Research article

The effect of *Mentha piperita* oil in improving serotonin level, physiological factors, antioxidants and fertility markers in male rabbits on opioid oxycodone withdrawal

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ABSTRACT

Introduction and Aim: Medicinal plants have shown to be effective in treatment of different stages of addiction with lower side effects, including reducing the severity of withdrawal symptoms that occur when an opioid-dependent patient stops using opioids. The current study aimed to evaluate the effective role of *Mentha piperita* oil on alleviating the symptoms of oxycodone withdrawal, and to elucidate the mechanisms by which it does so.

Materials and Methods: Twenty-five healthy male rabbits divided into 5 groups (G1-G5) consisting of 5 rabbits each. The groups were divided as follows: G1-Control Group: G2-Oxycodone: G3-Oxycodone withdrawal: G4-Oxycodone withdrawal + *M. piperita*: G5-Oxycodone withdrawal +*M. piperita*. Blood was drawn from each animal and the serum obtained was analyzed for physiological (cortisol, serotonin, dopamine, CRP and T, LH levels), antioxidant parameters (LDH, SOD, GSH and MDA) and sperm activity.

Results: This study found statistically significant changes in most variables. G2 rabbits had higher serotonin and dopamine levels, higher body weight, and lower cortisol and CRP levels than the healthy control group G1. Sperm activity, efficacy, testosterone, luteinizing hormone, superoxide dismutase, glutathione, and malondialdehyde did not alter across generations (G2 and G1). Group 3 showed that oxycodone withdrawal negatively affected dopamine, testosterone, LH, SOD, GSH, and sperm activity with weight, compared to Groups 1 and 2. Without therapy, rabbits in G3 with fast withdrawal had considerably higher cortisol, CRP, LDH, and MDA than G2 and G1. Serotonin level decreased in G3 compared with G1 and G2. An improvement in most of the studied variables, stress factors and antioxidants, as well as fertility indicators in the G4 and G5 improved gradually depending on period of treatment with *M. piperita*.

Conclusion: *M. piperita* being rich in antioxidants can be adopted as one of the natural plant-based compound in patients undergoing oxycodone addiction withdrawal.

Keywords: Mentha piperita oil; oxycodone; serotonin; cortisol; antioxidant activity; sperm activity.

INTRODUCTION

Low doses of opioids produce drowsiness, but excessive doses can cause respiratory depression and cardiac arrest, potentially leading to death. Opioids are commonly prescribed to treat severe pain; nevertheless, the pleasurable feelings that users report after using the drug might increase their desire to use it again and again, which can lead to addiction. Opioids are commonly utilized as psychoactive chemicals all over the world. Some examples of opioids are morphine, heroin, oxycodone, codeine, methadone, and hydromorphone hydrochloride. Users who use these opioids for an extended period of time run the risk of developing a dependent on the substance despite the fact that these medications relieve pain, calm the user's mind, and provide euphoric feelings (1). Oxycodone is one of the opioids, and it is a highly effective, semi-synthetic opioid that is used in medicine to relieve severe pain. Despite this, it has a significant potential for addiction and is a common narcotic substance (2).

Opioid withdrawal syndrome is a disorder that can be life-threatening that is brought on by opioid addiction. Opioid addiction has repercussions not just for the individual who uses drugs but also for society as a whole. It contributes to an increase in the costs of medical care, as well as in rates of unemployment, tardiness, and early death. Opioid withdrawal, also known as detoxification, is the process in which a patient who is dependent on opioids stops taking opioids and begins to experience withdrawal symptoms. Medication is used in this process to minimize the intensity of withdrawal symptoms (3,4).

Analgesic, antispasmodic, and neurotonic effects can be found in the herb *Mentha piperita*, which belongs to the family Lamiaceae and is a perennial herb. Carvacrol and flavonoids found in *M. piperita* have analgesic effects, meaning they reduce pain by

activating kappa-opioid receptors and blocking the transfer of pain signals (5). In addition, menthol, menthone, menthofuran, isomenthone, (E) -caryophyllene, 1,8-cineole, linalool, limonene, carvone, pulegone, and terpineol are all examples of mono-sesqui-terpenes that are produced by plants and have a nice odor (6). There is an abundance of essential oils, enzymatic antioxidants, and non-enzymatic antioxidants within this plant family. All three types of antioxidants are known to have neurocognitive effects, and they do this by activating the body's natural antioxidant defense mechanism (7). Evidence also suggests that *M. piperita* volatile constituents have psychotropic properties. *M. piperita* oil is considered an opioid antagonist and shown to rapidly reverse an opioid overdose. Hence in this study we aimed to investigate the withdrawal effect of *M. piperita* oil on the physiological and psychological aspects of animals dependent on the opioid oxycodone.

