ORIGINAL ARTICLE

Ameliorative Effects of Olea europaea Oil on Morphine Withdrawal: Behavioural, Biochemical and Histological Evidence in Rats

Shariff Halim[a], Kara Rao LB[b], Sri Rejeki P[c], Indriati DW[de], Muhammad A[f], P. M. Ridzuan[g], Adam SH[h]

[a]Faculty of Health Sciences, Universiti Teknologi MARA Cawangan Pulau Pinang, Bertam Campus, Pulau Pinang, Malaysia.
[b]International Medical School, Management & Science University, Shah Alam, Selangor, Malaysia.
[c]Physiology Division, Department of Medical Physiology Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia.
[d]Department of Health, Faculty of Vocational Studies Universitas Airlangga, Surabaya, Indonesia.
[e]Research Center for Global Emerging and Re-emerging Infectious Disease, Universitas Airlangga, Surabaya, Indonesia.
[f]Faculty of Medicine, Universitas Muhammadiyah Purwokerto, Indonesia.
[g]Department of Research & Development, Dr. Ridz Research Centre, Kuala Nerus, Terengganu, Malaysia.
[h]Pre-clinical Department, Faculty of Medicine and Defense Health, Universiti Pertahanan Nasional Malaysia, Malaysia.

ABSTRACT

INTRODUCTION: Prolonged morphine use can trigger dependence and addiction leading to severe withdrawal symptoms upon cessation. Oxidative stress is a key factor in its pathogenesis. *Olea europaea* (olive) oil, rich in polyphenols and oleuropein, exhibits antioxidant, anti-inflammatory and neuroprotective properties. Thus, this study aimed to evaluate the effects of *Olea europaea* oil in alleviating morphine withdrawal symptoms in morphine-dependent rats. **MATERIALS AND METHODS:** A total of eighteen rats were randomly divided into three groups. The control group received normal saline, while the positive control group received intraperitoneal injections of morphine sulphate (2.5 mg/kg to 50 mg/kg) for seven days. The treatment group received the same morphine doses followed by oral administration of 250 mg/kg Olea Europaea oil for twenty-one days during morphine withdrawal period. Spontaneous morphine withdrawal behaviours of rats were observed. Subsequently the rats were sacrificed and the brain tissue was stained with H&E for histological markers. Blood serum and brain tissue were collected for Glutathione (GSH) level measurement using an ELISA kit. The findings were analysed using GraphPad Prism. **RESULTS:** The administration of Olea Europaea oil significantly reduced ($p<0.05$) spontaneous withdrawal symptoms. Findings show that Olea Europaea oil ameliorated histological brain signs of morphine toxicity and GSH levels in the brain tissue of the treated group were significantly higher ($p<0.05$) compared to the no-treatment group. **CONCLUSION:** This study demonstrates that Olea Europaea oil supplementation significantly alleviates morphine withdrawal symptoms and restores antioxidant capacity likely due to its potent antioxidant and neuroprotective properties.

Keywords
Antioxidant, Glutathione (GSH), Olea Europaea oil, Oxidative Stress, Morphine withdrawal

Corresponding Author
Ts. Dr. Mohamad Halim Mohamad Shariff
Faculty of Health Sciences, Universiti Teknologi MARA, Cawangan Pulau Pinang
Bertam Campus, Penang, Malaysia
E-mail: halimshariff@uitm.edu.my

