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Central Properties and Chemical Composition of *Ocimum basilicum* Essential Oil

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Abstract

Ocimum basilicum L. (Lamiaceae) is an Egyptian plant used as a folkloric remedy in Egyptian traditional medicine. In the current study, the aerial part of this plant was used, and its essential oil was obtained by hydrodistillation. The essential oil of *Ocimum basilicum* (OB) was screened for its composition and some CNS activities (viz., sedative, hypnotic, anticonvulsant, local anesthetic). When tested in mice, OB essential oil had no effect on motor activity up to a dose of 1.2 mL kg^{-1} at 90 min postadministration. However, higher doses produced motor impairment at all time intervals. Pentobarbitone sleeping time tested in mice was significantly increased by all doses of the essential oil higher than 0.2 mL kg^{-1}. Intraperitoneal administration of OB essential oil significantly increased in a dose-dependent manner the latency of convulsion and percent of animals exhibiting clonic seizures. Likewise, it reduced lethality in response to different convulsive stimulus used in this study. The ED_{50} values of the essential oil of OB were 0.61 mL kg^{-1}, 0.43 mL kg^{-1}, and 1.27 mL kg^{-1}, against convulsions induced by pentylenetetrazole, picrotoxin, and strychnine, respectively. A study of the local anesthetic activity of the OB essential oil by using a nerve block model employing in frog revealed that it had no local anesthetic effect. The LD_{50} of the essential oil was 3.64 mL kg^{-1} [correlation coefficient $r = 0.961$ and linear regression $y = 147\ \ln(x) - 141.7$]. Gas chromatography (GC)/mass spectrometry (MS) analysis of the essential oil revealed the presence of linalool (44.18%), 1,8-cineol (13.65%), eugenol (8.59%), methyl cinnamate (4.26%), *iso* caryophyllene (3.10%), and α-cubebene (4.97%) as the main components. The observed anticonvulsant and hypnotic activities in this study could be related to the presence of a variety of terpenes in the essential oil.

Keywords: Anticonvulsant, cineol, eugenol, linalool, local anesthetic, LD_{50}, mass spectrometry, *Ocimum basilicum*, sedative, terpenes.

Introduction

Ocimum basilicum L. (sweet basil) is a popular culinary herb belonging to the Lamiaceae family. It grows in several regions all over the world (Bariaux et al., 1992). Basil is well-known as a plant of a folk medicinal value and as such is accepted officially in a number of countries (Lawrence, 1985). The leaves of basil are used in folk medicine as a tonic and vermifuge, and basil tea taken hot is good for treating nausea, flatulence, and dysentery (Baytop, 1984). The oil of the plant has been found to be beneficial for the alleviation of mental fatigue, colds, spasm, rhinitis, and as a first aid treatment for wasp stings and snakebites (Baytop, 1984). There are usually considerable variations in the content of the major components within this species from different geographical origins (Akgul, 1989; Baritaux et al., 1992; Ozek et al., 1995). However, there is no report available for the chemical components of the essential oil obtained from species cultivated in Egypt as well as their pharmacological activities on the CNS. Therefore, the current study was undertaken to elucidate the chemical composition and central properties of the essential oil of sweet basil cultivated in Egypt.

Materials and Methods

Plant material

The fresh aerial parts of *Ocimum basilicum* were collected from plants cultivated in the Horticulture Department,

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Faculty of Agriculture, Cairo University (Giza, Egypt), in July 2005. *Ocimum basilicum* was authenticated through the herbarium of the Faculty of Science, Cairo University, where a voucher specimen (no. HCU/23451) has been deposited.

Preparation of the essential oil

The aerial parts of *Ocimum basilicum* (OB) were processed by hydrodistillation for 4 h in a Clevenger apparatus to obtain the essential oil with 1.7% (v/w) yield. The essential oil was diluted with sesame oil to obtain the desired doses and was immediately administered intraperitoneally (i.p.) to mice as a single dose expressed as milliliters of the essential oil per kilogram body weight.