MATERIALS AND METHODS

Experimental animals

In the course of the research that took place between August 8, 2020 and January 25, 2021, male rabbits that had been grown in the animal house of the Department of Biology in the College of Education at the University of Kirkuk in Iraq were used. All experimental animals were kept in cages measuring 100×80×50 cm and housed in a room (4×5 m) with an ambient temperature of 25-27^{o}C for four to six weeks. Each cage was cleaned and sterilized twice a week. The rabbits were fed with special food containing corn (25%), animal protein (10 %), dried milk (10%), and food salt (1%).

In the course of the experimental research, there were a total of 25 healthy male rabbits used, and these rabbits were each assigned to one of five groups (G1–G5). The following is a description of each of the five experimental groups:

G1: Control group: administration with standard food, water with 0.9% normal saline
G2: Oxycodone group: administration with (10 mg/kg /day) for four weeks
G3: Oxycodone withdrawal group: Animals received no dose for the first three days, after which they were given oxycodone (10 mg/kg/day) for four weeks
G4: Oxycodone withdrawal + *M. piperita* group: Male rabbits given *M. piperita* (200 mg/kg body wt.) in combination with oxycodone for four weeks
G5: Oxycodone withdrawal + *M. piperita* group: Male rabbits given *M. piperita* (200 mg/kg body wt.) in combination with oxycodone for six weeks

All animals were dosed orally (daily for 4-6 weeks) using a special oral-pharyngeal cannula. Oxycodone dose 10 mg/kg was based on the Human Equivalent Dose, while dose of *M. piperita* were (200 mg/kg body wt., which is as reported in our previous study. After the end of the prescribed experimental period, the treated and controlled male rabbits were sacrificed after starving for a period of 10-11 hours and 5 ml of venous blood was withdrawn. After separating the serum using centrifugation at a rate of 3000 revolutions per minute for ten minutes, the serum was stored at a temperature of 0°Celsius so that physiological and hormonal tests could be carried out on it.

The blood samples obtained from each experimental group were subjected to determination of serum

- Cortisol via making use of an ELISA kit, which is a device that was developed for the precise measurement of cortisol in animal blood serum (8).
- Serotonin: Serotonin level by Assay Kit (9)
- CRP: determined by Assay Kit (10)
- Glutathione levels were determined by adapting a method used by Sedlak and Lindsay (11)
- Malondialdehyde: Assessment of plasma peroxidation levels (12)
- Testosterone: Assessment of testosterone in blood serum (13)
- LDH: Assessment of lactate dehydrogenase in blood serum (14)
- Dopamine: Dopamine was measured by commercial enzyme-linked immunosorbent assay (ELISA). Dopamine (DA) was purchased from Sigma-Aldrich- USA) and
- Estimation of sperm activity in studied groups (15)

Statistical analysis

The results of a statistical analysis carried out with SPSS (version 25, IBM Corporation, USA) on the collected data are reported in terms of the mean and standard error. The Duncan Multiple Range test was utilized in order to do statistical analysis on the differences in group means. When p was less than 0.05, we regarded the differences in mean values of the various parameters to be statistically significant.

RESULTS

Estimation of cortisol, serotonin, dopamine, CRP levels and body weight

According to our findings, there was a significant decrease in cortisol and CRP concentrations in G2 when compared to the control group, when compared to the control group, both cortisol and CRP levels were shown to be higher in the experimental group G3. Comparing data from groups G4 and G5 with those from G3, it can be seen that both cortisol and CRP levels in rabbits that were given *M. piperita* decreased (Table 1). Both dopamine levels and body weight significantly increased in Group 2, while dopamine levels dramatically fell in Group 3, when the two groups were compared to the control group.

