appraisal of anti-arthritic and anti-inflammatory potential of folkloric medicinal plant *Peganum harmala*. Endocr Metab Immune Disord Drug Targets. 2022;22(1):49-63. DOI: 10.2174/187.153.0321666.210.208211310

[49] Wang L, Wang Q, Wang W, Ge G, Xu N, Zheng D, Jiang S, Zhao G, Xu Y, Wang Y, Zhu R, Geng D. Harmine alleviates titanium particle-induced inflammatory bone destruction by immunomodulatory effect on the macrophage polarization and subsequent osteogenic differentiation. Front Immunol. 2021;12:657687. DOI: 10.3389/fimmu.2021.657687

[50] Liu W, Cheng X, Wang Y, Li S, Zheng T, Gao Y, Wang G, Qi S, Wang J, Ni J, Wang Z, Wang C. *In vivo* evaluation of the antitussive, expectorant and bronchodilating effects of extract and fractions from aerial parts of *Peganum harmala* Linn. J Ethnopharmacol. 2015;162:79-86. DOI: 10.1016/j.jep.2014.12.046

[51] Benzekri R, Bouslama L, Papetti A, Hammami M, Smaoui A, Limam F. Anti HSV-2 activity of *Peganum harmala* (L.) and isolation of the active compound. Microb Pathog. 2018;114:291-298. DOI: 10.1016/j.micpath.2017.12.017

[52] Moradi MT, Karimi A, Rafieian-Kopaei M, Fotouhi F. *In vitro* antiviral effects of *Peganum harmala* seed extract and its total alkaloids against Influenza virus. Microb Pathog. 2017;110:42-49. DOI: 10.1016/j.micpath.2017.06.014

[53] Keihanian F, Moohebati M, Saeidinia A, Mohajeri SA, Madaeni S. Therapeutic effects of medicinal plants on isoproterenol-induced heart failure in rats. Biomed Pharmacother. 2021;134:111101. DOI: 10.1016/j.biopha.2020.111101

[54] Huang J, Liu Y, Chen JX, Lu XY, Zhu WJ, Qin L, Xun ZX, Zheng QY, Li EM, Sun N, Xu C, Chen HY. Harmine is an effective therapeutic small molecule for the treatment of cardiac hypertrophy. Acta Pharmacol Sin. 2022;43(1):50–63. DOI: 10.1038/s41401.021.00639-y

[55] Yavari N, Emamian F, Yarani R, Reza Mohammadi-Motlagh H, Mansouri K, Mostafaie A. In *vitro* inhibition of angiogenesis by heat and low pH stable hydroalcoholic extract of *Peganum harmala* seeds via inhibition of cell proliferation and suppression of VEGF secretion. Pharm Biol. 2015;53(6):855-861. DOI: 10.3109/13880.209.2014.946057

[56] Ruan S, Jia F, Li J. Potential antitumor effect of harmine in the treatment of thyroid cancer. Evid Based Complement Alternat Med. 2017; 2017(4):1-7. DOI: 10.1155/2017/9402615

[57] Hamsa T, Kuttan G. Studies on anti-metastatic and anti-invasive effects of harmine using highly metastatic murine B16F-10 melanoma cells. J Environ Pathol Toxicol Oncol. 2011;30(2):123-137. DOI: 10.1615/jenvironpatholtoxicoloncol.v30.i2.40

[58] Yalçın D, Bayraktar O. Inhibition of catechol-O-methyltransferase (COMT) by some plant-derived alkaloids and phenolics. J Mol Catal B Enzym. 2010;64(3):162-166. DOI: 10.1016/j.molcatb.2009.04.014

[59] Rezaei M, Nasri S, Roughani M, Niknami Z, Ziai SA. *Peganum harmala* L. extract reduces oxidative stress and improves symptoms in 6-hydroxydopamine-induced Parkinson's disease in rats. Iranian J Pharm Res. 2016;15(1):275-281.

[60] Singh VK, Mishra V, Tiwari S, Khaliq T, Barthwal MK, Pandey HP, Palit G, Narender T. Anti-secretory and cyto-protective effects of peganine hydrochloride isolated from the seeds of *Peganum harmala* on gastric ülcers. Phytomedicine. 2013; 20(13):1180-1185. DOI: 10.1016/j.phymed.2013.06.017

[61] Niu X, Yao Q, Li W, Zang L, Li W, Zhao J, Liu F, Zhi W. Harmine mitigates LPS-induced acute kidney injury through inhibition of the TLR4-NF-κB/NLRP3 inflammasome signalling pathway in mice. Eur J Pharmacol. 2019;849:160-169. DOI: 10.1016/j.ejphar.2019.01.062

[62] Singh AB, Chaturvedi JP, Narender T, Srivastava AK. Preliminary studies on the hypoglycemic effect of *Peganum harmala* L. seeds ethanol extract on normal and streptozotocin induced diabetic rats. Indian J Clin Biochem. 2008;23(4):391-393. DOI: 10.1007/s12291.008.0086-3