Identification of the essential oil composition

The constituents of OB essential oil were analyzed by gas chromatography (GC) coupled to mass spectrometry (MS). GC analysis was carried out using a Shimadzu GC chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) with a Rtx-5 Ms column (30 m × 0.320 mm i.d., film thickness 0.5 μm). Temperature was programmed at 5°C/min from 50°C to 250°C. Helium was used as carrier gas; injector temperature was adjusted at 240°C, detector temperature at 230°C, and ionization energy was 70 eV. Relative percentage amounts were calculated from peak total area by apparatus software. The compounds were identified by comparing their retention times and mass spectra with those obtained from the authentic samples and/or the MS library.

Drugs

Picrotoxin, pentylenetetrazole, strychnine, pentobarbitone sodium, and lignocaine were obtained from Sigma (Poole, UK). Diazepam was obtained from Memphis Pharmaceutical Company (Cairo, Egypt) as 10 mg mL^{-1} ampoules (Neuril), sodium valproate was obtained as capsules of 150 mg (Convulex) from Gerot (Vienna, Austria), and ethosuximide was obtained as 250 mg capsules (Zarontin) from Parke Davis (Morris Plains, NJ, USA). Sesame oil was obtained from a local market. All drugs were freshly prepared by dissolving in distilled water. All i.p. or s.c. injections were done in volumes of not higher than 10 mL kg^{-1} of the body weight for mice.

Animals

Swiss Albino mice of either sex (25–30 g) were used throughout this study, and frogs were used to study local anesthetic effects. Mice were obtained from the laboratory animal colony (Helwan, Egypt). The animals were kept in groups of five in polyethylene cages in 12 h dark/12 h light control, with food and water *ad libitum* for at least 15 days before the experiment. The protocol of this study was reviewed and approved by Cairo University's Institutional Animal Care and Use Committee.

Assessment of motor impairment

The rotarod test according to Lima et al. (1993) was used to determine the effect of *Ocimum basilicum* essential oil on motor coordination. This test used a custom-built apparatus that consisted of an elevated rod (diameter 2 cm) that rotated at a constant speed (10 rpm). Mice were trained to walk continuously on the rod for a period of 120 s. The animals were then evaluated for motor coordination at 30, 60, 90, and 120 min after i.p. administration of 0.2, 0.4, 0.8, 1.2, 1.6, 1.8, 2.0, and 2.2 mL kg^{-1} of the essential oil. The time each animal could walk continuously on the rod was recorded. Control groups were used with mice only receiving distilled water or sesame oil (10 mL kg^{-1}, i.p.).

Pentobarbital sleeping time in mice

The effect of *Ocimum basilicum* essential oil on pentobarbitone sleeping time was studied in mice as described previously (Gilani & Janbaz, 1995). In this experiment, the mice having sleeping times between 38 and 42 min with pentobarbitone sodium (50 mg kg^{-1}; i.p.) were screened out 2 days prior to the experiment.

Different doses of the essential oil (0.2, 0.4, 0.8, 1.2, 1.4, 1.6, 1.8, 2.0, and 2.2 mL kg^{-1}), sesame oil, distilled water (10 mL kg^{-1}), or diazepam (5 mg kg^{-1}) were given intraperitoneally 1 h before administration of pentobarbitone sodium (50 mg kg^{-1}; i.p.). The sleeping time was recorded as the difference between time of awakening and the onset of sleep.

Anticonvulsant activity

Pentylenetetrazole-induced seizures

The experiment used the method described by Goodman et al. (1953). In these experiments, groups of mice (n = 10) were treated i.p. with *Ocimum basilicum* essential oil at doses of 0.2, 0.4, 0.8, and 1.2 mL kg^{-1}, distilled water or sesame oil (10 mL kg^{-1}) as negative control, or ethosuximide (150 mg kg^{-1}, i.p.) as positive control, 30 min before the administration of pentylentetrazole (85 mg kg^{-1}; i.p.). The time before the onset of clonic seizures (latency time), percent of animals showing convulsion, and percent of lethality during the following 24 h were registered.

Picrotoxin-induced seizures

The method is as described by Abdul-Guani et al. (1987). Groups of mice (n = 10) were treated i.p. with *Ocimum basilicum* essential oil at doses of 0.2, 0.4, 0.8, and

Table 1. Chemical composition of *Ocimum basilicum* essential oil obtained by GC/MS.