Received: 26th September 2024; Accepted: 22th July 2025

Doi: https://doi.org10.31436/imjm.v24i04.2721

INTRODUCTION

Morphine is known for its potent analgesic effects in managing patients experiencing severe or chronic pain. However, repeated prolonged or abused administration of morphine can trigger the development of dependence and addiction, subsequently leading to a difficult withdrawal syndrome upon abrupt cessation.[1] Upon the cessation of chronic morphine use, patients can experience severe withdrawal symptoms including tremors, anxiety, muscle aches, sweating, gastrointestinal distress, and more.[2] Managing the withdrawal symptoms remain the most difficult challenge in treating opioid addiction.[2] Despite the pharmaceutical treatments used today such as methadone, buprenorphine, clonidine, and naltrexone are effective, they frequently have serious adverse effects that increase the risk of relapse.[3] Herbal medicines have drawn more attention in recent times as viable therapeutic choices for a range of medical ailments. Herbal remedies have long been used to relieve withdrawal symptoms, and

new studies suggest they could offer a promising alternative or addiction to conventional treatment. This strategy could improve patient outcome and lower the chance of relapse by effectively relieving symptoms with fewer side effects.[4]

Olea europaea commonly known as olive is an abundant tree along the Eastern Mediterranean coastline, Western Asia and Northern Iran near the Caspian Sea which has been traditionally used in folk medicine to treat a variety of conditions.[5] Due to its wide availability in local markets and low cost, *Olea europaea* oil presents a practical and economical option for therapeutic application. Emerging research demonstrates that *Olea Europaea* is enriched with a variety of polyphenolic antioxidants including oleuropein, hydroxytyrosol, tyrosol, and oleocanthal which can scavenge free radicals and attenuate oxidative stress implicated in multiple disease states. Several studies have linked oxidative stress to the development of both morphine tolerance and withdrawal.[6,7] However, limited studies are available on the effectiveness of *Olea Europaea* oil to mitigate morphine dependence and withdrawal symptoms by restoring redox homeostasis. Therefore, the current study aimed to analyse the efficacy of *Olea europaea* oil supplementation in alleviating morphine withdrawal symptoms using a rat model of morphine dependence.

MATERIALS AND METHODS

Olea europaea oil

Olea europaea oil was purchased from the local market in Selangor.

Animals

This study included eighteen (n=18) Sprague-Dawley rats weighing between 180-220 grams on average. The sample size is calculated using the following formula:

E = total number of animals – total number of groups

E = [6 x 3] – 3

E = 15

A sample size is deemed acceptable if it maintains E between 10 and 20. Thus, when the E is 15, the number of rats employed in this experiment is deemed appropriate.[8] The rats were kept in the animal house at Management & Science University under carefully monitored circumstances including 23 ± 1°C, a 12-hour light/dark cycle, a generally humid atmosphere (30%–40%) and unrestricted access to food and water. The rats were acclimatized for one week before the study begin.

Induction of morphine dependence rats

Eighteen (n=18) rats we exposed to morphine sulphate (10 mg/mL; Merck, Germany) to induce morphine dependency in rats. Based on a study conducted by Sabuee et al. with some modifications, morphine was delivered intraperitoneally twice daily for seven days with gradually increasing doses to induce dependency.[9] Morphine sulphate was administered incrementally from 2.5 mg/kg to a maximum dosage of 50 mg/kg. The doses were titrated as follows: Day 1–2.5 mg/kg, Day 2–5 mg/kg, Day 3–10 mg/kg, Day 4–20 mg/kg, Day 5–30 mg/kg, Day 6–40 mg/kg, and Day 7–50 mg/kg. When induction of morphine was completed, rats in the treatment group were given *Olea europaea* oil orally for 21 days. Withdrawal behaviour was observed on Days 1, 7, 14 and 21. After completing behavioural testing, the animals were sacrificed by cervical dislocation.

Animal grouping

The rats were divided into 3 groups consisting of 6 rats each as shown in Table I.

