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Research Article

Evaluating the Pharmacological Dose (Oral LD_{50}) and Antibacterial Activity of Leaf Extracts of *Mentha piperita* Linn. Grown in Kingdom of Saudi Arabia: A Pilot Study for Nephrotoxicity

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Abstract

The clinical usefulness of gentamicin is limited due to the development of nephrotoxicity. Several natural agents have been used to ameliorate drugs toxicity. The survey of literature reveals that the *Mentha piperita* Linn. is found to be used in the traditional system of medicine. In the course of an ongoing UOH-project evaluate the effects of *M. piperita* L. on nephrotoxicity in rat model. So, the present study was designed to determine the pharmacological dose (oral LD_{50}) and antibacterial activity of *M. piperita* leaf extracts for nephrotoxicity study. Freshly prepared ethanolic and aqueous extracts of *M. piperita* (EMPet and AMPet) at the following concentrations, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 g kg^{-1} b.wt., were orally administered to rats to find out the LD_{50} values of them. The LD_{50} was calculated by both arithmetically and graphically according to the method of Ghosh. The antibiotic activities of both extracts were tested against a variety of Gram-positive and Gram-negative bacteria. The LD_{50} of EMPet was found to be 3.7 and 3.6 g kg^{-1} b.wt., by arithmetic and graphical method, respectively. Similarly, AMPet were 4.8 and 4.69 g kg^{-1} b.wt., by arithmetic and graphical method, respectively. The inhibition zone for both Gram-negative and Gram-positive bacteria range from 5.0-20 mm and the lowest minimum inhibitory concentrations values were found in *Staphylococcus. hominis*. In conclusion, this pilot study revealed that EMPet and AMPet administered at a dose of 300 and 400 mg kg^{-1} b.wt., were effective, respectively. The active chemical compounds present in *M. piperita* have potential antibacterial activity.

Key words: *Mentha piperita*, gentamicin, acute toxicity studies, LD_{50}, antibacterial activity, nephrotoxicity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mentha piperita, the peppermint plant belongs to the Family Lamiaceae. It is an aromatic and carminative herb cultivated throughout all regions of the world (Saharkhiz *et al*., 2012) have traditionally been used in folk remedy or in complementary and alternative medical therapy. The peppermint is widely used as flavoring, additive in foods, the preparation of toothpaste, chewing gum, mouthwash, soaps, sweets, balms or creams and cough medicine (Iwu *et al*., 1999; Georgiev and Stoyanova, 2006; Cragg and Newman, 2001; Sharafi *et al*., 2010) and other hygienic products and in pharmaceutical formulations (Simoes and Spitzer, 2000). A literature study reveals that peppermint has been ascribed a variety of biological properties, viz., antiallergenic (Inoue *et al*., 2002), antibacterial (Shapiro *et al*., 1994), anti-inflammatory (Inoue *et al*., 2002), antimycotic (Pattnaik *et al*., 1996), antitumor (Ohara and Matsuhisa, 2002), antiviral (Yamasaki *et al*., 1998), gastrointestinal protective (Mahmood *et al*., 2003), hepatoprotective (Akdogan *et al*., 2003) and chemopreventive (Samman *et al*., 1998). Several other studies have shown that it has antioxidant, antiperoxidative properties (Krishnaswamy and Raghuramulu, 1998; Al-Sereiti *et al*., 1999; Dorman *et al*., 2003). It is also used for antimutagenic purpose (Hossain *et al*., 2012) and symptomatic relief of the common cold (Stojanova *et al*., 2000). The formulation products from peppermint are used to decrease symptoms of irritable bowel syndrome and decrease digestive symptoms such as dyspepsia, nausea (Sharafi *et al*., 2010; Hossain *et al*., 2009) and used as an analgesic and to treat headache (Samarth *et al*., 2006). *Mentha piperita* contains active ingredients, such as menthol, menthone and menthyl acetate flavonoids, polymerized polyphenols, carotenes, tocopherols, saponin and choline (Saharkhiz *et al*., 2012; Iwu *et al*., 1999; Georgiev and Stoyanova, 2006; Cragg and Newman, 2001; Sharafi *et al*., 2010) together with several other minor constituents, including pulegone, menthofuran and limonene (Nair, 2001) and some of its constituents may have immunomodulating properties (Juergens *et al*., 2004, 2003; Raphael and Kuttan, 2003; Hamada *et al*., 2002) and effective in conditions such as arthritis and rheumatism (Darshan and Doreswamy, 2004).