Retention time (R_t) (min)	Components	Relative percentage
5.22	α-Pinene	0.69
5.59	Camphene	0.88
6.31	β-Pinene	1.11
6.68	β-Myrcene	0.82
7.89	1,8-Cineol	13.65
8.29	*trans*-β Ocimene	1.61
8.59	γ-Terpin	1.81
11.38	Linalool	44.18
12.55	Camphor	0.67
12.71	Myrtenol	1.33
16.08	α-Cubebene	4.97
19.23	Eugenol	8.59
19.57	Methyl cinnamate	4.26
19.99	*iso*-Caryophyllene	3.10
20.9	α-Caryophyllene	1.75
21.8	Azulene	1.31
22.9	α-Farnesene	1.70
23.5	Germacrene B	1.62
23.9	Germacrene D	0.82
24.6	Naphthalene	2.01
	Compounds not identified	3.12

1.2 mL kg^{-1}, distilled water or sesame oil (10 mL kg^{-1}) as negative control, or ethosuximide (150 mg kg^{-1}, i.p.) as a positive control, 30 min before the administration of picrotoxin (6 mg kg^{-1}; i.p.). Latency of convulsions, percent of animals showing convulsion, and lethality during the following 24 h were recorded.

Strychnine-induced seizures

The method described by Vohara et al. (1990) was used. Groups of mice (n = 10) were treated with *Ocimum basilicum* essential oil at doses of 0.2, 0.4, 0.8, and 1.2 mL kg^{-1}, distilled water or sesame oil (10 ml kg^{-1}) as negative control, or sodium valproate (232 mg kg^{-1}, i.p.) as a positive control, 30 min before administration of strychnine (2 mg kg^{-1}, s.c.). Latency of convulsion, percent of animals showing convulsion, and percent of lethality during the following 24 h were recorded.

Determination of ED_{50} for anticonvulsant activities

The values of ED_{50} for anticonvulsant activities against pentylenetetrazole, picrotoxin, or strychnine were calculated according to the method of Litchfield and Wilcoxon (1949) using the curve of protected animals (%) in function of log of the doses.

Local anesthetic activity

Nerve block anesthesia was carried out on frogs according to the method described by Bulbring and Wajda (1945). The brain and upper eighth of the spinal cord was pithed and both the sciatic nerves were exposed; after waiting for 5 min the reflex time for each foot was recorded by dipping it in 0.1 M HCl solution. The foot was rinsed with 0.7% saline after each exposure to the acid solution. A small cotton pledget soaked in test drug solution/saline was laid on the nerve for 2 min. The test for reflex time was repeated, using lignocaine (1%) as standard drug.

Determination of LD_{50}

Groups of mice (n = 10) were given different doses of *Ocimum basilicum* essential oil (i.p., 2.4, 2.8, 3.2, 3.6, 4.0, and 4.4 mL kg^{-1}) to determine the median lethal dose (LD_{50}). These animals were observed during a 48-h period. The number of animals, which died during this period, was expressed as percentile, and the LD_{50} was determined by a Probit test using death percent versus dose according to the method of Thompson and Weil (1952).

Table 2. Effect of i.p. administration of *Ocimum basilicum* (OB) essential oil on rotarod test endurance time in seconds at different time intervals: 30, 60, 90, and 120 min postadministration (n = 5).

		Mean (± SE)			
Treatment	Dose	30 min	60 min	90 min	120 min
Sesame oil	10 mL kg^{-1}	119 ± 7.1	120 ± 6.9	120 ± 6.4	118 ± 6.3
OB essential oil	0.2 mL kg^{-1}	115 ± 7.2	113 ± 5.5	113 ± 5.3	107 ± 7.7
OB essential oil	0.4 mL kg^{-1}	113 ± 6.2	112 ± 8.4	109 ± 5.5	105 ± 6.4
OB essential oil	0.8 mg kg^{-1}	110 ± 7.1	108 ± 4.9	106 ± 5.6	103 ± 6.3
OB essential oil	1.2 mg kg^{-1}	109 ± 5	102 ± 6.3	95 ± 5.5*	91 ± 4.9*
OB essential oil	1.6 mg kg^{-1}	85 ± 6.4**	82 ± 5.5**	78 ± 4.9**	70 ± 4.2***
OB essential oil	1.8 mg kg^{-1}	66 ± 5.1***	59 ± 3.6***	54 ± 4.4***	51 ± 3.2***
OB essential oil	2.0 mL kg^{-1}	36 ± 3.1***	21 ± 1.9***	16 ± 1.1***	15 ± 1.1***
OB essential oil	2.2 mL kg^{-1}	5 ± 0.5***	3 ± 0.2***	3 ± 0.1***	1 ± 0.08***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with sesame oil group.