Table I: Animal grouping

Group	Exposure
Negative control (n=6)	Normal saline (200 µL/rat, i.p) daily for 7 days.
Positive control (n=6)	Morphine sulphate solution (i.p) twice daily for 7 days at incremental dose from 2.5 mg/kg up to 50 mg/kg, twice daily. The doses were titrated as follows: Day 1–2.5 mg/kg, Day 2–5 mg/kg, Day 3 – 10 mg/kg, Day 4–20 mg/kg, Day 5–30 mg/kg, Day 6–40 mg/kg, and Day 7–50 mg/kg. Withdrawal symptom observed for 21 days following morphine dependency.
Treatment group (n=6)	Morphine sulphate solution (i.p) twice daily for 7 days at incremental dose from 2.5 mg/kg up to 50 mg/kg, twice daily. The doses were titrated as follows: Day 1–2.5 mg/kg, Day 2–5 mg/kg, Day 3–10 mg/kg, Day 4–20 mg/kg, Day 5 – 30 mg/kg, Day 6–40 mg/kg, and Day 7–50 mg/kg. When morphine dependency has been established, oral Olea europaea oil (250 mg/kg) was once given daily for 21 days. Withdrawal symptom observed for 21 days following treatment.

Evaluation of spontaneous morphine withdrawal behaviour

Following the induction of morphine dependency, the rats were placed in cages for 30 minutes on Days 1, 7, 14 and 21, to observe signs of spontaneous morphine

withdrawal namely, i) wet dog shakes, i) writhing, iii) face grooming, and iv) sniffing. After allowing 5 minutes for the rats to acclimatise to the cages, the above-mentioned withdrawal signs were continuously monitored and recorded on video for 30 minutes and the scores were averaged for each behavioural test.

Measurement of antioxidant level by ELISA test

All 18 rats were sacrificed upon completing the study. An enzyme-linked immunosorbent assay (ELISA) (Elabscience, USA) kit was used to quantify glutathione (GSH) in the blood and brain tissue. Blood was drawn via cardiac puncture before sacrificed and centrifuged at 3000 rpm for 10 minutes. To measure GSH levels, the serum was isolated and kept at -80°C. The rat brain was removed for tissue analysis, and rinsed with ice-cold phosphate buffer saline (0.01 M, pH 7.4) to eliminate excess blood. The GSH levels in the supernatant were ascertained, using the GSH ELISA test kit following the manufacturer's instructions.

Haematoxylin and eosin (H&E) staining of rat tissue sections

To assess neuronal integrity, histological analysis focused on identifying morphological changes including neuronal degeneration, fragmentation, and hyperchromatic features particularly in the hippocampal CA1 region. These structural characteristics were evaluated as potential indicators of apoptosis associated with escalating morphine dosages. Brain tissues were fixed in 10% neutral phosphate buffered formalin then dehydrated and stored in liquid paraffin. Tissue samples were sectioned at 5 μm thickness, deparaffinized, rehydrated and staining with haematoxylin and eosin (H&E).

Data analysis

For each group, the data are shown as the mean ± SEM. One-way ANOVA was used for statistical analysis, and Tukey's post hoc test was used for pairwise comparisons of group means. A P-value of less than 0.05 was deemed statistically significant.

RESULTS

Effects of *Olea europaea* oil on Morphine withdrawal

The effects of morphine and *Olea europaea* oil on withdrawal were investigated by observing exposed animals following the cessation of morphine. Withdrawal behaviour namely i)wet dog shakes, ii)writhing, iii)face grooming, and iv)sniffing were scored over a 30-minute period. Based on the results, the group receiving *Olea europaea* oil (Treatment Group) showed a significant improvement in the morphine withdrawal signs as compared to the untreated group (Positive Control) ($p<0.05$), as depicted in Figure 1.

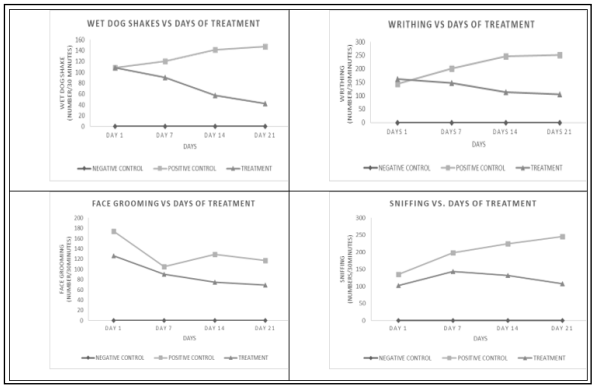

Figure 1: The effects of *Olea europaea* oil on morphine withdrawal signs after 21 days of morphine cessation. The data is expressed as the mean ± SEM.

