https://doi.org/10.48047/AFJBS.7.1.2025.398-409

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Acute Toxicity Study of *Mentha suaveolens* Ehrh Aqueous Extract in Swiss Albino Mice

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Volume 7, Issue 1, Jan 2025

Received: 15 Nov 2024

Accepted: 20 Dec 2024

Published: 05 Jan 2025

doi:10.48047/AFJBS.7.1.2025.398-409

ABSTRACT

The genus Mentha L. (Lamiaceae) is distributed all over the world and can be found in many environments. The study evaluated the acute toxicity of the aqueous extract from the leaves of *Mentha suaveolens* in Swiss mice. The mice were administered single oral doses of 2000 mg/kg and 5000 mg/kg and were monitored for death and body weight for the first four hours, as well as over a period of 24 hours and at least once daily for 14 days. The results indicated that the LD_{50} (lethal dose at which 50% of the population dies) was found to be greater than 5000 mg/kg, suggesting that the plant extract is relatively safe. Furthermore, there were no significant variations in body and organ weights between the control group and the group treated with the extract after 24 days of treatment. Biochemical analysis of the mice showed no differences in the examined parameters. Additionally, histological examination did not reveal any changes in the organs of the treated mice compared to the control group. These findings indicate that the aqueous extract demonstrated drug safety in mice. These results suggest that the aqueous extract of *Mentha suaveolens* is safe for use in mice.

Key words: *Mentha suaveolens*, aqueous extract, acute toxicity, biochemical analysis, body weight.

INTRODUCTION

Medicinal plants and their derived compounds (phytochemicals) have been considered of pharmacological significance since ancient times. The use of plants in medicine dated back to 60,000 years ago, before the birth of civilization [1].Use of these natural antimicrobials for food preservation. This is due to the strong consumer demand for more natural foods that still possess a long shelf-life. Moreover, there are new concerns about the increasing occurrence of new food-borne disease outbreaks caused by pathogenic microorganisms [2]. Antioxidants are compounds that can delay, inhibit, or prevent the oxidation of oxidizable matters by scavenging free radicals and diminishing oxidative stress. Plants contain a wide variety of antioxidant phytochemicals or bioactive molecules, which can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress and reactive oxygen species (ROS). The intake of natural antioxidants has been associated with reduced risk of cancer, cardiovascular disease, diabetes and diseases associated with ageing. Studies on dietary free radical scavenging molecules have attracted the attention to characterize phenolic compounds and other naturally occurring phytochemicals as antioxidants [3]. Phenolics belong to an important class of compounds responsible for the antioxidant activity of mint. Previous studies have found the total polyphenolic content of peppermint leaves to be approximately 19–23% with total flavonoids of 12% [4].

Mentha is a genus belonging to the family Lamiaceae, whose plants are among the most aromatic and spread in diverse environments worldwide [5]. , having simple, characteristic leaves with pleasant scent. Mentha taxonomy is highly complicated and includes about 42 species and 15 hybrids, with hundreds of subspecies and cultivars [6]. Among its species include Mentha suaveolens which located in North Africa, Europe, America, and Japan [7]. *Mentha* spp. have been utilized for culinary applications for many years [8]. These groups of plants are mainly used to cure gastrointestinal complaints, however, their medical effectiveness is broad [9]. Moreover, the consumption of teas made from parts of Mentha spp. has many benefits to human health. In folk medicine the plant is used to treat nausea bronchitis, anorexia, ulcerative colitis and liver problems [10]. This plant is known to possess anti-inflammatory, antiemetic, antispasmodic and analgesic properties [11]. Mentha plants are rich in flavonoids, particularly in flavones and flavanones. Luteolin and its derivatives are the main flavones described in Mentha species [12]. Species of the genus *Mentha* have been reported to contain a range of components, including cinnamic and

organic acids, aglycon, glycoside or acylated flavonoids, sterols and steroidal glycosides, alkaloids, lignans, hydrocarbons, fatty acids, tocopherols, proteins, free sugars [13,14,15,16,17,18]. Terefore, the present study aimed at investigating acuteoral toxicity effects of aqueous extract of *Mentha suaveolens*,

MATERIALS AND METHODS

Plant material

Mentha suaveolens plant material was collected from Bougaa région, Wilaya of Sétif Northeast of Algeria.