Table 3. Effect of i.p. administration of *Ocimum basilicum* (OB) essential oil on pentobarbitone sleeping time (mean ± SE) in mice (n = 5).

Treatment	Dose	Sleeping time (min)	
		Initial	After treatment
Distilled water	10 mL kg^{-1}	40 ± 1.2	39 ± 1.3
Sesame oil	10 mL kg^{-1}	41 ± 1.6	40 ± 1.4
Diazepam	5 mL kg^{-1}	40 ± 1.1	130 ± 6.9***
OB essential oil	0.2 mL kg^{-1}	41 ± 1.2	45 ± 1.7
OB essential oil	0.4 mL kg^{-1}	43 ± 1.1	48 ± 2.9**
OB essential oil	0.8 mL kg^{-1}	42 ± 1.2	50 ± 2.4**
OB essential oil	1.2 mL kg^{-1}	39 ± 1.5	55 ± 3.2***
OB essential oil	1.4 mL kg^{-1}	40 ± 1.2	61 ± 2.9***
OB essential oil	1.6 mL kg^{-1}	40 ± 1.2	67 ± 3.2***
OB essential oil	1.8 mL kg^{-1}	41 ± 1.3	71 ± 3.7***
OB essential oil	2.0 mL kg^{-1}	42 ± 1.7	79 ± 4.1***
OB essential oil	2.2 mL kg^{-1}	39 ± 1.6	82 ± 3.9***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with the initial sleeping time of same group.

Statistical analysis

Results were analyzed using the ANOVA test. For comparison between control group (given distilled water or sesame oil) and treated group, Student's *t*-test was used. The data are expressed as mean ±SE. The results with $p < 0.05$ were considered significant.

Results

Composition of the essential oil

Analysis of the essential oil of *Ocimum basilicum* by GC/MS revealed that terpenes are the most abundant components in the essential oil (Table 1). The major terpenes present are linalool (44.18%), cineole (13.65%), eugenol (8.59%), isocaryophyllene (3.10%), methyl cinnamate (4.26%), and α-cubebene (4.97%).

Effect on motor function

Ocimum basilicum essential oil did not induce a statistically significant disturbance in motor coordination up to a dose of 1.2 mL kg^{-1} at 60 min postadministration period (Table 2). However, a dose of 1.2 mL kg^{-1} of the essential oil 90 and 120 min after administration, and also doses higher than 1.2 mL kg^{-1} up to 2.0 mL kg^{-1}, produced a significant difference in motor coordination. As a dose of 2.2 mL kg^{-1} produced severe sedation (endurance time on rotarod ≤ 5 s).

Effect on pentobarbitone sleeping time

Ocimum basilicum essential oil significantly increased pentobarbitone sleeping time (Table 3) at doses higher than 0.2 mL kg^{-1}.

Anticonvulsant activity

The essential oil of *Ocimum basilicum* increased, in a dose-dependent manner, the latency time for the onset of clonic seizures (Tables 4 and 5), decreased the percentage of animals showing convulsion, and percentage of lethality in response to i.p. injection of pentylenetetrazole and picrotoxin. The ED_{50} values (Figs. 1 and 2) of the *Ocimum basilicum* essential oil against convulsion induced by pentylenetetrazole was 0.615 mL kg^{-1}

Table 4. Effect of i.p. administration of *Ocimum basilicum* (OB) essential oil on pentylenetetrazole (85 mg kg^{-1}, i.p.) induced seizures in mice (n = 10).