Effects of *Olea europaea* oil on Glutathione (GSH) level in the brain and blood

An analysis of GSH concentration in the brain tissues and blood is displayed in Figure 2. The results demonstrate that the GSH levels in the brain tissue of the treatment group are significantly greater than those in the untreated group, and there is no significant difference in the GSH concentration of blood in all groups.

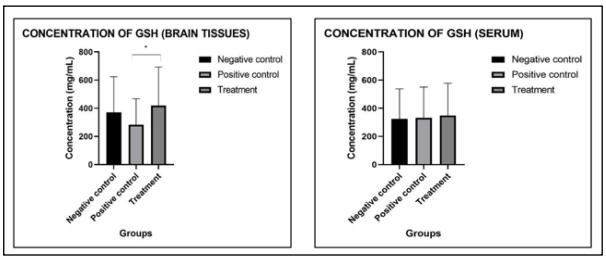

Figure 2: The effects of *Olea europaea* oil on GSH level in blood and brain tissue. Data are shown using the mean ± SEM.

Effects of *Olea europaea* oil on the histological changes in hippocampus

Figure 3 illustrates the effects of *Olea europaea* oil on the hippocampal region in brain sections. In this comparative histological study, the CA1 area of the hippocampus in the positive group exhibits tiny, fragmented hyperchromatic neurones during the examination of the effects of escalating morphine dosages (50 mg/kg), which suggests cellular death. In contrast, the group that received *Olea europaea* at 250 mg/kg showed noticeably reduced neuronal damage with more preserved neuronal morphology and fewer activated microglia, similar to the brain structure observed in the negative control group (saline only).

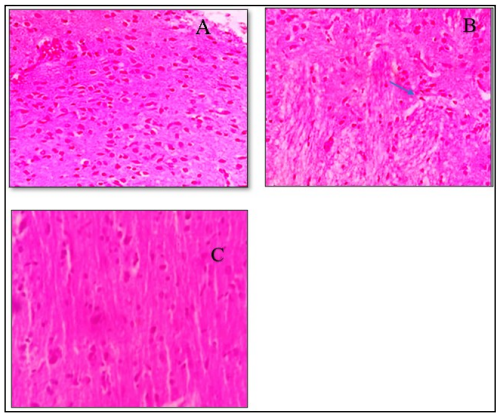

Figure 3: Histological section of neuron with haematoxylin and eosin at 40x magnification. (A) Negative control (normal saline only), (B) Positive control group (morphine sulphate only), (C) Treatment group (morphine sulphate and *Olea europaea* oil -treated with 250mg/kg). Neurons that have degenerated can be identified by their shrunken appearance, with condensed and darkly stained nuclei (blue arrow). The microglia present in this region are characterized by their small size, featuring elongated nuclei surrounded by minimal cytoplasm.

