Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Potential Antidepressant Constituents of *Nigella sativa* Seeds

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ABSTRACT

Background: *Nigella sativa* Linn. is well known seed in the Middle East, Asia, and the Far East as a natural remedy for many ailments and as a flavoring agent proclaimed medicinal usage dating back to the ancient Egyptians, Greeks, and Romans. An authentic saying of the Prophet Muhammad (Peace Be Upon Him) about black seed is also quoted in Al-Bukhari. **Objective:** This study was carried out to evaluate the antidepressant effect and isolate the potential antidepressant constituents of the polar extract of *N. sativa* seeds. **Materials and Methods:** The antidepressant effect was evaluated through the immobility duration in tail suspension and forced swim tests (FSTs). Albino mice were orally treated with *N. sativa* polar extract and its RP-18 column chromatography fractions (50 and 100 mg/kg,). **Results:** The polar extract and two of its sub-fractions were significantly able to decrease the immobility time of mice when subjected to both tail suspension and FSTs, the effects are comparable to standard drug (Sertraline, 5 mg/kg). However, these treatments did not affect the number of crossings and rearing in the open field test. Phytochemical investigation of the two active fractions led to the isolation of quercetin-3-O-α-L-rhamnopyranoside 1, quercetin-7-O-β-D-gluco- pyranoside 2, tauroside E 3, and sapindoside B as the potential antidepressant constituents.

Key words: Antidepressant constituents, forced swim test, *Nigella sativa*, tail suspension test

SUMMARY

- Phytochemical and biological evaluation the antidepressant constituents in Nigella sativa using the tail suspension and forced swim methods afforded the isolation and identification of quercetin-3-O-α-L rhamnopyranoside, quercetin-7-O-β-D gluco pyranoside, tauroside E, and sapindoside B as the potential antidepressant constituents in the polar extract of N. sativa. The isolated compounds were identified through extensive NMR analysis (1D, 2D, ESI MS).

Abbreviations used: TST: Tail suspension test, FST: Forced swim test, OFT: An Open field test.

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DOI: 10.4103/0973-1296.176118

Access this article online
Website: www.phcog.com
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INTRODUCTION

Most people feel anxious or depressed at times. Many difficult situations can lead a person to feel sad, lonely, scared, nervous, or anxious. These feelings are normal reactions to life's stressors. However some people experience these feelings daily or nearly daily for no apparent reason, making it difficult to carry on with normal life. These people may have an anxiety disorder, depression, or both.[1,2] Depression is a common disorder of the modern life. There are more than 350 million people of all ages suffer from depression worldwide. It was estimated that 1 million suicides occur due to depression every year.[3] Despite the availability of antidepressant drugs as; tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A, or selective serotonin reuptake inhibitors (SSRIs), approximately 50% of the people experiencing depression not receiving any treatment.[4] Although the vast majority of the drugs used in treating depression are synthetic, they have various adverse effects ranging from drowsiness and ataxia (benzodiazepines) to insomnia and libido (selective SSRIs). Drugs of natural sources seem to have fewer adverse effects while having the curing ability in the same way as their synthetic equivalents. Recently, the search for novel drugs from medicinal plants for the psychiatric disorder has stepped forward signifi antly disclosing promising pharmacological effici cy of a variety of plants used in complementary and alternative medicines for management of mood disorders.[5]

In view of the previously mentioned, the seeds of *Nigella sativa* were selected to evaluate their potential antidepressant constituents. *N. sativa* have been shown to be rich in diverse phytoconstituents including volatile and fi ed oils, monoterpenes, alkaloids, triterpenes, and saponins.[6-11] In addition to the wide spectrum of activity, such as antioxidant, antitumor, antiinflammatory, analgesic, antiaflatoxin, smooth muscle relaxant, cytotoxic, melanogenic, immunostimulant, and antidiabetic activities.[12-20] However, previous studies showed that the aqueous extract of *N. sativa*, fi ed and volatile oils produced antianxiety effect,[21,22] in this paper we try to explore the major constituents responsible for the antidepressant effect in the polar extract.

MATERIALS AND METHODS

Plant material

The seeds of *N. sativa* L. were purchased from the local herbal market in Makah.

