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THE EFFECT OF *NIGELLA SATVIA* AQUEOUS EXTRACT ON THE ANXIETY AND DEPRESSION IN MICE

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ABSTRACT

In this study we investigated the effect of *Nigella sativa* (NS) seeds by two behavioral tests intended to determine their anxiety and depression in rodents. The pharmacological test used for studying the anxiolytic effect was the light/dark exploration model while the depression was measured with Forced test swimming (FTS). The aqueous extract of NS, administered orally in mice at 1, 10, 100 and 500 mg/kg did not affect spontaneous motor activity in the forced swimming test, the extract (at 1 and 2 mg/kg) possesses a significant anti-anxiolytic activity by increase the transition number and the time spent by mice in the illuminated side of the light–dark test. These preliminary results on the potential antipsychotic NS can be confirmed by other neuropsychopharmacology tests.

Keywords: *Nigella sativa; anxiolytic effect; depression; Forced test swimming; light/dark exploration.*

INTRODUCTION

Actually a number of the world population suffers from depression and anxiety, World Health Organization envisaged that depression will become the second leading cause of premature death or disability worldwide by the year 2020 (WHO, 2001). There are also a large number of herbal medicines whose therapeutic potential has been assessed in a variety of animal models, and whose mechanisms of actions have been investigated through neurochemical approaches [1,2,3]. These studies have provided useful information for the development of new

pharmacotherapies from medicinal plants for use in the treatment of neurological disorders. The Mediterranean region holds a wide variety of indigenous plant species, still offering the possibility to discover very interesting new natural products with potential therapeutic value. Most of the African population depends on traditional medicine for primary health care, it has been reported that most patients with a mental disorder sought herbal medicine treatment for somatic problems rather than for their mental and emotional symptoms and the best example is somatic symptoms of depression [4].

Nigella sativa belongs to the Ranunculaceae family and Ranunculales order, known as "black seed" or "black cumin" The seeds of this plant are believed to have galactagogue, carminative, laxative and antiparasitic properties [5,6]. *N. sativa* have been subjected to a range of pharmacological investigations in recent years. These studies have showed a wide spectrum of activities such as a antibacterial [7,8,9], antitumor [10], anti-inflammatory [11,12], hypotensive [13,14]., hepatoprotective [15,16,17] and immunomodulatory effects [18,19,20] . Thus, *N. sativa* seed is a promising source for active ingredients that would be with potential therapeutic modalities in different clinical settings. The efficacy of the active ingredients, however, should be measured by the nature of the disease [21, 22].

The data concerning the anti-depressive and antipsychotic effects of NS are limited, and there has not been any report of its effect on anxiety and depression which are also pathological conditions of the nervous system. The present study analyzed the behavioral effects of *N. sativa* aqueous extract in mice during light/dark exploration model, and forced swimming following acute treatment.

MATERIALS AND METHODS

Plant material and preparation of crude extract

Nigella sativa (NS) was collected from villages around the region Rabat-Salé-Zemour-Zaers, on June 2012. A voucher specimen (N° RAB10359) was deposited in the Herbarium of Botany Department of Scientific Institute. University Mohammed V, Rabat - Morocco. Aqueous extract was prepared by adding 500 ml of distilled water to 250 g of powdered plant's seeds and macerated within a period of 48 h. The respective aqueous extract was separated from its residues by gravity filtration and then lyophilized (TELSTAR, France). The final extract was black semi-solid in percentage dry weight 15% (w/w) and was kept at +4°C until use.

Drugs and Pharmacological Procedures

NS aqueous extract was dissolved in a normal solution of 0.9% sodium chloride. An equivalent preparation of saline solution was used as a vehicle control. Fluoxetine (FXT, 32 mg/kg), Diazepam (DZP, 0.5 mg/kg) were suspended in distilled water containing and normal saline 0.9%.