Gentamicin (GM) is widely applied in human clinical practices for treatment of life threatening Gram-negative infections (Nagai and Takano, 2004; Tavafi, 2012). The antibiotics also cause drug induced a dose-dependent nephrotoxicity in 10-20% of therapeutic courses. Therefore, the clinical usefulness of this drug is limited due to the development of nephrotoxicity (Cuzzocrea *et al*., 2002). Thus, a therapeutic approach to protect or reverse renal damage would have very important clinical consequences. Several natural agents have been used to ameliorate some toxic and carcinogenic and drugs toxicity. The survey of literature reveals that the *Mentha piperita* Linn. are found to be used in the traditional system of medicine as a liver tonic. Many studies shows that various oral dose of *M. piperita* extracts were used viz g kg^{-1} b.wt. (Sharma *et al*., 2007; Samarth and Samarth, 2009) and 100 mg kg^{-1} b.wt. (Thangapandiyan *et al*., 2013). However nephroprotective activity of *M. piperita* has not been scientifically investigated. In the course of an ongoing UOH-project (CM4 2013) to evaluate the effects of *M. piperita* L. on nephrotoxicity in rat model. So, the present study was design to determine the LD_{50} and antibacterial activity of *M. piperita* leaf extracts.

MATERIALS AND METHODS

Preparation of plant extracts: Separated leave of *M. piperita* (Fig. 1a) was washed with tap water to remove the dust and other foreign materials (Fig. 1b). Washed leaves were dried under shade for one week (Fig. 1c). Approximately about 500 g of air-dried whole leaves were pulverized into powdered form (Fig. 1d) by using heavy duty commercial blender.

Preparation of ethanolic *Mentha piperita* extracts (EMPet): The powder samples (50 g) were extracted with 95% ethanol (1:3 w/v) by using Soxhlet extractor at 37°C for two days. The total yield was 4.67 g (9.34% w/w) of dark greenish extract. The EMPet from *M. piperita* was reconstituted to a final concentration of 5% (w/v) using aqueous solution of gum acacia 5%, (Fig. 1e) for further treatments.

Preparation of aqueous *Mentha piperita* extracts (AMPet): The aqueous extracts of *M. piperita* leaves were prepared according to the method of Hossain *et al*. (1992). The *M. piperita* leaves yielded 13% light greenish semisolid which was stored at 0-4°C until used.

Acute toxicity studies: Male Wistar albino rats weighing 130-140 g (7-8 weeks of age) were used for acute toxicity studies. The animals were divided into number of experimental groups (lower doses and higher doses groups) 10 animals for each group. All animals were allowed to fast by withdrawing the food and water for 18 h. Freshly prepared EMPet and AMPet at the following concentrations, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 g kg^{-1} b.wt., were orally administered to rats to find out the LD_{50} values of them. The animals were provided with food and water immediately after the plant drugs administration. The LD_{50}

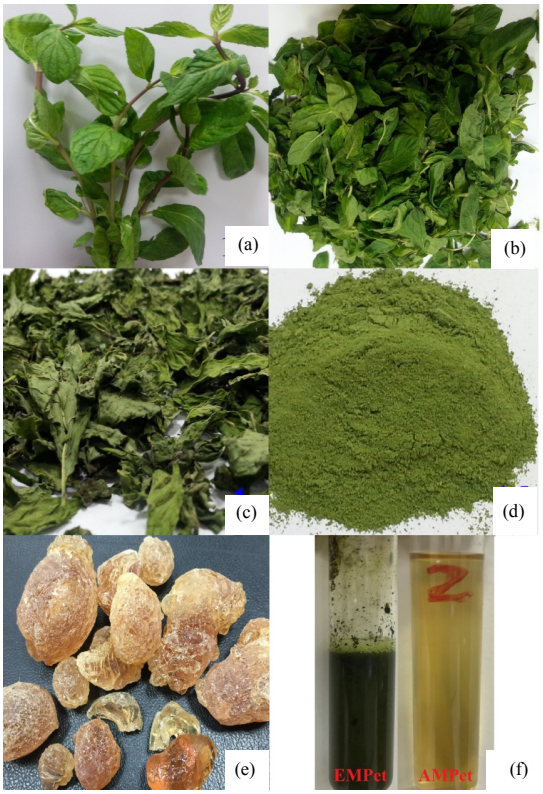

Fig. 1(a-f): Various stages of extraction of *M. piperita* leaves, (a): Fresh *M. piperita* L, (b): Separated cleaned leaves, (c): Dried leaves under shadow, (d): Powdered leaves, (e): Gum acacia and (f): Final extracts of *M. peperita* (EMPet and AMPet)

value of the plant extracts was calculated by both arithmetically and graphically according to the method of Ghosh (1984). For the interpretation of the toxicity data, the observed percentage mortality was converted into probit by referring to Table 1 (Ghosh, 1984). The LD_{50} of the plant extracts was calculated by the following formula:

$$LD_{50} = \text{Maximum dose (100\% dead)} - \frac{\text{Product (a} \times \text{b)}}{\text{No. of animals in each group}}$$