There was no discernible variation in the levels of dopamine in G4, however there was an increase in both dopamine levels and body weight in G5 in comparison to G3. When compared to group G1 in the control population, serotonin levels considerably increased in group G2, but they fell in group G3. The levels of serotonin in Groups 4 and 5 were higher when compared to Group 3, but they were unchanged when compared to the Control Group 1 (Table 1).

Estimation of TLH, LDH, SOD, GSH and MDA in experimental groups

The levels of testosterone, luteinizing hormone (LH), superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) in group G2 were not significantly different from the levels in group G1, and the levels of LDH decreased substantially when compared to the control group (Table 2). When compared to the control group, group G3 had significantly lower levels of testosterone, LH, SOD, and GSH, but higher levels of LDH and MDA. In comparison to G3, administration of *M. piperita* (G4 and G5 group) showed a significant increase in testosterone, LH, SOD GSH levels and a significant decrease in LDH and MDA level (Table 2).

Estimation of sperm activity in experimental groups

According to the results of our research, there was no discernible difference between groups G1 and G2 in terms of the concentration or activity of sperm. In contrast, in the G3 group, which included the rabbits that were going through oxycodone withdrawal, there was a significant decrease in sperm activity, as measured by sperm concentration, sperm motility, sperm viability, and sperm grade motility. This was in comparison to the group that served as the control. Comparing G3 with G4, there was not a significant difference in the effect that *M. piperita* had on the activity of sperm in rabbits. In group G5, the majority of the sperm activity markers exhibited a considerable rise with time, in contrast to the activity of the sperm in group G3 (Table 3).

Table 1: Levels of cortisol, serotonin, dopamine, CRP and body weight estimated in experimental groups

Parameter / Groups	Cortisol µg/dl	Serotonin ng/ml	Dopamine pg/ml	CRP Mg	Body weight (g)
G1	4.9±0.31^{bc}	92± 14.2^{ab}	26.2± 4.17^{ab}	4.05±0.6^{b}	985±26^{c}
G2	2.6±0.17^{c}	112± 15.7^{a}	52.9± 12.02^{a}	3.01±0.6^{b}	2300±38^{a}
G3	28.0±4.40^{a}	80± 15.6^{b}	10.5± 2.03^{b}	8.2±2.5^{a}	1047±15^{b}
G4	22.2±4.01^{ab}	85±11.80^{b}	10.7± 2.52^{b}	4.1±2.0^{b}	1450±15^{ab}
G5	10.1±2.68^{b}	91± 16.2^{ab}	28.5± 4.57^{ab}	4.07±0.8^{b}	1980 ±21^{ab}

'a' and 'b' show significant difference at the level ($p \leq 0.05$)

Table 2: Levels of testosterone, LH, LDH, SOD, GSH and MDA in experimental groups

Parameter / Groups	Testosterone ng/ml	LH IU/L	LDH	Superoxide dismutase	GSH µmol/L	MDA µmol/L
G1	2.92 ±1.01^{a}	3.53 ±1.3^{a}	166 ±8.5^{bc}	12.4 ±1.8^{a}	10.5 ±2.5^{a}	5.33 ±0.8^{b}
G2	2.65 ±1.06^{a}	2.09 ±1.1^{ab}	155 ±8.1^{c}	12.2 ±1.5^{a}	10.3 ±2.4^{a}	5.24 ±0.8^{b}
G3	0.20 ±0.72^{b}	0.01 ±1.2^{b}	304 ±25.0^{a}	2.9 ±0.3 ^{b}	1.0 ±1.10^{c}	19.3 ±3.6^{a}
G4	0.25 ±0.70^{b}	0.08 ±2.21^{b}	244 ±20.8^{ab}	5.8 ±0.5 ^{b}	4.6 ±1.90^{b}	14.5 ±1.5^{b}
G5	1.13 ±0.78^{ab}	0.46 ±1.4^{b}	207 ±20.1^{b}	8.5 ±0.7^{ab}	9.0 ±1.90^{ab}	11.1 ±1.8^{b}

'a' and 'b' show significant difference at the level ($p \leq 0.05$)