[63] Singh AB, Khaliq T, Chaturvedi JP, Narender T, Srivastava AK. Anti-diabetic and anti-oxidative effects of 4-hydroxypipecolic acid in C57BL/KsJ-db/db mice. Hum Exp Toxicol. 2012;31(1):57-65. DOI: 10.1177/096.032.7111407227

[64] Al-Saikhan FI, Ansari MN. Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of *Peganum harmala* L. in Wistar albino rats. Saudi J Biol Sci. 2016;23(6):749-775. DOI: 10.1016/j.sjbs.2016.01.025

[65] Berrougui H, Marhuenda E, Haddad W, Hmamouchi M, Khalil A, El-Bouadili A, Herrera MD. Comparative study on the vasorelaxant effects of three alkaloids-rich extracts from seeds of *Peganum harmala* L. J Nat Remedies. 2012;12(1):87-91. DOI: 10.18311/jnr/2012/42

[66] Khoshzaban F, Ghaffarifar F, Koohsari HRJ. *Peganum harmala* aqueous and ethanol extracts effects on lesions caused by *Leishmania major* (MRHO/IR/75/ER) in BALB/c mice. Jundishapur J Microbiol. 2014;7(7):e10992. DOI: 10.5812/jjm.10992

[67] Shang XF, Guo X, Li B, Pan H, Zhang J, Zhang Y, Miao X. Microwave-assisted extraction of three bioactive alkaloids from *Peganum harmala* L. and their acaricidal activity against *Psoroptes cuniculi in vitro.* J Ethnopharmacol. 2016;192:350-361. DOI: 10.1016/j.jep.2016.07.057

[68] Bourogaa E, Jarraya RM, Damak M, Elfeki A. Hepatoprotective activity of *Peganum harmala* against ethanol-induced liver damages in rats. Arch Physiol Biochem. 2015;121(2):62-67. DOI: 10.3109/13813.455.2015.1016974

[69] Derakhshanfar A, Oloumi MM, Mirzaie M. Study on the effect of *Peganum harmala* extract on experimental skin wound healing in rat: pathological and biomechanical findings. Comp Clin Pathol. 2010;19:169-172. DOI: 10.1007/s00580.009.0848-1

[70] Abolhassanzadeh Z, Aflaki E, Yousefi G, Mohagheghzadeh A. Randomized clinical trial of *Peganum* oil for knee osteoarthritis. Evid Based Complement Alternat Med. 2015;20(2):126-131. DOI: 10.1177/215.658.7214566867

[71] Hafshejani ZK, Lorigooini Z, Deris F, Akbari N, Farahbod F, Fazelian S, Moradi F, Dehghan M. Effect of oral capsule of *Peganum harmala* seeds on bone density in menopausal women prone to osteoporosis. J Shahrekord Univ Med Sci. 2021;23(3):139-145. DOI: 10.34172/jsums.2021.23

[72] Boroujeni MS, Soureshjani SH, Hafshejani ZK. Impact of oral capsule of *Peganum harmala* on alleviating urinary symptoms in men with benign prostatic hyperplasia: a randomized clinical trial. J Ren Inj Prev. 2017;6(2):127-131. DOI: 10.15171/jrip.2017.25

[73] Shakeri N, Mehrabi S, Mehrabi A, Jahanabad HM. Comparison efficacy of oral *Peganum harmala* seed versus tamsulosin on pain relief and expulsion of renal and ureteral stones; a randomized clinical trial. J Nephropathol. 2020;9(4):e40. DOI: 10.34172/jnp.2020.40

[74] Panahi Y, Saadat A, Seifi M, Rajaee M, Butler AE, Sahebkar A. Effects of spinal-Z in patients with gastroesophageal cancer. J Pharmacopuncture. 2018;21(1):026-034. DOI: 10.3831/KPI.2018.21.004

[75] Tabrizizadeh M, Kazemipoor M, Hakimian M, Maleksabet M, Kazemipoor M, Zandi H, Poorrajab F, Che C, Cordell GA. Effects of *Peganum harmala* (Zygophyllaceae) preparation for root canal disinfection. Phytotherapy Research. 2018;32(4):672-677. DOI: 10.1002/ptr.6015

[76] Boeira JM, da Silva J, Erdtmann B, Henriques JA. Genotoxic effects of the alkaloids harman and harmine assessed by comet assay and chromosome aberration test in mammalian cells in vitro. Pharmacol Toxicol. 2001;89(6):287-294. DOI: 10.1034/j.1600-0773.2001.d01-162.x

[77] Boeira JM, Viana AF, Picada JN, Henriques JA. Genotoxic and recombinogenic activities of the two beta-carboline alkaloids harman and harmine in Saccharomyces cerevisiae. Mutat Res. 2002;500(1-2):39-48. DOI: 10.1016/s0027-5107(01)00294-9

[78] Benbott A, Bahri L, Boubendir A, Yahia A. Study of the chemical components of *Peganum harmala* and evaluation of acute toxicity of alkaloids extracted in the Wistar albino mice. J Mater Environ Sci. 2013;4(4):558-565.