Determinations of antimicrobial activity: Antibiotic activity of EMPet and AMPet were tested against a variety of Gram-positive and Gram-negative clinical isolates according to Kirby-Bauer method as described by Hudzicki (2009). One plate of each test microorganism was taken and colonies were transferred into normal saline under aseptic conditions. Density of each microbial suspension was adjusted to be equal to that of 10^6 CFU mL^{-1} (standardized by 0.5 McFarland standard). The bacterial suspensions were then spread uniformly with sterile swab stick on Nutrient Agar (NA) plates. Sterile filter paper disks were then placed onto the bacterial culture thus spread on the NA plates maintaining uniform distance from each other with a sterile forceps. Different concentrations (5-20 µL) of the plant extract from a 1% (w/v) solution were then delivered onto the filter paper disks. The plates were then kept at room temperature for 15 min. Then the plates were incubated at 37°C for 24 h. The zones of inhibitions around the disks were measured and recorded.

RESULTS

The LD_{50} of the *M. piperita* leaves extracts was calculated by using the formula:

$$LD_{50} = \text{Maximum dose (100\% dead)} - \frac{\text{Product (a} \times \text{b)}}{\text{NO. of animals in each group}}$$

The LD_{50} of EMPet was found to be 3700 mg kg^{-1} b.wt., by arithmetic method (Table 2) and also it was found 3.6058 g kg^{-1} b.wt., by graphical method (Fig. 2). Similarly, the LD_{50} of AMPet was found to be 4800 mg kg^{-1} b.wt., by arithmetic method (Table 3) and also it was found 4.6989 g kg^{-1} b.wt., by graphical method (Fig. 3). Then 1/10th of the LD_{50} values of both EMPet and AMPet were fixed as pharmacological dose. From both arithmetic and graphical methods shows the EMPet administered at the dose of 300 mg kg^{-1} b.wt. and AMPet administered at the dose of 400 mg kg^{-1} b.wt., were effective than the rest of the doses (Table 4). The antibacterial activity of EMPet and AMPet were evaluated according to their zones of growth inhibition against various pathogens measured in mm (Fig. 4). The inhibition zone for both Gram-negative and Gram-positive bacteria range from 5.0-20 mm and the lowest minimum inhibitory concentrations values were found for the *S. hominis*. All the tested microorganisms EMPet showed more potential antibacterial activity compared with AMPet.

DISCUSSION

The aim of the present study was to calculating the LD_{50} values for the EMPet and AMPet, given orally in rats, because of wide differences in the reported results from other studies (Sharma *et al*., 2007; Thangapandiyan *et al*., 2013; Samarth and Samarth, 2009). The dose dependent studies were carried out to find out effective pharmacological dose of the plant extracts for further experimental studies. The LD_{50} of *M. piperita* leaves extracts were then fixed 1/10th as

Table 1: Transformation of percentage mortalities to probits

Transformation (%)	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	6.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	6.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 2: Results of the lethal doses determination after oral ingestion of EMPet (n = 10)

			Arithmetic				Graphical method		
Groups	Dose (mg kg^{-1})*	Number of dead animals	Dose difference (a)	Mean mortality (b)	Product (a×b)	Log dose	Dead (%)	Corrected (%)#	Probits
1	2500	0/10	-	-	-	0.3979	0	2.5	3.04
2	3000	2/10	500	1.0	500	0.4771	20	20	4.16
3	3500	4/10	500	3.0	1500	0.5441	40	40	4.75
4	4000	6/10	500	5.0	2500	0.6021	60	60	5.25
5	4500	9/10	500	7.5	3750	0.6532	90	90	6.28
6	5000	10/10	500	9.5	4750	0.6989	100	97.5	6.96
Total (a×b) = 13000									

*: The data below 2.5 g kg^{-1} b.wt. and above 5.0 g kg^{-1} b.wt., were omitted for calculation, #: Corrected formula for 0% dead = 100×0.25/n for 100% dead = 100× (n-0.25)/n, where n is the number of animals in each group LD_{50} of EMPet = 5000-(13,000/10) = 3700 mg kg^{-1} b.wt.

