

Chemical and Pharmacological Study of *Cymbopogon proximus* Volatile Oil

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Abstract: The volatile oil of *Cymbopogon proximus* was prepared by hydrodistillation method and analyzed chemically by GC/MS. The chromatogram showed 8 peaks corresponding to eight components with piperitone representing 72.44% of the oil's composition. Oral and intraperitoneal (i.p.) administration of the volatile oil to male, female rats and mice resulted in LD₅₀ values in the range of 1.9- 2.6 mL kg⁻¹ with an oral absorption of 80-90%. I.p. administration of the oil to anaesthetized rats (0.2-1.6 mL kg⁻¹) decreased the arterial blood pressure in a dose-dependent manner without significant changes in the heart rate except in the largest dose tested where a 16% decrease was observed. The induced decreases were not antagonized by atropine or mepyramine but were significantly reduced by indomethacin. The oil did not induce significant changes in the ECG. I.p. administration of the oil (1.2 mL kg⁻¹) to mice before induction of convulsions with electric shock, pentylenetetrazole, picrotoxin and strychnine resulted in complete protection only against the electrically induced convulsions. I.p. administration of the oil to pigeons in doses of 0.4 and 0.8 mL kg⁻¹ significantly protected against ouabain-induced vomiting. The results of these studies pointed to the involvement of prostaglandins in the oil-induced cardiovascular depressant effects and a probable antidopaminergic and ant glutamic-aspartic acids in the antiemetic and anticonvulsant effects, respectively.

Key words: *Cymbopogon proximus*, oil, GC/MS, cardiovascular system, convulsions, vomiting

INTRODUCTION

Halfabar, the common name of *Cymbopogon proximus*, is a common weed with strong aromatic odour grows in Southern Egypt and Northern parts of Sudan (Boulos, 1999). In Egypt, the plant is widely used in the folk medicine as an effective renal antispasmodic and diuretic (Boulos, 1983; Batanouny *et al.*, 1999). GC/MS analyses were performed on the oil prepared from samples obtained from Burkina Faso and Sudan and showed significant composition differences. Piperitone was identified as the major component in samples obtained from Burkina Faso while those of Sudan were free from that compound (Menut *et al.*, 2000; Siddiqui *et al.*, 1980). The volatile oil showed ovicidal, larvicidal and antioxidant activities (Menut *et al.*, 2000; Bassole *et al.*, 2003). The literature lacks data about the pharmacological effects of the oil on different organs. In this study the effects of the oil on the cardiovascular system, convulsions and vomiting induced to experimental animals were studied.

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MATERIALS AND METHODS

Plant Material

The plants of *Cymbopogon proximus* (Hochst. ex A. Rich.) Stapf Fam. Poaceae (Gramineae) were purchased from the local market in Alexandria-Egypt (August, 2005). The plant material was identified by Saniya Kamal, Prof. of Taxonomy, College of Science, Alexandria University. A voucher specimen (#CP15) was deposited at the herbarium of the College of Science.

Preparation and Analysis of the Oil

The dry powdered plants of *Cymbopogon proximus* (250 g) were subjected to hydrodistillation for 5 h (Egyptian Pharmacopoeia, 1984). The oil was separated and the aqueous layer was extracted with ether, the oil and ether were dried over anhydrous sodium sulphate. The ether was evaporated under reduced pressure and the residue left was combined with the oil. The yield of volatile constituents was 5.44% w/w. The GC/MS analysis was carried out on Gas Chromatography Mass Spectrometer GC-17A Shimadzu, Auto injector-AOC-201, Shimadzu, Japan, using capillary column of fused silica (30 m×0.25 mm i.d.) coated with 5% phenyl methyl polysiloxane (DB-5). Helium ultra pure was used as carrier gas at flow rate 25 mL min⁻¹. Oven temperature was programmed at 60-200°C at 10°C min⁻¹, the injection mode split ratio was 1:50, column inlet program was 25.5 cm sec⁻¹ and the MS detection routine was from 9-60 min. The scan of m/z was from 80-550 and the Start-Stop masses were from 40-600. Identification of the constituents was performed by aid of the computer library search (CAS No. 5989-27-5, Entry 8747, LIB# 1).

Source of the Animals

Experimental Animals Care Center, King Saud University, Riyadh, Kingdom of Saudi Arabia. The animals were housed in suitable metabolic polypropylene cages under controlled hygienic conditions at room temperature 23±2°C, 55% relative humidity and normal light cycle of 12 h light and 12 h dark. The animals were allowed free access to rodents' chow pellets and water. The experiments and the procedures used in this study were approved by the Ethical Committee of the College of Pharmacy, King Saud University.

