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Antioxidant Activity and Acute Oral Toxicity of the Methanol Extract from Mentha Longifolia L. ssp. in Iraq

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ABSTRACT

Background: Recently, researchers have shown an increased interest in traditional herbal medicine. The leaves of *Mentha longifolia* are cultivated all over Iraq. Although, the plants have a wide range of reputed therapeutic properties for treating stomach problems and intestinal disorder, there is insufficient knowledge about this plant makes it difficult to warrant its effectiveness and safety.

Objective: The current study is designed to assess the antioxidant activities and acute oral toxicity of methanol extract from *Mentha longifolia* leaf in Wistar albino rats.

Materials and Methods: The antioxidant activity of *Mentha longifolia* leaf was examined using TLC- bioautography assay based 2,2-Diphenyl-1-picrylhydrazyl (DPPH). In acute toxicity evaluation, the mortality, toxicity signs, feeding and water consuming parameters were daily assessed for 14 days. This followed oral single dose administration of *Mentha longifolia* extract to rats at doses ranging from 10 to 70 mg/kg body weight. The oral single-dose effect of this plant extract on serum level of liver biochemical parameters (AST, ALT, ALP) was also estimated.

Results: The results revealed that the methanolic extracts of *Mentha longifolia* plant possess good antioxidant activity and free radical scavenging capacity with IC_{50} value to neutralize the 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) were 30 mg/ml. *Mentha longifolia* did not exhibit any toxicity, mortality and significant changes in food/water consumption by rats at 70mg/kg body weight. Furthermore, no significant differences were observed in the serum AST, ALT and ALP activities between the treated groups and control group.

Conclusion: The results suggested that the *Mentha longifolia* leaves extract shows good antioxidant properties. Therefore, it might be a promising natural source of candidate compounds to develop new drugs. In addition, the acute oral dose administration of *Mentha longifolia* methanolic leaves extract was nontoxic and safe in single dose.

Keywords: Mentha longifolia L., LD_{50}, DPPH, liver enzymes,

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INTRODUCTION

Recently, dietary supplements produced by various fruits, vegetables and herbs have role as a natural curative and protective strategy against disease and poor health. Insufficient information is available in developing countries about the nutritive value of several native plants, which could be beneficial for different purposes. Herbal plants and their natural extracted products can offer promising treatments for different diseases [1, 2]. *Mentha longifolia* L. [Lamiaceae] is a perennial herb that known as horsemint or wild mint. It is one of the most potent natural products that revealed to have various therapeutic benefits. Although, South East Asia is the native habitat of these plants, it is also grown in Iran where it is called “Pooneh” or “Fudanaj” [2,3]. Traditionally, *Mentha longifolia* was used as herbal medicine to treat wide range of diseases including respiratory disorders, gastrointestinal illnesses, infectious diseases, and inflammatory diseases alongside with menstrual disorders in different cultures around the world. Externally, this plant is also used to treat injuries and swollen glands [4].

In Iraq, *Mentha longifolia* leaves are broadly used as a folk remedy for relief of several cases including sore throat, minor mouth throat irritation, aches, sprains, and in nasal decongestants as well as stomach problems, and intestinal disorder [5]. They are also used for nutritional purposes and consumed as a green vegetables or food additive whilst the dry or fresh leaves of this plant are added to the tea as a flavor. Furthermore, it has been reported that *Mentha longifolia* and its phytochemicals have pharmacological properties, and biological activities [6].

Antioxidants are a group of compounds that have ability to protect the cells against free radicals, which can help to decrease different disorders such as aging and cancer [7]. Various studies have identified the antioxidants in *Mentha* species [8,9,10]. It has been stated that some *Mentha longifolia* ssp extracts and essential oils such as M. spicata and M. suaveolens have antimicrobial and antioxidant properties [8, 11, 12,]. People from different cultures believed that using medicinal plants for healing purposes are beneficial and free from adverse effects. Thus, they are widely utilizing this type of plant. Nevertheless, the basis of using these medicinal plants has mostly depended on long-term clinical experience with limited scientific facts concerning their safety and efficacy. Studies of toxicity can conclude whether a new medication would be adopted for clinical application or not. Dosage is a key factor that affects toxicity [13]. It has been proposed that acute toxicity test could be used to determine the toxic effect immediately by consuming large quantity of the medicinal plants in a single dose. Therefore, this study was designed to evaluate the antioxidants activities, safety and efficacy of methanol extracts from *Mentha longifolia* to increase its benefits for humans.