DISCUSSION

Effects of *Olea europaea* oil on morphine withdrawal

In agreement with our previous studies, administering daily intraperitoneal injection of increasing dosage until the maximum of 50 mg/kg morphine was effective in sustaining rats in a state of morphine dependence at Day 7.[7] Small modifications were made to the morphine dependence model originally described by previous study.[9] In the previous study, rats were treated with morphine at a higher dosage of 180 mg/kg on Day 6. However, in this study, rats that had been treated at Day 7 with a lower dosage (50 mg/kg) were enough to elicit the full spectrum of withdrawal signs. Reducing both the duration of treatment and the dosage significantly contributed to a notable decrease in the mortality rate. Withdrawal symptoms became evident around 20 hours after the last morphine dose. In these recent investigations, various indicators of morphine withdrawal were employed to verify the physical dependence of rats on morphine. These included alterations in well-characterised and commonly observed behaviours associated with withdrawal, namely i)wet dog shaking, ii)writhing, iii)face grooming, and iv)sniffing. Rats that had been deprived of morphine for 20 hours displayed other characteristic signs of opioid withdrawal, such as physical withdrawal symptoms. Notably, these withdrawal indicators remained consistent throughout the duration of the study. The present study evaluated the impact of *Olea europaea* oil on the symptoms of morphine withdrawal syndrome in rats, using dosages of 250 mg/kg of the oil. Results showed that dosages of 250 mg/kg of *Olea europaea* oil decreased the amount of withdrawal-related wet dog shakes. Also, this dose reduced the number of writhing, sniffing, and face grooming due to withdrawal syndrome significantly.

Studies had demonstrated that calcium channel blockers, which function via mechanisms involving both the central and peripheral neural systems could mitigate the symptoms associated with morphine withdrawal.[10] A previous study has demonstrated that T-type voltage-gated calcium channels played a crucial role in the development of morphine dependence and the manifestation of withdrawal symptoms.[11] It was proven that the symptoms of morphine withdrawal were lessened by calcium channel blockers which inhibited L-type voltage-gated calcium channels.[12] Another researcher had found that in rats, mecamylamine-precipitated nicotine withdrawal syndrome was lessened by calcium channel blockers. The development of tolerance and dependence as well as the antinociception induced by morphine and nicotine appeared to have been mediated by comparable calcium-dependent pathways. In the current study, we proposed *Olea europaea* oil could alleviate morphine withdrawal syndromes owing to its calcium channel blocking properties. Studies had revealed that olive leaf extract blocked calcium channels. This effect of the leaf extract from *Olea europaea* oil had been connected to oleuropein, which is the primary component of the extract.[13] Oleuropein, which made up 35.6% of the extract was likewise the main ingredient of olive leaf

extract according to high-performance liquid chromatography (HPLC) studies.[14]

Antioxidant

Changes in the cellular antioxidant state revealed the oxidative stress induced by morphine in specific brain areas. Further research had demonstrated that morphine reduced the hippocampal enzymatic activity of glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx).[15,16] Results from this study showed that the quantities of GSH were much lower in the brains of rats that were given morphine alone (Positive control group) which was consistent with another research. This was because the administration of morphine caused an excessive number of free radicals to be produced, which led to oxidative stress and a reduction in antioxidant status. Thus, the levels of antioxidants like GSH were substantially diminished, signalling their depletion in neutralising free radicals.[17] Morphine could alter intracellular GSH levels, which could therefore affect cellular redox state, S-adenosylmethionine levels, and ultimately global DNA methylation.[18]

A growing amount of research indicated that oxidative stress contributed to the development of addiction to a variety of drugs, including morphine and methamphetamine.[19,20] In addition to causing the generation of free radicals, such as reactive oxygen species (ROS) or reactive nitrogen species (RNS), morphine, which was known to activate opioid receptors, also reduced the actions of antioxidants in target cells. Rats that were dependent on morphine demonstrated increased oxidative stress in the hippocampus and prefrontal cortex.[21] The ratio of pro-oxidants to antioxidants determined the oxidative state of cells. Reactive oxygen species (ROS), another name for pro-oxidants, were divided into two groups: radicals and nonradicals. Radicals were very reactive because they could easily accept or give electrons in order to stabilize. Excess ROS was produced under oxidative stress in cells, and this excess ROS has the ability to oxidize DNA, lipids, and proteins. Damage to organs and cell death resulted from this oxidation. The present results indicated an elevation in brain GSH levels in response to the influence of morphine. This observation aligned with previous evidence demonstrating that *Olea Europaea* oil had the capacity to boost GSH levels.[22]