Treatment	Dose	Latency time (min) mean ± SE	% of convulsion	% of mortality
Distilled water	10 mL kg^{-1}	5.0 ± 0.4	100	100
Sesame oil	10 mL kg^{-1}	5.4 ± 0.4	100	100
Ethosuximide	150 mg kg^{-1}	N	0***	0***
OB essential oil	0.2 mg kg^{-1}	6.2 ± 0.51	90	80
OB essential oil	0.4 mg kg^{-1}	8.5 ± 0.7***	70	50*
OB essential oil	0.8 mg kg^{-1}	10.5 ± 0.8***	40**	30**
OB essential oil	1.2 mg kg^{-1}	13.4 ± 0.6***	30**	20***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with sesame oil group; N, not applicable.

Table 5. Effect of i.p. administration of *Ocimum basilicum* (OB) essential oil on picrotoxin (6 mg kg^{-1}, i.p.) induced seizures in mice (n = 10).

Treatment	Dose	Latency time (min) mean ± SE	% of convulsion	% of mortality
Distilled water	10 mL kg^{-1}	17 ± 1.1	100	100
Sesame oil	10 mL kg^{-1}	17.6 ± 0.9	100	100
Ethosuximide	150 mg kg^{-1}	N	0***	0***
OB essential oil	0.2 mg kg^{-1}	21.9 ± 1.5*	80	70
OB essential oil	0.4 mg kg^{-1}	23.6 ± 1.3**	50*	40**
OB essential oil	0.8 mg kg^{-1}	27.9 ± 2.1***	30**	20***
OB essential oil	1.2 mg kg^{-1}	32.8 ± 1.7***	20***	10***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with sesame oil group; N, not applicable.

[correlation coefficient, r = 0.997 and linear regression, $y = 36.19 \ln(x) \pm 67.58$] and against convulsions induced by picrotoxin was 0.436 mL kg^{-1} [correlation coefficient, r = 0.99 and linear regression, $y = 38.26 \ln(x) \pm 81.75$).

Doses of 0.8 and 1.2 mL kg^{-1} of the essential oil significantly alter the latency time, percentage of animals showing convulsion, and percentage of lethality in response to s.c. injection of strychnine (Table 6). The ED_{50} for this effect was 1.27 mL kg^{-1} (Fig. 3) [correlation coefficient, r = 0.98 and linear regression, $y = 29.6 \ln(x) \pm 42.8$].

Local anesthetic effect

The essential oil of *Ocimum basilicum* failed to exhibit any local anesthetic activity in the model employed.

LD_{50}

The LD_{50} determined for the essential oil was 3.64 mL kg^{-1} (Fig. 4) with correlation coefficient, r = 0.961 and linear regression, $y = 147.8 \ln(x) - 141.2$.

Discussion

The current study investigated the sedative, hypnotic, anticonvulsant, and local anesthetic effects of *Ocimum basilicum* essential oil. Results of the current work showed that OB essential oil induces a disturbance in motor coordination. However, this effect was not significant at the doses in which the anticonvulsant activity was observed.

The sedative/hypnotic effect of *Ocimum basilicum* essential oil shown by using a rotarod test was confirmed by the significant increase in pentobarbitone sleeping time after pretreatment with the essential oil at doses higher than 0.2 mL kg^{-1}. OB essential oil blocked the clonic seizures induced by pentylenetetrazole, picrotoxin, and strychnine. However, the ED_{50} values for these anticonvulsant activities indicate that *Ocimum basilicum* essential oil has 2-and 3-fold greater potency against clonic seizures induced by pentylenetetrazole and picrotoxin than that induced by strychnine. Convulsions induced by pentylenetetrazole and picrotoxin can be prevented by drugs that