Chemicals and instruments

Sertraline hydrochloride (Modapex®, APEX Pharmaceuticals, Egypt) was used as a reference standard for antidepressant activity. Vacuum

Cite this article as: Elkhayat ES, Alorainy MS, El-Ashmawy IM, Fat'hi S. Potential antidepressant constituents of *Nigella sativa* seeds. Phcog Mag 2016;12:S27-S31.

liquid chromatography (VLC) and column chromatography were carried out using silica gel 60, 0.04–0.063 mm mesh size (Merck). Reversed phase silica gel for column chromatography, RP-18 (0.04–0.063 mm Merck). Sephadex LH-20 (0.25–0.1 mm, Merck). Precoated silica gel 60 F_{254} plates (Merck) were used for thin layer chromatography (TLC). The TLC plates were visualized by spraying with *p*-anisaldehyde/H_2SO_4 reagent and heating at 110°C for 1–2 min. ^{1}H and ^{13}C-NMR spectra were recorded on a JEOL-JNM-EX-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Mass spectra electrospray ionization mass spectrometry (ESI-MS) were recorded on a Finnigan MAT TSQ-7000 triple stage quadrupole mass spectrometer. All solvents for extraction and isolation were distilled prior to use. NMR grade solvents (Merck) were used for NMR analysis.

Extraction and isolation

The evaluation done by Raza *et al.* showed that the aqueous extract had high antidepressant activity. Thus, the study was planned to use the bioassay-guided fractionation for the polar extract of *N. sativa* seeds in order to isolate and identify the potential antidepressant constituents. *N. sativa* seeds (300 g) were crushed into fi e powder and were extracted with chloroform, then dried, and extracted with 70% MeOH (3 L × 1 L) at room temperature till exhaustion to get the polar extract. The combined methanol extract was filtered and then evaporated under reduced pressure to get a dark brown oily residue of the polar extract (19.1 g). The preliminary assay using the forced swimming model (FSM) and tail suspension test (TST) confi med the antidepressant–like activity of the extract. Then the dried extract was subjected to chromatographic fractionation using RP-18 column (0.04–0.063 mm; 750 mm × 35 mm, i.d.) eluted sequentially with H_2O, H_2O/MeOH (8:2, 1:1, 3:7) and fi ally with MeOH 100% to get five fractions N1-5, the TLC analysis revealed similarity in the composition of fractions N-1 and 2, thus N-1 and 2 were combined into one fraction (N-1). The preliminary antidepressant assay using FSM and TST revealed that only fractions N-3 and N-4 have antidepressant–like activity. Thus, these two fractions were subjected for the phytochemical investigation to isolate and identify the potential antidepressant components.

Isolation of compounds from fraction N-3

Fraction N-3 was subjected to RP-18 column chromatography (0.04–0.063 mm; 500 mm × 25 mm, i.d.) eluted with H_2O/MeOH (4:6). The TLC analysis of the eluents combined them into 4 sub-fractions (N-3a – N-3d). TLC analysis of sub-fraction N-3c showed a major component ($CHCl_3$/MeOH/H_2O 65:30:5), which was isolated after silica gel column chromatography eluted with $CHCl_3$/MeOH/H_2O (65:25:4) to get compound 1 (Quercetin-3-O-α-L-r hamnopyranoside).[23] Sub-fraction N-3b was subjected to repeated silica gel column chromatography eluted with $CHCl_3$/MeOH/H_2O (60:30:5) to get compound 2 (Quercetin-7-O- β-D-glucopyaranoside).[24]

Isolation of compounds from fraction N-4

Fraction N-4 was subjected to VLC, on silica gel and eluted successively with $CHCl_3$/MeOH gradients (85:15); (7:3); (1:1) and MeOH 100%, to get four sub-fractions. The TLC analysis ($CHCl_3$/MeOH/H_2O, 65:30:5), showed the similarity of the main composition of the obtained sub-fractions; hence they were combined in two sub-fractions N-4a and N-4b. VLC on silica gel eluted $CHCl_3$/MeOH gradients of sub-fraction N-4a afforded five fractions, fraction N4-a3 was further purifi d by silica gel column chromatography (700 mm × 35 mm, i.d.), eluted with $CHCl_3$/MeOH 8:2 to get compound 1 (Tauroside E).[25] Sub-fraction N-4b was passed through Sephadex LH-20 column (750 mm × 30 mm, i.d.) eluted with MeOH, followed by silica gel column chromatography eluted with $CHCl_3$/EtOAC gradient to get compounds 2 (Sapindoside B).[26]