Table 3: Estimation of sperm activity in experimental groups

Parameters / Groups	Sperm Concentration	Percentage of sperm motility %	Percentage of Normal sperm	Percentage of sperm viability	Sperm Grade Motility
G1	84.5 ±9.29^{a}	85 ±12.12^{a}	77.3 ±3.46^{a}	72 ±10.22^{a}	2.3 ±0.77^{a}
G2	83.2 ±9.03^{a}	84 ±10.17^{a}	75 ±3.09^{a}	70 ±10.01^{a}	2.0 ±0.61^{a}
G3	40.2 ±4.11^{b}	50 ±3.11^{b}	40.8 ±1.95^{b}	28 ±2.21^{b}	1.01 ±0.01^{b}
G4	40.4 ±425^{b}	50 ±3.12^{b}	40.5 ±3.51^{b}	30 ±2.70^{b}	1.01 ±0.04^{b}
G5	48.7 ±4.20^{ab}	59 ±3.22^{b}	49.01 ±3.11^{b}	38 ±2.62^{b}	1.00 ±0.10^{b}

'a' and 'b' show significant difference at the level ($p \leq 0.05$)

DISCUSSION

Chronic opioid dependence and addiction can result in brain abnormalities which hampers basic life functions (16).

Serotonin and dopamine are both neurotransmitters. While serotonin is known to influence emotions, appetite, and sleep in humans, dopamine is linked to feelings of reward and motivation. Results in this study showed the serotonin and dopamine levels to increase when animals were administered with the opioid oxycodone which agrees with earlier studies that have shown the levels of these hormones are greatly affected by opioid consumption (17, 18). On the other hand, Patients who are going through the process of drug substance withdrawal, on the other hand, show a significant and ongoing increase in their cortisol levels (19). An increase in cortisol levels was also observed throughout the course of this research, despite the fact that these levels significantly dropped after treatment with *M. piperita*. Animals treated with *M. piperita* exhibited lower levels of stress-induced anxiety than untreated animals did. The administration of an extract of *M. piperita* leaves for a period of five weeks was sufficient to reverse the impact, which manifested as a reduction in the levels of circulating plasma corticosterone and an increase in the levels of serotonin and dopamine. Our findings agree with those of an earlier study that showed how *M. piperita* oil, when taken orally for a period of four weeks, can reverse the effect of stress on plasma corticosterone levels as well as the brain's serotonin and dopamine metabolism (20).

In addition, the levels of testosterone, luteinizing hormone (LH), lactate dehydrogenase (LDH), superoxide dismutase (SOD), and glutathione (GSH) were shown to be significantly reduced in the rats treated with *M. piperita* for opioid withdrawal. The findings of this study are consistent with those of a prior study that found the phytochemicals in *M. piperita* to function by scavenging free radicals created by medicines, hence reducing the rate at which free radicals are formed (21).

Across a wide range of different chemical tests, *M. peperita* demonstrated a level of anti-oxidative activity that was consistent with what was anticipated. These results are consistent with those of other research that have related mint's antioxidant activity to its primary monoterpenoids, such as menthol, menthone, carvone, and 1,8-cineole. Previous studies have linked mint's antioxidant activity to its primary monoterpenoids (22,23). Other minor components of mint with active methylene group chemistry, such as terpinolene, -, and -terpinene, have been found to have significant antioxidant activity in addition to gamma-tocopherol, which is the most well-known of these compounds. It has been demonstrated that the menthol and menthone that are present in the essential oil of *M. piperita* have beneficial effects on both mental exhaustion and cognitive performance (23).

Results for sperm activity in this study is in agreement with earlier studies by Luo *et al.*, (24) and Soltani *et al*., (25), who report that the essential oils in *M. peperita* are a vital substrate for the sperm through the oxidation of fatty acids as a source of energy, which contributes to increasing the effectiveness of the sperm and their maturation as well as in maintaining their concentration, composition and activity (24, 25). It can be recommended to apply *M. peperita* in the treatment of anti-testicular antibodies in wide ratio of infertile males (26). Thus, our study shows a positive role for *M. peperita* oils, in withdrawal of opioid oxycodone addiction.

CONCLUSION

According to the findings of the study, *M. piperita* plays a significant role in lowering oxycodone addiction withdrawal symptoms and can be used as one of the natural plant-based chemicals for this purpose.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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