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Research Article

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Primary Phytochemical Identification and some Biochemical Parameters Study of Ethanolic Extract of *Mentha spicata* Leaves in Mice

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ABSTRACT

The objective of the present study was to investigate the presence of phytochemicals constitution and some biochemical parameters of ethanolic leaves extract of Mentha spicata plant. Soxhlet apparatus was used for the organic solvent extraction. Solvents used were ethanol-water (80:20) respectively. Qualitative analysis of phytochemical constituents of (alkaloids, phenols, glycosides, tannins, saponins, flavonoids, steroids and terpenoids) and some biochemical parameters of ethanolic extract by measuring (Blood Sugar, Blood Urea, Serum Creatinine, Cholesterol, Triglycerides, Total Serum Bilirubin). The results shown the phytochemical screening revealed the extract richness in alkaloids, tannins, saponins, flavonoids, phenols, glycosides and steroids. While the terpenoids not detectable. The results of blood sugar and serum creatinine showed significantly increased within (T2) through exposure to sodium nitrite at a level (P<0.01), while (T3) show significantly increased at a level (P<0.05), but (T4) did not showed any significant difference. Blood urea show significantly increased within (T2) at a level of (P<0.01), while (T3, T4) did not showed any significant difference. Cholesterol results showed significantly increased within (T2) at a level of (P<0.01), while (T3, T4) showed significant difference at a level (P<0.05). Triglycerides showed significantly increased within (T2, T4) at a level of (P<0.01), while (T3) did not showed any significant difference. Serum bilirubin did not showed any significant difference of (T2, T3 and T4) in comparison with (T1). To conclude, ethanolic extract of Mentha spicata contains; alkaloids, phenol, steroid, flavonoids, saponins, glycosides and tannins.

Keywords: *Mentha spicata*, Blood urea, Phytochemical, Creatinine, Flavonoids.

INTRODUCTION

Lamiaceae (Labiatae) is an important plant family that has been investigated for its medicinal properties due to its large amounts of phenolic acids, flavonoids and essential oils. *Mentha* is a member of family *Lamiaceae* and is a popular plant in Iraq [1], Europe, Australia and South Africa [2,3] and is frequently consumed in form of hot beverage. Various biological activities have been reported for species of *Mentha*, such as antibacterial [4,5], antifungal [6] and insecticidal properties [7,8]. *Mentha* spp possesses antioxidant properties due to the presence of active constituents like menthone, menthol, flavonoids, rosmarinic acid and carvone [9]. Plants are naturally gifted tool and the extractions, characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic values [10]. Herbal extracts can act in a synergistic manner within the human body and provide unique therapeutic properties with minimal or no undesirable side effects [11]. Medicinal activities of plants have long been associated with the production of secondary metabolites which include tannins, terpenoides, coumarins, alkaloids and flavonoids.

These products help plant to carry out various activities like defense and pollination. However, their antioxidant, antimicrobial and other medicinal properties are widely exploited for the benefit of mankind regarding healthcare. Certain biological assays are conducted in order to assess the phytochemicals and antimicrobial potentials of a plant [12]. Nitrite salts are added to meats, poultry and fish in minute quantities as a means of preservation, this has been a

common practice for many centuries. Sodium nitrite has been reported to have adverse health effects due to increased oxidative stress that could be harmful to different organs [13].

The addition of sodium nitrite ($NaNO_2$) as a food additive to our foods may react with amines of the foods in the stomach and produces nitrosamines or large numbers of free radicals. Such products may increase lipid peroxidation which can create many harmful hazards to the different body organs [14]. The reactive nitrogen species that are produced by exposure to nitrite have many toxic effects including hepatotoxicity, nephrotoxicity and dysregulation of inflammatory responses, tissue injury [15], haematotoxicity [16,17], and hyperlipidemic effects [18]. Therefore, a study was conducted to evaluate the phytochemicals constitution and some biochemical parameters.

EXPERIMENTAL SECTION

Collection of sample

Fresh leaves of *Mentha spicata* plant were obtained from local market which was identified, authenticated by Assist. Prof. (Dr) Khazal Dh. Wadi, College of Sciences, Diyala University. The leaves washed, cleaned then dried in room temperature at shade. The samples were then crushed into powder by using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

Preparation of extracts

Organic solvent extraction of the *Mentha spicata* leaves was carried out by using ethanol. This was done by using Soxhlet apparatus. The extraction was carried out for (24) h by heating temperature that kept the solvent at (50-60) ^oC until a clear and colorless solvent appeared in the extracting unit. After that, the extract was dried by using an electric oven at temperature (40-45) ^oC until dry extract was obtained. The dry extract was placed in an incubator under (38-40) ^oC for complete dryness of the sample .The final extract was kept frozen at (–20) ^oC until use. This experiments design was approved by the ethical committee at the Department of Physiology and Pharmacology /College of Veterinary Medicine/ Diyala University.