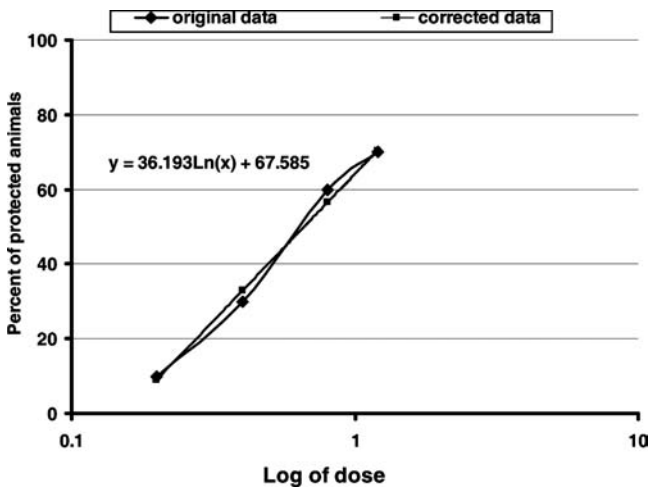

Figure 1. Plot of log of the dose of *Ocimum basilicum* essential oil versus percent of protected animals (original data and corrected values obtained by linear regression) against convulsions induced by pentylenetetrazole (85 mg/kg b.w.).

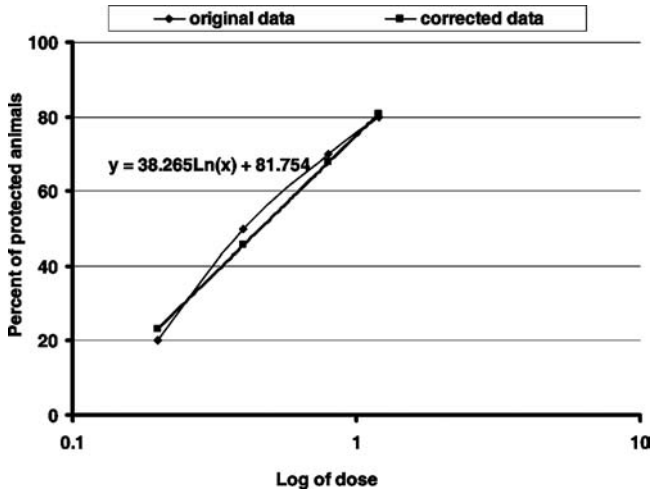

Figure 2. Plot of log of the dose of *Ocimum basilicum* essential oil versus percent of protected animals (original data and corrected values obtained by linear regression) against convulsions induced by picrotoxin (6 mg/kg b.w.).

Table 6. Effect of i.p. administration of *Ocimum basilicum* (OB) essential oil on strychnine (2 mg kg^{-1}, s.c.) induced seizures in mice (n = 10).

Treatment	Dose	Latency time (min) mean ± SE	% of convulsion	% of mortality
Distilled water	10 mL kg^{-1}	1.1 ± 0.11	100	100
Sesame oil	10 mL kg^{-1}	1.2 ± 0.1	100	100
Valproate	232 mg kg^{-1}	N	0***	0***
OB essential oil	0.2 mg kg^{-1}	1.2 ± 0.1	100	100
OB essential oil	0.4 mg kg^{-1}	1.4 ± 0.1	90	80
OB essential oil	0.8 mg kg^{-1}	1.9 ± 0.14***	70	60
OB essential oil	1.2 mg kg^{-1}	2.4 ± 0.16***	50*	40**

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with sesame oil group; N, not applicable.

reduced T-type Ca^{2+} currents such as ethosuximide. It also can be prevented by drugs that enhance gamma amino butyric acid type A (GABAA) receptor-mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital (Macdonald & Kelly, 1995). Furthermore, activation of *N*-methyl-D-aspartate receptor appears to be involved in the initiation and generalization of the pentylenetetrazole- and picrotoxin-induced seizures (Nevins & Arnolde, 1988; Velisek et al., 1990).

The inhibition of Renshaw cells (interneurons) is related to convulsions when glycine receptors are blocked. Strychnine is a glycine receptor antagonist; convulsions induced by strychnine could be prevented by glycine agonist (mephensin), benzodiazepine, or barbiturates (Gilman & Limbird, 1996).

Ocimum basilicum essential oil inhibited convulsions induced by strychnine at higher doses, close to those associated with motor impairment. Thus, the anticonvulsant activity of OB essential oil is close to that of members of barbiturates or benzodiazepines, which possess multiple mechanism of action and display broad anticonvulsant activity (MacDonald & Kelly, 1995; Hobbs et al., 1996).