Structure elucidation of the isolated components 1–4

Elucidation of the chemical structures of the isolated compounds [Figure 1], was based on one-dimensional (1D) and 2D-NMR spectroscopic analysis (^{1}H, ^{13}C-NMR, HMBC, and COSY), in conjunction with ESI-MS and comparison with compounds' previously published data.

Animals

Th s study was approved by the Ethics Committee. All experiments were performed in accordance with Good Clinical Practice, as revealed by Helsinki Declaration and approved by the World Health Association; the guidelines were adhered during the whole experiment.

Swiss albino mice of either sex weighing between 20 and 25 g were used for the study. The animals were maintained under standard environmental conditions (25°C ± 2°C and relative humidity of 45–55%) and were fed with standard pellet diet and water were provided *ad libitum*, animals observed daily for signs of toxicity and behavioral changes. All experiments were carried out between 10:00 and 17:00 h.

Drug administration

Animals were distributed randomly on experiment day into control and experimental groups; each group is six animals. Tested materials were dissolved in distilled water to the desired dosages; animals of the control group received distilled water. Solutions of the standard drug, extract and tested fractions were administered orally via gastric intubation 1 h before experiment in case of acute study, and administration once a day at 10.00 am for 21 days in case of subchronic study. The animals were acclimatized 1 h before behavioral tests. Before running the TST and forced swim tests (FST) experiments, animals were observed in the open fi ld test (OFT) in order to exclude the influence of motor alterations on the animal performance.

Acute toxicity study

Mice were fasted for 3 h prior to the experiment. An acute toxicity study was carried out by giving 4 doses (0.25, 0.50, 0.75 and 1.00 g/kg) of the extract to different mice groups (n = 5). The mortality and general behavior of the mice were observed for 48 h with special attention to

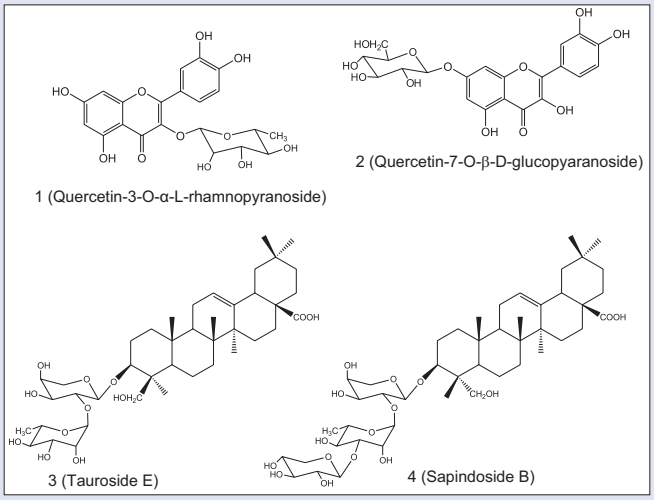

Figure 1: Structures of the isolated compounds

the fi st 30 min and the fi st 4 h after the single oral administration then periodically during the 48 h and daily for a total of 2 weeks.

Open field test

The OFT was used as a complement for the TST and FST to reject nonspecific responses of the antidepressant materials. The OFT was carried out in a glass container (transparent walls and dark fl or, 40 cm × 40 cm × 25 cm), subdivided into 10 equal areas. Respective treatment was given to the animals 60 min before the experiment and the animals were individually placed in the center of the open fi ld and allowed to explore it freely. The following parameters were observed for the last 10 min of a 20 min testing period; Ambulation (number of squares crossed with all 4 paws) and number of grooming and rearing.[27] The open fi ld was cleaned carefully with 70% alcohol between tests to eliminate odors. Mice were divided equally into four groups (n = 6), and treated for 3 weeks as follow, the fi st group given distilled water (10 ml/kg, control), the second and third groups given the polar extract 100 and 200 mg/kg respectively, and the fourth group was given sertraline (5 mg/kg).