Determination of the Lethal Dose 50 Values (LD₅₀) of the Volatile Oil of *Cymbopogon proximus* in Rats and Mice

Weight of the Rats and Mice

All of the rats used were 150±2 g and all the mice used were 25±0.6 g.

Doses for Determination of LD₅₀

For both rats and mice: 0.75, 1.5, 2 and 3 mL kg⁻¹ (i.p.) and orally.

Number of Animals per Group

This was indicated under each Table on line with the significance value.

Determination of the Absorption Percentage

$$\frac{(\text{i.p.})\text{LD}_{50} \text{ dose}}{\text{Oral LD}_{50} \text{ dose}}$$

Initially, the oil was mixed with 0.25% aqueous solution of sodium carboxymethyl cellulose (NaCMC) in the ratio of 1:3 (oil: NaCMC). Pilot experiments were performed to find the minimal

lethal dose in male rats and mice via intraperitoneal route injection. The obtained doses were taken as a guide for the design of the experiments using 4 doses, the 4th dose being the dose that induces death in all animals.

To start the experiment, male and female Wistar rats, male and female Swiss albino mice were divided into 16 groups eight for rats (males and females) and 8 for mice (males and females) with 10 animals in each group. Each subgroup was administered a dose of the volatile oil in the range of 0.75-3 mL kg⁻¹ (i.p.). In another 16 groups the volatile was administered in a similar dose range orally. The animals were observed closely and continuously for 2 h. Any live animals were kept and observed frequently for 72 h. At the end of this period, the % of death was calculated in each group. During the whole period of observation, the animals were checked for any changes in behaviour or any symptoms in any of the observable body systems. The percentage deaths were converted into probits and the LD₅₀ values were calculated using linear regression as described by Ghosh (1984).

Preparation of the Animals for Measurement of the Arterial Blood Pressure and Heart Rate

Male Wistar rats (150 g body weight) were anaesthetized and prepared for the measurement of the arterial blood pressure and heart rate as described before (El Tahir *et al.*, 1991, 1993). In brief, the rats were anaesthetized using 25% (w/v) aqueous urethane in doses of 1.25 g kg⁻¹ (i.p.). The carotid artery was cannulated and connected to an ITT blood pressure transducer for measurement of the arterial blood pressure. The heart rate was calculated from the blood pressure pulse by increasing the rate of recording from 0.05 to 1 cm sec⁻¹ in the Narco Biosystems (USA) physiograph used for recording. All injections of test drugs were injected intraperitoneally. Changes in the arterial blood pressure were quantified using the calibration system built-in the physiograph. Changes in blood pressure were reported in mmHg and changes in the heart rate were reported as % change from the pre-dosing rate.

Effect of Receptor Blockers and Enzyme Synthesis Inhibitors

To investigate the effect of the receptor blockers on the induced changes of blood pressure and heart rate atropine (2 mg kg⁻¹) or mepyramine (5 mg kg⁻¹) were injected (i.p.) 10 min before administration of a submaximal dose of the volatile oil. However, the non-selective COX enzyme inhibitor-indomethacin was administered (i.p.) at a dose of (25 mg kg⁻¹) 30 min before the submaximal dose of the volatile oil. The % antagonism was then calculated.

Preparation of Mice for Recording the ECG

Swiss albino mice (30 g body weight) were prepared for recording of the ECG as previously described for rats (El Tahir *et al.*, 1995; Bakheet *et al.*, 1999). In brief, the animals were anaesthetized with aqueous urethane 25% (w/v) in doses of (2 g kg⁻¹ i.p.). The mice were placed on their backs, the limbs at the fingers sites were fixed with an adhesive tape and the ECG needles were inserted subcutaneously to pick up lead II using ECG instrument. The speed of recording was set at 25 mm sec⁻¹ and sensitivity of 10 mm/mV. The heart rate was counted from the QRS complexes per unit time. Changes in the heart rate were calculated as % change compared with the predosing rate.

Induction of Convulsions in Mice

Experimental convulsions were induced in groups of Swiss albino mice (25 g body weight) via electrical shocks and chemicals. To induce electrical convulsions in mice, a Ugo Basile ECT unit 7801 instrument was used. The two electrodes of the instrument were attached to the left and right ears of each mouse. The mouse was then placed under a large glass funnel (diameter 30 cm) and an electric shock was applied using a frequency of 75 Hz, an electric current of 75 mA, a pulse width of 2 m sec and a shock duration of 2 sec. To investigate the effect of the oil on the induced convulsions, naïve mice

were initially treated with the oil in the indicated doses (i.p.). Ten minutes later, an electric shock using the above parameters was applied and its result evaluated regarding the extension of the lower limbs (clonic convulsions) and death of the shocked animals.