GC/MS analysis showed that most of the essential oil was composed of three main terpenes including linalool, 1,8-cineol, and eugenol. It has been reported that terpenes have a protective effect against pentylenetetrazole- and picrotoxin-induced convulsions (Librowski et al., 2000; Brum et al., 2001). Modulation of glutamergic and GABAergic transmission are mechanisms indicated for anticonvulsant action of monoterpenes (Wie et al., 1997; Szabadiss & Erdelyi, 2000). The most abundant constituent of the essential oil analyzed in the current study was linalool (44.18%). Linalool has anticonvulsant activity against pentylenetetrazole-induced convulsion (Elisabetsky et al., 1995). Anticonvulsant activity of linalool is through inhibition of glutamergic transmission (Silva, 2001) and through suppression of voltage-gated currents (Narusuye et al., 2005).

Additionally, it has been reported that linalool has a locomotor inhibitory action as well as hypnotic action (Robbers et al., 1996). Cineole constitutes (13.65%) the second major terpene present in the essential oil of *Ocimum basilicum*. It has been reported that 1,8-cineole

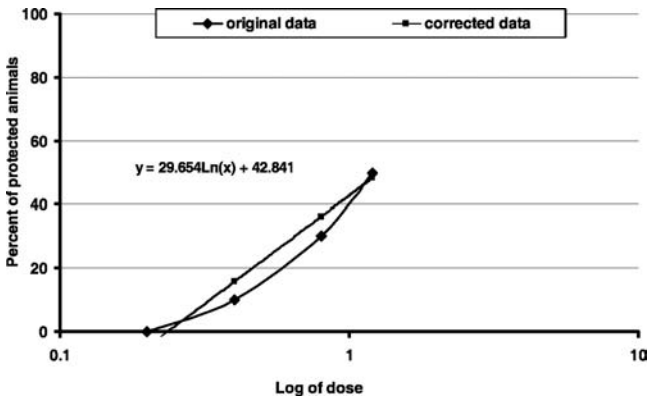

Figure 3. Plot of log of the dose of *Ocimum basilicum* essential oil versus percent of protected animals (original data and corrected values obtained by linear regression) against convulsions induced by strychnine (2 mg/kg b.w.).

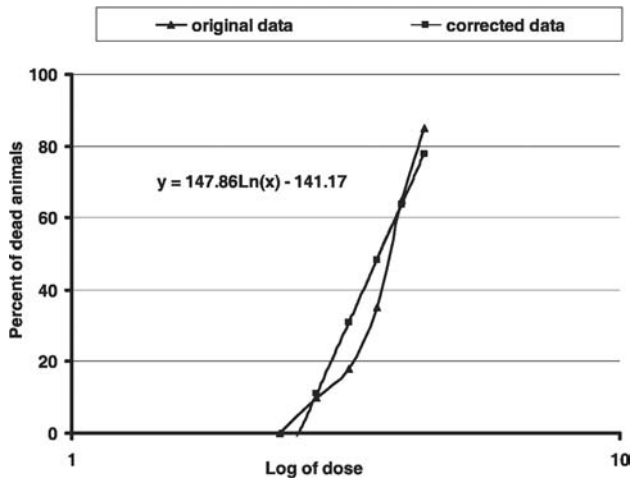

Figure 4. Plot of log of the dose of *Ocimum basilicum* essential oil versus percent of dead animals (original data and corrected values obtained by linear regression).

exerts anticonvulsant activity, potentiates phenobarbitone sleeping time, and has an inhibitory effect on locomotor activity (Santos & Rao, 2000). Eugenol comprised 8.59% of OB essential oil. Eugenol is reported to exert an anticonvulsant effect in mice (Boissier et al., 1967). Aoshima and Hamamoto (1999) reported that eugenol exerts anticonvulsant activity through potentiation of binding of GABA to its receptor and by increasing the affinity of these receptors to bind GABA. Additionally, it has been reported that eugenol has anesthetic, sedative, and muscle relaxant effects (Boissier et al., 1967; Dallmeier & Carlini, 1981). In conclusion, the sedative, hypnotic, and anticonvulsant activities reported for *Ocimum basilicum* essential oil could be attributed to terpenes as the major constituents.

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