The protective ability of *Olea europaea* oil came from its polyphenols, which were known to be highly antioxidant. These polyphenols could neutralise ROS and other free radicals by donating electrons to them, hence protected cells from being damaged by the attack of ROS and free radicals.[23,24] Phenolic acids, phenolic alcohols, flavonoids, and secoiridoids, were the four primary categories of phenolic chemicals identified in olive fruit. While many fruits and vegetables from other plant groups included phenolic acids, alcohols, and flavonoids, only the Oleaceae family contained secoiridoids. Many phenolic acids, including vanillic, syringic, caffeic, ferulic, p-coumaric, gallic, p-hydroxybenzoic, protocatechuic, and sinapic acids, were found in olive fruit. Tyrosol [(p-hydroxyphenyl) ethanol] and hydroxytyrosol [(3,4-dihydroxyphenyl) ethanol] were the most prevalent phenolic alcohols in olive fruit.[25] Flavonoids found in olive fruit include anthocyanins like cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside, as well as flavonol glycosides such luteolin-7-glucoside and rutin.

Histopathology

The histological examination of the hippocampus after administering morphine (50 mg/kg) revealed signs of cellular apoptosis or programmed cell death. Particularly, in the hippocampal CA1 region, tiny, broken up, and hyperchromatic neurons were seen, which was indicative of neuronal death. *Olea europaea* oil treatment, however, did not result in these apoptotic alterations, indicating that *Olea europaea* oil could inhibit morphine-induced neuronal death. Apoptosis, or cell death, in nerve cells could be induced by amphetamines and heroin, as multiple investigations had shown. It had been established by earlier studies that these medications might have caused differentiated neurons to undergo apoptosis, which could have led to changes in intercellular dopamine levels.[26] It had been demonstrated that long-term or chronic morphine usage causes had caused oxidative stress, inflammation, and death in brain neurons,

especially in the hippocampus region of lab animals.[27]

It had been suggested that the antioxidant polyphenol oleeuropein which was present in olive leaf extract scavenged free radicals. Oleuropein was a phenolic molecule that had been shown in several studies to have a variety of pharmacological actions, including antioxidant, hypotensive, vasodilatory, anti-inflammatory, and neuroprotective activities.[28-30] Furthermore, studies had demonstrated that oleuropein administration could have reduced oxidative stress and cognitive impairment caused by specific anaesthetic medications in the rat CA1 area of the hippocampal hippocampus.[31] Oleuropein or olive leaf extract has been demonstrated in a number of earlier investigations to have anti-apoptotic effects in a variety of cell types, especially in the presence of oxidative stress. Furthermore, oleuropein administration following spinal cord injury has been shown to have a neuroprotective effect in rats, as shown by an increase in glutathione levels and a decrease in several apoptotic markers (Bax/Bcl2 ratio).[32] According to previous study, oleuropein had lowered cleaved caspase-3 activation and the Bax/Bcl2 ratio, which in turn lowers the production of reactive oxygen species (ROS) and apoptosis in an in vitro model of Parkinson's disease.[33] Additionally, studies have demonstrated that oleuropein had neuroprotective effects against colchicine-induced cognitive impairment in the rat hippocampus CA1 region. These effects had been attained via lowering oxidative stress and activating caspase-3.[28]

CONCLUSION

Our finding demonstrates that *Olea europaea* oil markedly alleviated withdrawal symptoms while augmenting cerebral antioxidant activity. Histological study revealed a decrease in apoptotic cells following 21 days of treatment. While *Olea europaea* oil exhibits therapeutic potential for detoxification syndrome, its underlying mechanisms require additional exploration.

CONFLICT OF INTEREST

The authors declare no conflict of interest

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

All animal studies were performed according to the guidelines endorsed by the Ethics Committee of MSU [MSU-RMC-02/FR01/08/L3/108].

ACKNOWLEDGEMENTS

We the authors of this submission would like to thank International Medical School, MSU and UiTM Cawangan Pulau Pinang Bertam Campus for their support.