Experimental animals

The male Swiss albino mice (20–30 g) were fed ad libitum with standard food and water except when fasting was required in the course of the study. They were kept in a temperature and humidity controlled environment (23 ± 2 ºC and 70±5 %) with a 12 h light-dark cycle in proper ventilation. The animals were acquired from the animal laboratory of Medicine and Pharmacy Faculty, Mohammed V Souissi University, Rabat. Care of the mice was in compliance with the guidelines of the guide for the care and use of laboratory animals. (Commission on life science, national research council 1996). Prior to the experiments, animals were fed with standard diet for one week in order to be familiar with laboratory conditions. Animals were randomly assigned to administration acute regime, for the acute treatment groups, a single dose was administered 1 h before testing.

Experimental Procedures

Light–dark test (LDT)

The apparatus consisted of a Plexiglas box with two compartments (L: 32 cm, W: 22 cm, H: 18 cm), one of which was illuminated with a white light while (100 W) the other remained dark (40 W red bulb). It's separated by a connecting gate (W: 5 cm, H: 5 cm) located at the base, in the middle of the partition wall. The black and white box is supplied with a weight transducer system for automated animal detection. (Panlab, Harvard Appartus, Spain). Each animal was placed at the center of the illuminated compartment, facing one of the dark areas. The time spent in illuminated and dark places, as well as the number of entries in each space, was recorded for 5 min. This test was conducted in silence and darkness at an ambient temperature of 22±1°C and allowed to habituate for at least 1 h before beginning the behavioral tests. The black and white box allows easy and quick evaluation of the animal anxious behavior and its modification by assessing the animal displacements in two compartments [23]. The animals were divided into four groups of six animals each as follows:

- Group I (n=6) : Control, received distilled water, by per os administration (PO)

- Group II (n=6) : Standard, Diazepam 0.5 mg/kg, intraperitoneal administration (IP)
- Group III (n=6) : NS aqueous extract 250 mg/kg, PO
- Group IV (n=6) : NS aqueous extract 500 mg/kg, PO

Forced swimming test

The forced swimming test (FST) is one of the most widely used preclinical tests for detecting antidepressant-like activity as proposed by Porsolt et al. (1977) [24]. Mice were individually forced to swim in open glass cylinder (30 cm in diameter and 50-cm high) containing fresh water to a height of 40 cm and maintained at 23 ± 1°C, forcing the mice to either swim or float. The time spent immobile during 6 min of the test was recorded. Water in the chamber was changed after subjecting each animal. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel dried and returned to their housing conditions. The animals were divided into four groups of ten animals each as follows:

- Group I (n=10) : Control, received distilled water, PO
- Group II (n=10) : Standard, received fluoxetine 32 mg/kg, IP
- Group III (n=10) : received NS aqueous extract 250 mg/kg, PO
- Group IV (n=10) : received aqueous extract 500 mg/kg, PO

Statistical analysis

All the data are expressed as mean ± standard error of mean (S.E.M., n = number of experiments). The statistical analyses were obtained by the one way analysis of variance (ANOVA), followed by the Dunnett's test or Bonferroni post tests where necessary. $P<0.05$ was considered significant. The statistical analysis was carried out using software SPSS 13.0.

RESULTS AND DISCUSSION

Effect of NS aqueous extract on the light-dark test in mice

Light/dark box is also widely used in rodents as a model for screening anxiolytic or anxiogenic drugs; it has the advantages of being quick and easy to use. The administration of different doses of NS aqueous extract in mice induced a significant increment of the time spent by mice on the illuminated side of the light–dark apparatus.