For induction of chemical convulsions, the following chemicals were used with some modifications regarding the published methods: Pentylene tetrazole was administered (i.p.) into mice using doses of (100 mg kg⁻¹) (Banziger and Hane, 1967). Picrotoxin was administered (i.p.) in doses of (12 mg kg⁻¹) (Bowser-Riley *et al.*, 1988). Strychnine was administered in dose of (15 mg kg⁻¹ i.p.) (Bowser-Riley *et al.*, 1988). Following the injection of each convulsing drug, each mouse was placed under a large glass funnel (diameter 30 cm) and observed continuously for the onset of clonic convulsions in case of pentylene tetrazole and picrotoxin, time of death and the frequency of convulsions per 10 min starting from the time of onset of action. For strychnine the times of onset for the tonic convulsions and death were recorded.

Induction of Vomiting

To examine the effect of the volatile oil on the vomiting process, pigeons (*Columba livia livia*) (body weight 250 g) were used. Vomiting was induced using a cardiac glycoside as described by (Hazlik, 1929) using ouabain, the water soluble cardiac glycoside, instead of digoxin. Aqueous ouabain was administered intravenously via a wing vein using a 22 gauge needle in doses of (100 µg kg⁻¹). Each animal was then placed under a large glass funnel (diameter 30 cm) and observed continuously and via a video camera recording for the onset of down-head movement, flopping of wings and the expulsion of gravel. To investigate the influence of the volatile oil on ouabain-induced vomiting, the oil was administered (i.p.) into naïve animals and 10 min later ouabain was injected i.v. and the animals observed for the previously indicated parameters.

Statistical Analysis

All values reported in this study are mean±SE mean with N = number of experiments performed. Significant differences between the control and different treatments were calculated using paired or unpaired t-test or analysis of variance (ANOVA) as appropriate. p-values of <0.05 were considered significant.

RESULTS

Volatile Oil Components

GC/MS study revealed the presence of eight components in the volatile oil of *Cymbopogon proximus*. Summary of the GC/MS analysis is shown in Table 1. The compounds were identified by comparison of their retention time and mass spectra by aid of the computer library search (CAS No. 5989-27-5, Entry 8747, LIB# 1). The obtained results were comparable with the data reported for the oil samples obtained from Burkina Faso with some quantitative differences (Menut *et al.*, 2000).

Table 1: Identified volatile constituents of *Cymbopogon proximus* determined by GC/MS

Peak No.	Compound name	RT	M+	Base peak	Relative (%)
1	α-Terpinene	13.07	136	93	4.64
2	Carene	16.19	136	43	1.77
3	Limonene	16.43	136	59	2.40
4	Piperitone	17.39	152	82	72.44
5	p-Menth-2-en-1-ol	17.83	154	124	0.49
6	Eucamol	21.99	222	59	9.43
7	Bulnesol	23.20	222	189	2.11
8	Eudesmol	23.48	222	59	6.60

Table 4: Effect of the volatile oil of *Cymbopogon proximus* (1.2 mL kg⁻¹) on the electrically-and chemically-induced convulsions in mice

Convulsing agent	Treatment	Time of onset of convulsions	Frequency of convulsions per 10 min	Time of death following administration
Electrical shock	None (control)	<1 sec	1	2 sec
	Oil	None*	None*	None*
Pentylene tetrazole (100 mg kg ⁻¹ i.p.)	None (control)	1.1±0.05 min	11.3±1.7	17.4±1.6 min
	Oil	20.4±1.3*	3.1±1.7*	2.6±0.5* h
Picrotoxin (12 mg kg ⁻¹ i.p.)	None (control)	4.7±2.3 min	4.5±1.2	15.3±3.1 min
	Oil	30.7±1.9* (min)	1*	3.4±1.1* h
Strychnine (i.p.) 1.5 mg kg ⁻¹	None (control)	2.1±0.9 min	1	2.7±0.4 min
	Oil	7.3±1.6* (min)	1	31.0±1.9* min

*: p<0.001, N = 12 compared with the corresponding Non-treated controls

death of the animals within 2 sec (Table 4) pre-treatment of the animals with the volatile oil in a fixed dose of 1.2 mL kg⁻¹ (i.p.) for 10 min completely prevented the electrical shock-induced extension of the lower limbs and death (N = 12).

Intraperitoneal administration of pentylene tetrazole in a dose of 100 mg kg⁻¹ induced clonic convulsions after 1.1±0.05 min and death 17.4±1.6 min following administration of the drug. The frequency of convulsions was 11.3±1.7 per 10 min (N = 12) (Table 4). Pre-treatment of mice with the oil in doses of 1.2 mL kg⁻¹ (i.p.) for 10 min before administration of pentylene tetrazole significantly prolonged the above parameters (Table 4) except the frequency of convulsions which was decreased.