REFERENCES

1. Quezada M, Ponce C, Berríos-Cárcamo P, et al. Amelioration of morphine withdrawal syndrome by systemic and intranasal administration of mesenchymal stem cell-derived secretome in preclinical models of morphine dependence. CNS Neurosci Ther. 2024;30(4): e14517.
2. Kosten TR, Baxter LE. Effective management of opioid withdrawal symptoms: A gateway to opioid dependence treatment. Am J Addict. 2019;28(2):55-62.
3. Sofuoglu, M., DeVito, E. E., & Carroll, K. M. Pharmacological and behavioral treatment of opioid use disorder. Psychiatric Research and Clinical Practice.2018; 1(1), 4-15.
4. Kamali M, Tajadini H, Mehrabani M, Moghadari M. Consequences of opioid abuse and their treatments in Persian medicine: A review study. Addict Health. 2020;12(1):46.
5. Rashed K. Phytocontent and Biological Effects of Olea Europaea L.: A Review. Plantae Scientia. 2022;8;5(2):36-44.
6. Zeng, X. S., Geng, et al. Morphine addiction and oxidative stress: The potential effects of thioredoxin-1. Frontiers in pharmacology.2020; 11: 82.
7. Halim S, Jasmi NA, Ridzuan PM, Anna D, Abdullah S, Sina T. Novel potential Centella asiatica extract in ameliorating neurotoxicity induced oxidative stress in chronic morphine dependant rat model. International Journal of Medical Toxicology & Legal Medicine. 2020;23(3and4):79-83.

8. Jeppu, A. K., Halim, et al. Renal protective effect of Trigonella foenum-graecum seed extract on morphine withdrawal in rats. Biomedicine. 2022; 42 (6), 1172-1177.
9. Sabuee, S., Ahmadi-Soleimani, S. M., & Azizi, H. Prolonged morphine exposure during adolescence alters the responses of lateral paragigantocellularis neurons to naloxone in adult morphine dependent rats. The Journal of Physiological Sciences.2021; 71 (1): 25.
10. Bozorgi, H., Pour, A. R., et al. (2023). Relief of Morphine Withdrawal Symptoms by Japanese Sake Yeast Supplement (Saccharomyces cerevisiae sake) Through Adenosine A1 Receptor Activation in Mice. Middle East Journal of Rehabilitation and Health Studies. 2023; 11(3).
11. Ahmadi, S., Majidi, et al. Alterations in the expression of calcium channels and neurotrophic factors in the cerebellum are linked to the induction of morphine dependence and withdrawal. Jentashapir Journal of Cellular and Molecular Biology.2024; 15(2).
12. Mishra, P. R., Barik, M., & Ray, S. B. Effect of nimodipine on morphine-related withdrawal syndrome in rat model: An observational study. Journal of Pediatric Neurosciences.2017;12 (1):7-14.
13. Zare, L., Esmaeili-Mahani, S., et al. Oleuropein, chief constituent of olive leaf extract, prevents the development of morphine antinociceptive tolerance through inhibition of morphine-induced l-type calcium channel overexpression. Phytotherapy Research. 2012; 26(11): 1731-1737.
14. Mohagheghi, F., Bigdeli, et al (2011). The neuroprotective effect of olive leaf extract is related to improved blood–brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia. Phytomedicine. 2011; 18(2-3): 170-175.
15. Abbasi, E., Mirzaei, F., et al. Effects of opium on hippocampal-dependent memory, antioxidant enzyme levels, oxidative stress markers, and histopathological changes of rat hippocampus. Biochemistry and Biophysics Reports. 2025; 42: 101993.
16. Ma, J., Zou, L., Lou, et al. 20-Deoxyingenol attenuate morphine-induced hippocampus neurotoxicity and memory impairments in rats. Heliyon. 2024;10(11).
17. Chaudhary P, Janmeda P, Docea AO et al. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in chemistry. 2023;10; 11:1158198.
18. Trivedi M, Shah J, Hodgson N, Byun HM, Deth R. Morphine induces redox-based changes in global dna methylation and retrotransposon transcription by inhibition of excitatory amino acid transporter type 3 –mediated cysteine uptake. Molecular pharmacology. 2014;1;85(5):747-57.
19. Halim S, Sina T, Ridzuan PM, et al. Antioxidant activities of barringtonia racemosaleaf extract against acute morphine dependence rat model. International Journal of Medical Toxicology & Legal Medicine. 2020;23(3and4):67-72.
20. Halim S, Roslan NQ, Jasmi NA, Oosterhout DA. Elucidating the effects of Olea Europaea in eliminating neurotoxicity in methamphetamine (Meth) induced rat. International Journal of Medical Toxicology & Legal Medicine. 2021;24(1and2):10-8.
21. Famitafreshi H, Karimian M. Socialization alleviates burden of oxidative-stress in hippocampus and prefrontal cortex in morphine addiction period in male rats. Current molecular pharmacology. 2018;1;11(3):254-9.
22. Sarbishegi M, Charkhat Gorgich EA, Khajavi O, Komeili G, Salimi S. The neuroprotective effects of hydro-alcoholic extract of olive (Olea Europaea L.) leaf on rotenone-induced Parkinson's disease in rat. Metabolic Brain Disease. 2018; 33:79-88.
23. Halim S, Ismail UU. Potential therapeutic effects of Olea Europaea (olive) fruit oil as neuroprotective agent against neurotoxicity induced opioid. J Cell Mole Anesthes. 2018; 1;3(4):159-.
24. Kouka P, Chatzieffraimidi GA, Raftis G et al. Antioxidant effects of an olive oil total polyphenolic fraction from a Greek Olea Europaea variety in different cell cultures. Phytomedicine. 2018;1; 47:135 -42.
25. Tekaya, M., Chehab, H., et al. Study of phenolic composition of olive fruits: validation of a simple and fast HPLC-UV method. OCL. 2022; 29: 35.