Animal material

Male *Albino Wistar* mice weighing between 25 and 40 g were used for acute toxicity. The animals were obtained from Pasteur Institute (Algiers, Algeria). These animals were kept in the animal house of the faculty of Nature and Life Sciences, University of Sétif, at a temperature of 20°C and a photoperiod cycle of 12 hours light/dark. The animals were housed in plastic cages (3 mice per cage) and had free access to standard commercial diet and tap water.

Preparation of aqueous extract

The areal parts were washed in running water, dried and powdered. 50 g of powder was boiled in 500 ml of water for 15 minutes, the resulting was filtered using wattman filter paper and then evaporated in rotary vacuum evaporator.

Acute toxicity study

The acute oral toxicity of extract was evaluated using the procedures described by Organization for Economic Co-operation and Development 425 guidelines, the animals were divided into three groups with 3 animals (3 males). The control group was given normal saline. The second and third groups were given a single dose of 2000 mg/kg and 5000 mg/kg of aqueous extract respectively. The animals were fasted (4 h) with free access to water prior to administration of single doses of

the extract dissolved in distilled water. The general behavior of the mice was continuously monitored after dosing, periodically during the first 24 h (with special attention given during the first 4 hours), and then daily thereafter, for a total of 14 days.

At the end of the treatment, animals were fasted overnight, but allowed access to water and libitum. They were subsequently anesthetized with diethyl ether and blood samples were obtained by retro-orbital puncture and collected in tube containing heparin and centrifuged at 4000 r/min at 4°C for 15 minutes to obtain serum (stored at −20°C until analysis). The organs (kidneys, liver, lungs, heart, stomack and spleen) were weighed and fixed in 10% formalin for histopathological examination.

Body Weight, Food Consumption

The body weight of each mice was recorded once weekly and the amount of food consumed was measured from the quantity supplied and the amount remaining after 24 hours for 2 weeks of the study period.

Blood analysis

Biochemical analysis was performed using an automatic analyzer (Beckman). Parameters included: Créatinine (Créa); Urée ; aspartate aminotransferase (AST); alanine aminotransferase (ALT).

Organ weights

After the sacrifice of all animals, the kidneys, liver, heart, lungs, spleen and stomach were carefully removed and weighed individually (absolute organ weight).

Statistical analysis

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to assess differences between groups.

RESULTS

Mortality and signs of toxicity

The results of the acute toxicity study of *Mentha suaveolens* Aqueous Extrac.t, administered orally as single doses in Table 1, showed no mortality during the 7-day observation period after doses of 0, 2, and 5 g/kg of body weight. Additionally, no adverse effects were observed in the mice at these doses.

Table 1. Mortality and signs of toxicity of the *Mentha suaveolens* aqueous extract administered orally as one single dose in mice.

Dose (g/kg)	**Death (D/T)**	**Adverse effects**
0	**0/3**	**Normal**
2	**0/3**	**Normal**
5	**0/3**	**Normal**

Body weight changes

The results Table 2 suggest that the administration of *Mentha suaveolens* aqueous extract did not lead to significant changes in body weight in mice over the observation period, indicating stability in body weight compared to the control group.

Table 2: Effect of *Mentha suaveolens* aqueous extract on body weight in mice.

Day		**control**	**2000 mg/kg**	**5000 mg/kg**
1st Day Body Weight (g)		33,99 ± 16,71	30.06 ± 1.84	32.03 ± 1.91
7th Day Body Weight (g)		33,75 ± 13,32	33,11 ± 3.21	34.03 ± 5.1

14th Day Body Weight (g)		33,89 ± 10,76	31,16 ± 2.8	32.69 ± 6.91

Values are presented as mean ± SD; N= 3.

Biochemical analysis

Table 3 shows the results of the clinical biochemistry parameters assessed in the study investigating the effect of Mentha suaveolens aqueous extract on mice. The parameters measured were aspartate aminotransferase (AST), aminotransferase (ALT), creatinine (Créa), and urea.

Table 3 : *Effect of Mentha suaveolens aqueous extract on biochemical parameters of mice.*

Parameters	**Control**	**2000 mg/kg**	**5000 mg/kg**
ALT (UI/L)	29 ± 2,82	75 ± 4.24	62 ± 32
AST (UI/L)	119,66 ± 96,38	133 ± 26.28	166 ± 50.91
Créa (mg/L)	5,4 ± 3,09	2.92 ± 0,5	2.88 ± 0,62
Urée (mg/L)	0,45 ± 0,09	0.45 ± 0,14	0.63 ± 0,21

Values are presented as mean ± SD; N= 3.