Table 3: Results of the lethal doses determination after oral ingestion of AMPet (n = 10)

			Arithmetic method				Graphical method		
Groups	Dose (mg kg^{-1})*	Number of dead animals	Dose difference (a)	Mean mortality (b)	Product (a×b)	Log dose (x)	Dead (%)	Corrected (%)#	Probits
1	3500	0/10	-	-	-	0.5441	0	2.5	3.04
2	4000	2/10	500	1.0	500	0.6021	20	20	4.16
3	4500	4/10	500	3.0	1500	0.6532	40	40	4.75
4	5000	5/10	500	4.5	2250	0.6990	50	50	5.00
5	5500	8/10	500	6.5	3250	0.7404	80	80	5.84
6	6000	10/10	500	9.0	4500	0.7782	100	97.5	6.96
Total (a×b) = 12,000									

*: The data below 3.5 g kg^{-1} b.wt. and above 6.0 g kg^{-1} b.wt., were omitted for calculation, #: Corrected formula for 0% dead = 100×0.25/n for 100% dead = 100X (n-0.25/n), where, n is the number of animals in each group and LD_{50} of AMPet = 6000 - (12,000/ 10) = 4800 mg kg^{-1} b.wt.

Table 4: LD_{50} and pharmacological doses of EMPet and AMPet

	LD_{50} (g kg^{-1} b.wt.)		
Plant extract	Arithmetic method	Graphical method	Pharmacological dose (mg kg^{-1} b.wt.)
Ethanolic extract of *M. piperita* leaves (EMPet)	3.70	3.61	300
Aqueous extract of *M. piperita* leaves (AMPet)	4. 80	4.699	400

pharmacological doses. The EMPet administered at a dose of 300 mg kg^{-1} b.wt., were effective. Similarly, the AMPet administered at 400 mg kg^{-1} b.wt., were effective than the rest of the doses.

Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism (Kelmanson *et al*., 2000; Ahmad and Beg, 2001). These plant products have significant therapeutic application against human pathogens including bacteria. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Guleria and Kumar, 2006; Zakaria *et al*., 2007). In the present investigation, different extracts of *M. piperita* was evaluated for exploration of their antibacterial activity against certain Gram-negative and Gram-positive bacteria which was regarded as human pathogenic microorganism. The alcoholic extract of *M. piperita* showed significant

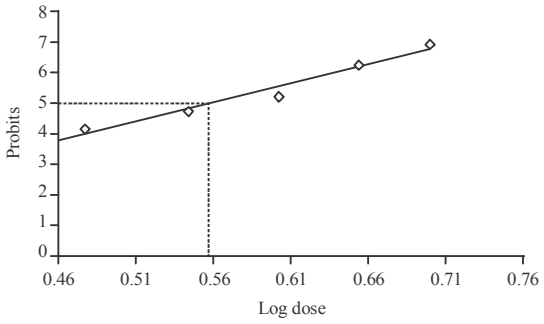

Fig. 2: Graphical representation of LD_{50} of EMPet LD_{50} = antilog 0.557 = 3.6058 g kg^{-1} b.wt.

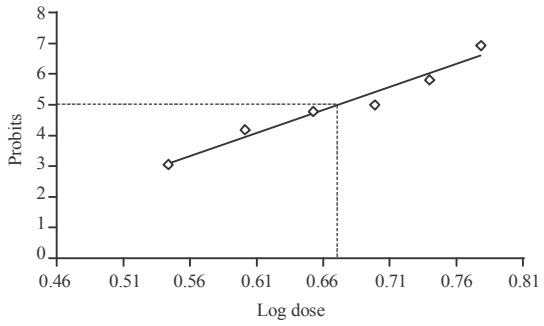

Fig. 3: Graphical representation of LD_{50} of AMPet LD_{50} = antilog 0.672 = 4.6989 g kg^{-1} b.wt.

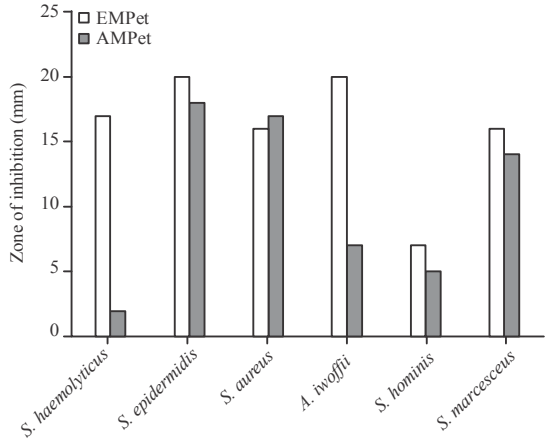

Fig. 4: Antibacterial effects of EMPet and AMPet on Gram-negative and Gram-positive bacteria strains

antibacterial activity against clinically isolated microorganisms than aqueous extract. It is clear indicates that the effectiveness of the extracts largely depends on the type of solvent used. This will support the synergistic efficacy to treat the Gram-negative bacteria with gentamicin with minimize nephrotoxicity.

CONCLUSION

In conclusion, this pilot study revealed that the ethanolic and aqueous extracts of Mentha piperita administered at a dose of 300 and 400 mg kg^{-1} b.wt., were effective respectively. Finally, it can be conclude the active chemical compounds present in *Mentha piperita* have potential antibacterial activity.

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