Tail suspension test

TST was performed according to the method described by Steru *et al.*[28,29] The test was conducted an hour after the last treatment in the dim lighted room. Animals were individually suspended by the tail using adhesive plaster 1 cm from the tip to the edge of a table 50 cm from the fl or. The suspension was continued for 6 min, the total period of immobility was recorded during the last 4 min. The animal was considered to be immobile when it did not make any struggling movement, any attempt to catch the adhesive tape, or body torsions.

Forced swim test

The test was carried out as proposed by Porsolt *et al.*[30-32] The test was conducted an hour after the last treatment in the dim lighted room. Mouse was individually forced to swim in open, transparent glass chamber (45 cm × 45 cm × 25 cm) containing fresh water to a height of 15 cm and maintained at 25°C ± 2°C. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind-paws or tail. Water in the chamber was changed after each animal. Duration of immobility was manually recorded during the last 4 min of 6 min testing period. The mouse was considered immobile when it ceased struggling and remained fl ating motionless on water, making only those movements necessary to keep their head above water.

Statistical analysis

All data were expressed as a mean ± standard error of the mean. Normal distribution of data was analyzed using the Student's t-test. The statistical signifi ance was evaluated by one-way analysis of variance. The values were considered to be statistically signifi ant different when $P < 0.05$.

RESULTS

Biologically guided isolation

Despite the previous results which conferred antidepressant effect of the aqueous extract and of *N. sativa*,[22] there was no phytochemical investigation concerned with the nature of compounds in this extract. Th s work was aimed to evaluate the antidepressant–like effect the polar extract of *N. sativa*, as well as isolation and identifi ation of the potential antidepressant components. The antidepressant assay using the TST and FST methods demonstrated that the polar extract of *N. sativa* in mice was able to induce antidepressant–like effect at dose levels of 100 and 200 mg/kg, where it showed immobility time reduction of 28.3 and 33.8% in the TST and 22.5 and 31.3% in the FST, respectively [Tables 1 and 2]. Chromatographic fractionation of the polar extract on RP-18 to yield 4 fractions (N-1 to 4), on evaluation of the antidepressant activities of the obtained fractions using the TST and FST experiments, it was evident that the antidepressant effect was restricted to fractions N-3 and 4, that could attributed to the type chemical constituents in these fractions. TLC analysis of the active factions (N-3 and N-4) revealed two major constituents in each of them. Therefore, N-3 and N-4 were individually subjected to further phytochemical investigations using repeated column chromatography in order to isolate the potential antidepressant constituents. Quercetin-3-O-α-L-rhamnopyranoside (1), and Quercetin -7-O-β-D-glucopyaranoside (2), were isolated from N-3, and tauroside E (3), and sapindoside B (4) were isolated from N-4. The isolated compounds were unambiguously identifi d through extensive NMR analysis (1D, 2D, ESI-MS) and comparison with previously published data.

Table 1: Effect of oral administration of different fractions of *Nigella sativa* on immobility time in the TST

Group (drug treatment)	Acute study		Subchronic study	
	Average immobility time±SEM	Reduction rate (%)	Average immobility time±SEM	Reduction rate (%)
Control (5 mg/kg)	203.5±2.89	-	200.5±2.71	-
Polar extract (50 mg/kg)	194.2±2.68	4.6	143.7±3.1	28.3
Polar extract (100 mg/kg)	186.6±3.01	8.3	132.8±2.68	33.8
N-3 (50 mg/kg)	183.4±2.54	9.9	145.2±2.91	23.1
N-3 (100 mg/kg)	183±3.1	10.1	154.5±3.02	37.8
N-4 (50 mg/kg)	189.2±2.47	7	145.7±2.96	27.3
N-4 (100 mg/kg)	183.2±3.07	11.1	142.2±2.57	29.1
Sertaline (5 mg/kg)	178.8±2.95	12.1	108.2±2.58	46