Experimental animals

Twenty male albino mice about three months of age and with body weight ranged between (20–25) g. mice were housed in plastic cages 20 × 50 × 75 c.m dimensions, placed in a special housing room belongs to the Department of Physiology and Pharmacology/College of Veterinary Medicine/ Diyala University for two weeks for adaptation. Standard rodent diet (Commercial feed pellets) and tap water were freely available. The animals were divided equally into four groups, five mice in each one.

1- **Group 1:** Control given water orally.
2- **Group 2:** Given sodium nitrite (70 mg/kg B.W.) orally for thirty days.
3- **Group 3:** Give *Mentha spicata* extract (500 mg/kg B.W.) orally for thirty days.
4- **Group 4:** Given sodium nitrite (70 mg/kg B.W.) and *Mentha spicata* extract (500 mg/kg B.W.) orally for thirty days.

Phytochemical analysis

Phytochemical screening was carried out to evaluate the qualitative chemical composition of various crude extracts to identify the major primary and secondary metabolites groups such as tannins, saponins, flavonoids, phenolic compounds, alkaloids, glycosides, steroids and terpenoids by using precipitation and coloration methods. This analysis revealed the presence or absence of these compounds in the crude extracts tested [19-23].

Statistical analysis

Data analysis was performed using T-test to find out the significance of differences between two groups that composed from continuous variables. Pearson test for correlation was used for non-categorical data. The level of significance was 0.05 (two-tail) in all statistical testing; significant of correlations include also 0.01 (two tail).

RESULTS AND DISCUSSION

Acute Toxicity Study Oral LD_{50} of Sodium Nitrite in Male Mice

Determination of acute toxic median lethal dose (LD_{50}) in albino mice was done by up and down method [24], the value of LD_{50} was (187.62) mg/kg B.W, and it was calculated according to the following equation result: Table (1).

$$\mathbf{LD_{50} = xf + kd}$$
$$\mathbf{= 180 + (0.381) \times 20 = 187.62 \quad mg/kg\ B.W}$$

Table 1: Calculation of LD_{50} for Sodium Nitrite by Up and Down Method

Range of doses	120 – 200 mg /kg B.W
Decrease or increase in dose	20 mg /kg B.W
Death or survival of animal after 24 hours	OOOOXXOO
Value of (K) table	0.381
Last used dose (xf)	180 mg /kg
Value of LD_{50}	187.62 mg/kg B.W.

O: survival, X: death

Table 2: Shows the Data of Orally Administration to Sodium Nitrite to Male Mice

MICE	WEIGHT (gm)	DOSE (mg/kg. B.W)	RESULTS (O or X)
1	19	120	O
2	21	140	O
3	22	160	O
4	19	180	O
5	24	200	X
6	19	180	X
7	22	160	O
8	29	180	O

o: survival, x: death

The table (3) is summarize the results of phytochemical screening of ethanolic extract of *Mentha spicata* leaves, the result of the preliminary phytochemical screening was carried out on the ethanolic extracts (80%) of the samples and revealed the presence of a wide range of phytoconstituents including alkaloids, saponins, flavonoids, tannins, steroids, phenol, glycosides but the terpenoid don't detectable in extract.

Table 3: Phytochemical Screening of Ethanolic Extract of *Mentha spicata* Leaves

No.	Test	80% Ethanolic Extract of *Mentha spicata* Leaves
1	**Alkaloid**	
	Mayer's	+
2	**Flavonoid**	+
3	**Steroid**	+
4	**Terpenoid**	–
5	**Phenol**	+
6	**Saponnins**	
	Stirring	+
	Silver Nitrate	+
	Mercuric Chloride	+
7	**Tannins**	
	Lead Acetate	+
	Ferric chloride	+
8	**Glycosides**	
	T. Benedict's	+

+ = Present, ─ = Absent

Table 4: Showed the changes in Blood Sugar, Blood Urea, Serum Creatinine, Cholesterol, Triglycerides and Total Serum Bilirubin