MATERIAL AND METHODS

- **Samples Collection and Preparation**

The *Mentha longifolia* leaves had been collected from a number of markets in Baghdad, Iraq. After collection, the *Mentha longifolia* leaves were taken to the lab of the chemistry department of the Science College at Al-Mustansiriyah University. In order to remove all traces of dust and insects, the leaves were rinsed and cleaned Afterword; the plants were squeezed, dried, and weighed. The dried *Mentha longifolia* leaves were kept in airtight bottles to be used for extraction [14, 15, 16].

- **Physico-Chemical Properties Determination**

Mentha longifolia physicochemical properties were determined following the procedure explained by A.O.A.C [17, 18].

- **Qualitative determination of free radical scavenging activity (TLC method)**

The DPPH radical scavenging assay was carried out using the TLC-method as explained by Rajan et al. [19]. TLC-bioautography assay was performed to assess the preliminary activity of *Mentha longifolia* extracts using silica gel as stationary phase and methanol as mobile phases. The antioxidant activity was determined by DPPH scavenging [20]. The sample of *Mentha longifolia* extract was dissolved in methanol, which was applied onto TLC plates (Merck, 10 x 10 cm2). The plates of TLC were developed through mobile phases consisting methanol. Once the active phytochemical compounds were separated and analyzed. Afterward, 0.05% methanol solution of DPPH was sprayed on the surface of TLC plates and subsequently incubated for thirty-minutes at a room temperature. The presence of active antioxidant pitaya and constituent was indicated by yellow to white spots where the intensity in color reflects the potent of activity of these compounds. The evaluation of DPPH radical scavenging activity was achieved as stated in Barros et al. [21]. The DPPH radical's reduction was determined using a UV/VIS spectrophotometer to measure the absorption at 517 nm. The activity of radical scavenging was calculated just as a percentage of DPPH by following equation:

$$\text{DPPH radical scavenging } \% = [(A_0 - A_1)/A_0] \times 100$$

Where:

A_0 = Absorbance of the DPPH solution (control).

A_1 = Absorbance of the sample.

The antioxidant activity is reported as 50% inhibition (IC_{50}), which represented the *Mentha longifolia* extracts concentration required to decrease the DPPH absorbance by half. IC_{50} was calculated by plotting the percentage of inhibition against extract concentration. Gallic acid was used as appositive references.

- **LD50 Examination**

In the current research, forty male adult albino rats were divided into eight groups including 5 rats (25-30g) in each one. The rats in the first groups (control) were orally received sterile water (3ml) whereas animals within other groups were treated with methanol extracts of *Mentha longifolia* at concentrations of 10-70 mg/kg body weight dose using gastric Gavage. A single dose of the *Mentha longifolia* extract was given to each rat. Clinical and behavioral signs of toxicity, mortality and diet besides water consuming of each animal were monitored for 24 hrs over 14 days period after treatment.

- **Biochemical analyses**

In this study, a number of indicative parameters were measured to investigate the hepatic function; those involved AST, ALT and ALP. The activities of these enzymes were estimated in serum using an autoanalyser and were assayed using commercial kits.

RESULTS AND DISCUSION

The first set of analyses examined the composition of *Mentha longifolia* juice. Table 1 shows some of the main content of *Mentha longifolia* extract involving 15.67% carbohydrate, 3.56% protein, 0.87% fat, 9.6% fiber and 75% energy.

Table 1. Proximate Composition of Juice *Mentha Longifolia*

Parameters	Per 100g
Crude Fibre %	9.6
Carbohydrate %	15.67
Protein %	3.56
Fat %	0.87
Energy	75

Previous research has established that the qualitative DPPH method on the plate of TLC may detect the compounds have antioxidant activity depended on their free radical scavenging activity [19]. The antioxidant capacity of *Mentha longifolia* leaves was screened using a TLC plate. After phytochemical compounds separated on plates of TLC by the mobile phase, the compounds with free radical scavenging activity were detected by reaction with 0.05% DPPH reagent. As shown in Figure 1, the active compounds of *Mentha longifolia* were indicated by producing yellow to white spots on the purple background after they had reacted with DPPH. The spots observed on the plates of TLC technique under visible light.