26. Suliman, N. A., Abu Bakar, N. A., & Othman, Z. A. Molecular Basis for Morphine Addiction. Malaysian Journal of Medicine & Health Sciences. 2023; 19:89-97.
27. Shibani F, Sahamsizadeh A, Fatemi I et al. Effect of oleuropein on morphine-induced hippocampus neurotoxicity and memory impairments in rats. Naunyn-Schmiedeberg's archives of pharmacology. 2019; 392:1383-1391.
28. Kouhakan, F., Ganjalikhan, S., Sarhadizadeh, A. et al. Oleuropein can Protect the Brain Against Deleterious Effects of Bile Duct Ligation in Male Mice. OBM Neurobiology. 2024; 8(2): 1-11.
29. Nediani C, Ruzzolini J, Romani A, Calorini L. Oleuropein, a bioactive compound from Olea Europaea L., as a potential preventive and therapeutic agent in non-communicable diseases. Antioxidants. 2019;22;8(12):578.
30. Ivanov M, Vajic UJ, Mihailovic-Stanojevic N et al. Highly potent antioxidant Olea Europaea L. leaf extract affects carotid and renal haemodynamics in experimental hypertension: The role of oleuropein. EXCLI Journal. 2018; 17:29.
31. Alirezaei M, Rezaei M, Hajighahramani S, Sookhtehzari A, Kiani K. Oleuropein attenuates cognitive dysfunction and oxidative stress induced by some anesthetic drugs in the hippocampal area of rats. The journal of physiological sciences.2017; 67:131-139.
32. Singh, R., Zahra, W., et al. Oleuropein confers neuroprotection against rotenone-induced model of Parkinson's disease via BDNF/CREB/Akt pathway. Scientific reports. 2023; 13(1): 2452.
33. Gonçalves, M., Vale, N., & Silva, P. Neuroprotective effects of olive oil: A comprehensive review of antioxidant properties. Antioxidants. 2024; 13(7): 762.