Organs weight

The study on the effect of *Mentha suaveolens* aqueous extract on organ weight in mice, as summarized in Table 4, revealed no significant variation among the groups. Here are the organ weight values for the different groups:

Table 4 : Effect of *Mentha suaveolens* aqueous extract on organ weight in mice.

Organ (g)	**Control**	**2000 mg/kg**	**5000 mg/kg**
Liver	2,31 ± 0,47	2.17 ± 0,31	1.94 ± 0,42
Kidneys	0,27 ± 0,04	0.34 ± 0,04	0,31 ± 0,06
Lungs	0,28 ±0,09	0,27 ± 0,19	0,30 ± 0,02
Heart	0,18 ± 0,03	0,21 ± 0,01	0,15 ± 0,02
Spleen	0,36 ±0,26	0,30 ± 0,1	0,22 ± 0,13

Values are presented as mean ± SD; N= 3.

DISCUSSION

Mentha suaveolens is an aromatic herb native to Southern and Western Europe. M.suaveolens is a perennial, herbaceous plant characterized by a sweet scent. The plant can grow up to 100 cm in height [19]. It has been used in traditional medicine in Mediterranean countries for a wide variety

of purposes, including cardiovascular effects, antibacterial properties, analgesic properties, anti-inflammatory properties, and antiviral properties [20]. The major phenolic compounds identified by HPLC in different Mentha varieties are caffeoylquinic acid, salvianic acid, rosmarinic acid, luteolin, salvigenin, chrysoeriol, thymonin, carnosol [21].

The acute toxicity study is utilized to check the harmful effects of an agent to the organism given as a single or short-term exposure [22]. The repeated dose toxicity tests provide information on toxic effects, identification of target organs, effects on animal physiology, hematology, the biochemical profile, and histopathology. These tests are required by regulatory agencies to characterize the toxicological potential of any substance [23]. In this study, during subacute exposure, all animals were active and responded positively to stimuli. No deaths and no clinical signs of local or systemic toxic effects were observed. The behavior of the animals was recorded daily (general health and clinical signs of toxicity) and no changes were found [24]. The behavior of all animals in all groups tested was framed as normal for the species. In the 14 day acute toxicity study, the aqueous *Mentha suaveolens* extract did not cause any significant signs of toxicity or death at any of the tested doses. The LD_{50} (lethal dose required to kill 50% of the test subjects) was found to be greater than 2000 mg/kg body weight, indicating that the extract is considered safe based on previous evidence [25].

The liver is the body's major organ for detoxification, and its damagemay result from the accumulation of toxic compounds due toinefficient metabolism and excretion. This damage is usually assessedby determining serum transaminases (ALT and AST) and total proteinin serum samples [26, 27]. Moreover, liver function and renal function tests were performed. Protein profile and metabolic biomarkers were also measured. Serum levels of three enzymes (ALT, AST, and ALP) are commonly used as clinical biochemistry markers associated with liver damage [28–29.30]. Among these enzymes, serum levels of ALT and AST of the groups 2000, and 5000 mg/kg/bw were statistically lower when compared to the control. Besides that, all the values found are within the normal range for the species [31]. Therefore, no changes in ALT and AST activities suggest that the chronic administration of extract from *Mentha suaveolens* extract did not alter the hepatocytes function and metabolism. Equally, there was also no significant change in creatinine, between the treated groups and the control group. Indeed, creatinine is known as a good indicator of renal function and any rise in creatinine levels is only observed if there is marked

damage to functional nephron [32]. Creatinine, urea, uric acid and renal clearance remain semiotic parameters for diagnosis of renal function [33]. An increase in the level of these parameters in the blood is associated with reduced renal function and increased renal failure. In this study, serum creatinine decreased at the dose of 2000 and 5000 mg/kg and renal clearance of creatinine increased at the dose of 2.92 ± 0,5 and 2.88 ± 0,62mg.

CONCLUSION

The results indicate that the extract from *Mentha suaveolens* is non-toxic in various doses, supporting its safe consumption as a culinary herb or for herbal medicinal purposes without exceeding a dose equivalent to 5000 mg/kg. The extract exhibited pharmacological activities such as central nervous system depressant action, analgesic effects, and anti-inflammatory properties. Additionally, the extract showed antioxidant activity and potential as a natural antioxidant, antifungal, and flavoring agent. The plant's chemical composition includes phenolic compounds like catechin tannins, flavonoids, and terpenes, contributing to its antioxidant properties

Conflicts of Interest

The authors declare that they have no competing interests.

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