TST: Tail suspension test; SEM: Standard error of mean

Table 2: Effect of oral administration of different fractions of *Nigella sativa* on immobility time in the FST

Group (drug treatment)	Acute study		Subchronic study	
	Average immobility time±SEM	Reduction rate (%)	Average immobility time±SEM	Reduction rate (%)
Control (5 mg/kg)	109.2±1.99	-	104.8±2.3	-
Polar extract (50 mg/kg)	94.4±3.19	13.6	81.2±3.62	22.5
Polar extract (100 mg/kg)	78.2±2.93	28.4	72±2.08	31.3
N-3 (50 mg/kg)	84.8±3.24	22.3	68.2±1.89	34.9
N-3 (100 mg/kg)	82.8±2.87	24.2	65.6±2.28	37.4
N-4 (50 mg/kg)	93±3.07	14.8	78.8±3.15	24.8
N-4 (100 mg/kg)	84.6±2.94	22.5	68±2.39	35.1
Sertaline (5 mg/kg)	78.6±2.12	28	64.2±1.97	38.7

FST: Forced swim test; SEM: Standard error of mean

Effect of the polar extract and fractions N-3 and 4 on the immobility period in forced swim tests and tail suspension test

Results showed that both the acute and subchronic administration [Tables 1 and 2] of the polar extract, as well as fractions 3 and 4 (50 and 100 mg/kg) produced a signifi ant dose dependant reduction in the immobility time in both forced swimming and TSTs comparable to sertraline (5 mg/kg), with the highest antidepressant–like activity at dose of 100 mg/kg. These treatments did not affect the number of crossing and rearing in the OFT.

DISCUSSION

Depression is a neurological disorder that becomes more prevalent in recent decades[33] Due to the complexity of depression mechanism, many currently available synthetic antidepressants show low therapeutic response and even more adverse effects.[34] Therefore, there is an exaggerated need for more effective antidepressants with a lower adverse effects. Many medicinal plants showed antidepressant–like effects, such as *Hypericum perforatum*,[35] *Passiflora edulis*,[36] *Plantago asiatica*,[37] and *Ginko biloba*.[38] In addition, certain plant constituents have been linked to attenuation of depression including flavonoids, oligo- and poly-saccharides, alkaloids, and terpenoids.[39] Th s study aimed to isolate and identify the potential antidepressant constituents in *N. sativa* seeds using the FST and TST experiments as behavioral model tests.

Effect on immobility times in tail suspension test and forced swim tests experiments

Evaluation of the antidepressant–like effect was based on the most widely used behavioral animal models that practiced the clinical efficacy of many antidepressant treatments which are therapeutically effective in human depression; TST and the FST.[30-33] The polar extract of *N. sativa* and two of its yielded chromatographic fractions (N-3 and N-4) exhibited antidepressant–like effect as shown in Tables 1 and 2. In order to detect any association between immobility in the tests with increase in locomotor activity, repeated administration of the tested materials did not increase the locomotor activity in the open fi ld at the doses which showed the antidepressant–like effect, revealing that the effect of the tested materials is a consequence of antidepressant activity.

The phytochemical investigations of the active factions N-3 and N-4 afforded the isolation of 2 flavonoid glycosides and two triterpenoidal glycosides, respectively. Thus, it seems that the antidepressant–like effect of the *N. sativa* polar extract is attributed to the presence of flavonoid and triterpene phytoconstituents. It was reported that triterpenoids exert antidepressant–like effect by inhibiting the uptake of norepinephrine, serotonin, and dopamine.[40,41] Meanwhile, flavonoids elicit the antidepressant–like effect through antioxidant protection against lipid peroxidation that leads to increase of 5-hydroxytryptamine and norepinephrine in the central nervous system.[34] Moreover, Zheng *et al*., reported that the flavonid extract of *Apocynum venetum* have reduced the immobility time in TST and FST showing antidepressant–like effect through increase of norepinephrin and dopamine along with their metabolites in the mice hippocampus.[42]

CONCLUSION

Th s work confi ms the antidepressant–like activity of the polar extract of *N. sativa* seeds, referring this activity to the presence of triterpenoid and flavonoid phytoconstituents.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no confli ts of interest.

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