The impact of the *Mentha longifolia* extracts on DPPH radical scavenging capacity was evaluated using the IC_{50} values determination and then compared to the gallic acid as positive control. It has been reported that the lower value of IC_{50} indicated high efficiency of antiradical scavenging [22]. Consequently, the results

in the DPPH radical scavenging assay reveal that *Mentha longifolia* extracts exhibit high activity of free radical scavenging, with an IC_{50} value of 30 mg/ml of methanol extract. This result may be explained by the fact that the existence of high content of phytochemical components such as the flavonoids and phenolic compounds within *Mentha longifolia* is responsible for its DPPH radical scavenging [23].

Figure 1: Chromatograms of methanol extracts of *Mentha longifolia*. TLC plate was sprayed with DPPH. TLC picture for 1-standard Gallic acid. 2- *Mentha longifolia*

In current acute toxicity study, there were no mortalities or toxic signs recorded in rats treated with oral single dose of methanol extract of *Mentha longifolia* leaves (70 mg/kg b.w.) for 14 days. The extract induced tremor in the animals received *Mentha longifolia* extract at 70 mg /kg dose. Although, these sings were repeatedly observed for a few minutes in a period of time, they disappeared immediately following treatment and all rat returned to normal state. A possible explanation for this might be related to the effect of abundance of various chemical components in the plant extract. Food and water consumption exhibited daily variations in the range of control, which indicates that the *Mentha longifolia* extract was harmless to a single dose of 70 mg/kg body weight. In acute toxicity test, *Mentha longifolia* leaves extract indicated that the LD_{50} is more than the level of tested dose.

Table 2: LD_{50} for *Mentha Longifolia* Extract

Dose of extract	No. Rat/group	No. Dead/No. Animals	Signs of animal treated with extract.
70 mg/kg b.w	5/8	0/40	Simple tremor, which takes few minute then disappeared.

The impact of oral single dose of the *Mentha longifolia* leaves extract administration on biochemical liver function parameters including alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is shown in Table 3. The treated rats exhibited a slight increase in the levels of serum ALT, AST, ALP in comparison to the control rats. Nevertheless, the level of these marker enzymes for both the treated and control group falls within the normal range, which indicated that they were not affected by the administration of *Mentha longifolia* leaves extract.

Table 3: Effects of Methanol Extract *of Mentha Longifolia* on Liver Enzymes in Male Wistar Rats.

Parameters	Normal control (3 ml/kg, b.w.)	*Mentha longifolia* 70 mg/kg, b.w.)	Normal range
AST (U/L)	76	78.8	74-143
ALT (U/L)	18	19	18-45
ALP (U/L)	80.43	152.19	62-230

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase.

CONCLUSION

Mentha longifolia are attracted traditional herbal to promote health and prevent illnesses. This study sets out to evaluate the antioxidant activities and acute toxicity of *Mentha longifolia* harvested in Iraq. The results of this investigation show that the local *Mentha longifolia* extract provide considerable antioxidant activity. DPPH radical-scavenging activity method was used to determine the antioxidant activity. The *Mentha longifolia* extracts apparently exhibited an effective DPPH radical scavenger with the lower IC_{50}. In terms of acute toxicity, there was no mortality or obvious clinical signs of toxicity (behavioral changes or poor food consumption) nor any evidence of hepatotoxicity promoted by biochemical data. The findings of such acute toxicity suggest that the oral-single dose of methanol extract of *Mentha longifolia* is well tolerated and safe if the dose was as much as 70mg/kg b.w. . *Mentha longifolia* extract is rich in natural antioxidants, which might become a valuable source of compounds to develop new drugs. Further studies of sub-acute and chronic toxicity are required to local *Mentha longifolia* extract to prove its safety and its therapeutic efficacy. As this study can act as a precursor for more research.

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CONFLICT INTEREST

There are no conflicts of interest

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