Administration of picrotoxin in a dose of 12 mg kg⁻¹ intraperitoneally induced clonic convulsions 4.7±2.3 minutes following administration of the drug and death 15.3±3.1 min following administration of the drug. The frequency of convulsions was 4.5±1.2 per 10 min (N = 12) (Table 4). Pretreatment of the mice with the volatile oil at doses of 1.2 mL kg⁻¹ intraperitoneally for 10 min before picrotoxin significantly prolonged the above parameters except the frequency and did not prevent death. The cumulative results are shown in Table 4.

Administration of strychnine 1.5 mg kg⁻¹ intraperitoneally into mice induced tonic convulsions following a delay of 2.1±0.9 minutes. The animals died 2.7±0.4 minutes after injection. The frequency of convulsions was 1 (N = 12) (Table 4). Pretreatment of the animals with the volatile oil at doses of 1.2 mL kg⁻¹ (i.p.) significantly prolonged the above parameters except the frequency. The cumulative findings are shown in Table 4.

Effect of Vomiting in Pigeons

Intravenous administration of ouabain at a dose of 100 µg kg⁻¹ into the wing vein of pigeons *Columba livia livia* resulted in a sequelae of vomiting starting with wing flopping 6.5±1.1 min and expulsion of gravel (real vomiting) 7.3±0.8 min following administration. The frequency of vomiting was 3.5±1.3 episodes per 20 min. Intraperitoneal administration of the volatile oil in doses of 400 and 800 µL kg⁻¹ 10 min before ouabain significantly delayed the onset and completely protected, respectively the pigeons against ouabain-induced vomiting.

DISCUSSION

The volatile oil yield from the whole plants of *Cymbopogon proximus* prepared by hydrodistillation method was 5.44% w/w. GC/MS analysis revealed that the oil contains only 8 components with piperitone forming 72.44% of the oil. Other minor components of the oil such as elemol and β-eudesmol are known constituents of the plant (Elgamal and Wolff, 1987). Analysis of the volatile oil sample from Burkina Faso showed that piperitone is the major component (55.6%) while Sudanese sample was free from that component (Menut *et al.*, 2000; Siddiqui *et al.*, 1980). It is most likely that this difference is the result of miss identification of the Sudanese sample.

The results of the pharmacological study revealed that the intraperitoneal and oral LD₅₀ values of the volatile oil of *Cymbopogon proximus* were comparable in both male and female rats and mice. This suggests that the oil was very well absorbed. The extent of absorption following the administration via gavage ranged from 84-90%. The cause of death in the animals seemed to be a generalized central depression together with respiratory failure.

Administration of the volatile oil to anaesthetized rats induced decreases in the arterial blood pressure and the heart rate. The effects were not antagonized by the non-specific muscarinic receptor blocker atropine (Barnes, 1990) or by the antihistaminic H₁ receptor blocker mepyramine (Niemegeers *et al.*, 1982). Thus, neither cholinergic muscarinic nor histaminergic mechanisms were involved in the observed cardiovascular depressant actions. However, pretreatment of the animals with indomethacin the non-selective COX enzyme inhibitor (Moncada and Vane, 1978), significantly blocked the observed effects. Thus, vasodilatory prostaglandins seemed to be involved in the induced hypotension and bradycardia. The most likely candidates are prostacyclin and PGE₂ (El Tahir and Ageel, 2002). The study did not deal in detail with regard to the component of the volatile oil that might have been involved in the induced hypotension and bradycardia.

The experiments performed using mice ECG recordings revealed the cardiac safety of the volatile oil and its components regarding direct effects on the cardiac muscles and conduction processes. However, they pointed to the slight bradycardiogenic effects of the volatile oil. The initial acute toxicity studies revealed the central nervous system depressant actions of the volatile oil. This directed the attention to investigate its effect against electrically and chemically induced seizures. The results clearly pointed to the inherent anticonvulsant effect of the volatile oil. The great effectiveness of the volatile oil against clonically-induced (via electric shocks, picrotoxin and pentylene tetrazole compared with the tonic convulsion induced by strychnine (Bowser-Riley *et al.*, 1988) suggest a potential role of the oil for treatment of petit-mal seizures. The antagonistic effect of the volatile oil may be due to suppression of release for action of some central stimulants such as glutamic and aspartic acids probably mediated via a prostaglandin releasing step.

The studies also revealed another central action for the volatile oil as revealed by its antagonism to ouabain-induced vomiting. Ouabain-induced vomiting is believed to involve dopamine release (Boireau *et al.*, 1998) thus, the anti-emetic effect of the volatile oil may have involved an inhibitory effect upon dopamine release at the site of the vomiting centre in the medulla.

In conclusion the results of this study clearly points to the potential of the volatile oil as a hypotensive, anticonvulsant and antiemetic agent.

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