Group / Parameter	T1	T2	T3	T4
Blood Sugar (mg/dl)	145.2 ± 1.85	184.2 ± 2.80**	154 ± 2.03 *	147.2 ± 2.93
Blood Urea (mg/dl)	26.2 ± 0.37	29.6 ± 0.51**	27 ± 0.70	26.6 ± 0.68
Serum Creatinine (mg/dl)	0.48 ± 0.03	0.88 ± 0.03**	0.68 ± 0.05*	0.54 ± 0.05
Cholesterol (mg/dl)	115 ± 1.84	150.6 ± 2.25**	124.2 ± 1.77*	123.6 ± 1.89 *
Triglycerides (mg/dl)	105.4 ± 1.78	155.8± 1.68**	100.8 ± 1.24	125.2 ± 1.50**
Total serum bilirubin (mg/dl)	0.5 ± 0.05	0.52 ± 0.05	0.51 ± 0.03	0.48 ± 0.03

The values are expressed in Mean ±SE; Significance at P < 0.05;** at P < 0.01 in comparison with T1 (control group) along experiment period*

The table above is summarize the results of blood sugar, blood urea, serum creatinine, cholesterol, triglycerides, total serum bilirubin .The study revealed that blood sugar was significantly increased within (T2) through exposure to sodium nitrite at a level (P<0.01), while (T3) show significantly increased in blood sugar and at a level (P<0.05), but (T4) did not showed any significant difference in comparison with (T1) control group. Blood urea show significantly increased within (T2) at a level of (P<0.01), while (T3, T4) did not showed any significant difference in comparison with (T1) control group.

The results of serum creatinine was significantly increased within (T2) at a level (P<0.01), while (T3) show significantly increased in serum creatinine at (P<0.05), but (T4) did not showed any significant difference in comparison with (T1). Cholesterol results showed significantly increased within (T2) at a level of (P<0.01), while (T3, T4) showed significant difference at a level (P<0.05) in comparison with (T1) control group. The results of triglycerides showed significantly increased within (T2, T4) at a level of (P<0.01), while (T3) did not showed any significant difference in comparison with (T1) control group. The results of total serum bilirubin did not showed any significant difference of (T2, T3 and T4) in comparison with (T1) control group.

Medicinal value of plants have assumes an important dimension in the past few decades. Plants produce a very diverse group of secondary metabolites with antioxidant potential. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases and in the aging process [25]. Our results were in agreement with [26] who observed the presence of alkaloids, saponins, phenols, flavonoids, terpenoids, cardioactive glycosides, tannins, and carbohydrates were present in ethanolic 80% extracts of three labiatae species, flavonoids and cardioactive glycosides present in *Osimum basilicum* in higher concentration followed by *Mentha longifolia* then *Mentha Piperita*, while alkaloids present in higher concentration in *Mentha longifolia* followed by *Mentha Piperita* and *Osimum basilicum*. Steroids and anthraquinone glycosides were found to be present in *Osimum basilicum* only and were absent in other plants.

Mint extracts are commonly used as food flavoring additive and are generally considered safe to use as they provide health benefits and good defense against oxidative damage [27]. It was shown previously that Saudi Arabia *M. longifolia* methanol extract has powerful antioxidant activity [28]. The results revealed high free radical scavenging capacity of the oil, which was found to be in correlation to the content of mainly monoterpein ketones and aldehydes. The *M. Longifolia* oil could serve as safe antioxidant and antiseptic supplements in pharmaceuticals. The antioxidant potential of mints greatly depends on the presence of phenolics. The major phenolic constituents of mints especially include rosmarinic acid and flavonoids, including flavones, flavanones and their glycosidic forms [29].

The phenolic and flavonoids present in the plants are secondary metabolites which act as antioxidants and help in free radical scavenging, metal ions chelation [30], and protects against human disease like cardiac-disorder, thrombosis, hepatotoxicity, anti-carcinogenic, anti-mutagenic, etc.[31]. Amongst the essential oils tested, *Mentha piperita* had the highest contents of total phenolic followed by *Mentha spicata*. Similar results were reported by [32,33]. Whereas, Derakhshani *et al*. (2012) [34] reported among mint species, spearmint had the highest TPC, though there was no significant difference between spearmint and peppermint. Mint extracts had good flavonoid content and total phenolic content [35]. The presence of tannins and flavanoids in the methanolic mint leaf extract. A correlative relationship has been reported between the phytochemicals such as tannins and flavanoids and the free radical scavenging activity and antibacterial activity [36]. Tannins and flavanoids have therapeutic uses due to their anti-inflammatory, anti-fungal, antioxidant and healing properties [37].

CONCLUSION

Phytochemical screening of ethanolic extract of *Mentha spicata* leaves had revealed the presence flavonoids, tannins, saponins, steroids, alkaloids glycosides and phenol by positive reaction with the respective test reagent. Results obtained in this investigation indicate that *Mentha spicata* leaf extract, rich in phenolics exhibited highest antioxidant and reducing activities. Total phenolic content had positive correlation with antioxidant capacity due to induce the stress on the organs of the body.

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