[79] Muhi-eldeen Z, Al-Shamma KJ, Al-Hussainy TM, Al – Kaissi EN, Al-Daraji AM, Ibrahim H. Acute toxicological studies on the extract of Iraqi *Peganum harmala* in rats. Eur J Sci Res. 2008;22(4):494-500.

[80] Lamchouri F, Settaf A, Cherrah Y, El Hamidi M, Tligui N, Lyoussi B, Hassar B. Experimental toxicity of *Peganum harmala seeds*. Ann. Pharm. Fr. 2002; 60: 123-129.

[81] Wang Y, Wang H, Zhang L, Zhang Y, Sheng Y, Deng G, Li S, Cao N, Guan H, Cheng X, Wang C. Subchronic toxicity and concomitant toxicokinetics of long-term oral administration of total alkaloid extracts from seeds of *Peganum harmala* Linn: A 28-day study in rats. J Ethnopharmacol. 2019;238:111866. DOI: 10.1016/j.jep.2019.111866

[82] Abderrahman SM, Soliman S, Mohammad MG. Genotoxic effects of *Peganum harmala* L. in relation to traditional use. JPP. 2018;10(9):167-173. DOI: 10.5897/JPP2018.0493

[83] Shapira Z, Terkel J, Egozi Y, Nyska A, Friedman J. Abortifacient potential for the epigeal parts of Peganum harmala. J Ethnopharmacol. 1989;27:319-325. DOI: 10.1016/0378-8741(89)90006-8

[84] Fathiazad F, Azarmi Y, Khodaie L. Pharmacological effects of *Peganum harmala* seeds extract on ısolated rat uterus. Iran. J. Pharm. Sci. 2006;2(2):81-86.

[85] El-Dwairi QA, Banihani SM. Histo-functional effects of *Peganum harmala* on male rat's spermatogenesis and fertility. Neuro Endocrinol Lett. 2007; 28(3):305-10.

[86] Bakim B, Sertcelik S, Tankaya O. A case of serotonin syndrome with antidepressant treatment and concomitant use of the herbal remedy (*Peganum harmala*). Klin Psikofarmakol B. 2012;22(4):359-36. DOI: 10.5455/bcp.201.207.29095402

[87] Liu CH, Chu WL, Liao SC, Yang CC, Lin CC. Syrian rue seeds interacted with Acacia tree bark in an herbal stew resulted in N,N-dimethyltryptamine poisoning. Clin Toxicol. 2019;57(10):867-869. DOI: 10.1080/15563.650.2019.1576877

[88] Durmaz Çelik N, Ger Akarsu F, Ozkan S, Özdemir AÖ, Aykac Ö, Memmedova F. Herbal teas can be harmful: a case of *Peganum harmala* induced coma in a patient with Parkinson's disease. Neurocase. 2021;27(2):117-119. DOI: 10.1080/13554.794.2021.1876092

[89] Ghizlane EA, Manal M, Ines HA, Soufiane D, Moussa L, Houssam B, Brahim H. Fatal poisoning of pregnant women by *Peganum harmala* L.: A case reports. Ann Med Surg (Lond). 2021;68:102649. DOI: 10.1016/j.amsu.2021.102649

[90] Vahabzadeh M, Mohammadi AB, Delirrad M. Abortion induced by *Peganum harmala* ingestion in a pregnant woman; a case report and literature review. Int J Med Toxicol. 2019;9(3):165-170. DOI: 10.32598/ijmtfm.v9i3.25910

[91] Ataee Z, Dadpour B, Najari F, Rahimpour M, Najari D. Case report: acute poisoning with *Peganum harmala*, esfand: a rare case report. Int J Med Toxicol Forensic Med. 2018;8(3):119-121. DOI: 10.22037/ijmtfm.v8i3(Summer).22342

[92] Mohammadi RS, Bidaki R, Mirdrikvand F, Yazdi SNM, Anari PY. *Peganum harmala* (Aspand) intoxication; a case report. Emergency. 2016;4(2):106-107.

[93] Berdai MA, Labib S, Harando M. *Peganum harmala* L. intoxication in a pregnant woman. Case Reports in Emergency Medicine. 2014;2014:783236. DOI: 10.1155/2014/783236

[94] Moshiri M, Etemad L, Javidi S, Alizadeh A. *Peganum harmala* intoxication, a case report. Avicenna J. Phytomedicine. 2013;3(3):288-292.

[95] Yuruktumen A, Karaduman S, Bengi F, Fowler J. Syrian rue tea: a recipe for disaster. Clin Toxicol. 2008;46:749-752. DOI: 10.1080/155.636.50701323205

[96] Mahmoudian M, Jalipour H, Dardashti PS. Toxicity of *Peganum harmala*: review and a case report. IJPT. 2002;1: 1-4.

How to cite this article: Gökkaya İ, Renda G, Subaş T, Özgen U. Phytochemical, Pharmacological, and Toxicological Studies on *Peganum harmala* L.: An Overview of the Last Decade. Clin Exp Health Sci 2023; 13: 664-678. DOI: 10.33808/clinexphealthsci.1125345