Anxious mice normally prefer to spend most of their time in closed and dark box. NS extract had significantly decreased the latency time spent in the dark area, at 250 mg/kg on 12±2.1 sec ($p<0.005$), and at 500mg/kg on 8±1.4 sec ($p<0.0005$), compared with control group. The animals treated with DZP were active to explore the light compartment, they stay only 6±2.5 sec in the dark compartment ($p<0.005$) (Figure 1). The NS extract dose-dependently increased the number of transitions between the two compartments and the time spent in light compartment. Respectively, at 250 mg/kg and 500 mg/kg of NS extract, the number of transitions between the two compartments; 150± 8.3 and 170±9.5 ($P<0.05$) versus 95±4 for control group and the time spent in the light area was 18±2 sec ($p<0.05$) and 23±1.5 sec ($p<0.005$) versus 10±1 sec for control group (Figure 2-3).

Diazepam 0.5 mg/kg increased very significantly the number of transitions between the two compartments and the time spent in light compartment ($p<0.0005$). The frequency and time spent in the open compartment is the major index of the anxiety, Clinical and preclinical evidence strongly implicates gamma-amino butyric acid (GABA) ergic dysfunction in anxiety [25]. In particular, the ionotropic GABAA receptors have been a key target for anxiolytic drug development, NS extract may produce a positive effect on this receptor by enhancing GABAergic transmission and/or action in brain, similar to benzodiazepine-like drugs. Reduction in the number of entries, in the light chamber is regarded as a marker of anxiety [26]. In the test, treated group at both doses showed promising anxiolytic potential by displaying increased number of travellings from dark chamber to light chamber compared to vehicle treated group (Figure 3).

Anxiety may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are novel. It has been established that there are lots of plant secondary metabolites being used in the treatment of psychotic disorder especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system, noradrenaline, serotonin, GABA and benzodiapzepine (BZD) neurotransmitters activities [27,28].

Pharmacological treatment of anxiety is principally based on 1,4-benzodiazepines (BDZs, diazepam and related drugs) or 5-HT1A receptor agonists and selective 5-HT reuptake inhibitors (SSRIs). However, these approaches have drawbacks because BDZs have a number of unwanted side effects, including tolerance, sedation, cognitive impairments, and alcohol

interaction and generally, the onset of action of 5-HT receptor ligands is slow [29]. Considering the limitations of the available conventional pharmacotherapeutic agents for treating the psychiatric conditions, herbal remedies offer an alternative for patients, especially for those with lingering conditions and intolerance to adverse effects [30].

NS aqueous extract and diazepam significantly and dose dependently decreased the latency time of mice in dark compartment and induced anxiolytic effect beginning at the lower doses employed. It has been assumed that the time mice spend in the illuminated side of the box is the most useful and consistent parameter of anxiety [31]. The extract increased the time spent by mice and the transition number into the illuminated compartment. The lack of dose-dependent effect could be attributed to the biological variability, as well as to the chemical complexity of the crude extract.

Forced Swimming Test

The present study was carried out to evaluate the antidepressant effect of aqueous extract of *Nigella sativa* in the mouse forced swimming test; this test is quite sensitive and relatively specific to all major classes of antidepressants [32]. In the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [33]. The results presented in Figure 4 show that the aqueous extract of NS did not affect or modify the mobility time of treated animals in the forced swimming test. The immobility time of mice in the control group is 190±6.5 s during a 6 min experimental session. The administration of the Fluoxetine (32 mg/kg) to the control group showed a significant decrease in the immobility time (100 s) during the same time interval ($p<0.001$). During 6 minutes, the animals treated with two doses of the NS extract 250 and 500 mg/kg gives immobility time in mice of respectively 155±3.5 sec and 170±5 sec. Depression is defined clinically as a pathological complex of psychological, neuroendocrine and somatic symptoms that cannot be reproduced in animals and especially in mice. The disorder was characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation. Various plants are being used in alternative medicines for the search for new antidepressant agents. Drugs obtained from natural sources have good efficacy, least risk and low side effects profile. In this study antidepressant effect of *N. sativa* seeds extract have

been studied. The aqueous extract of seeds of this plant showed a moderate antidepressant effect because their immobility time is close to the control group.

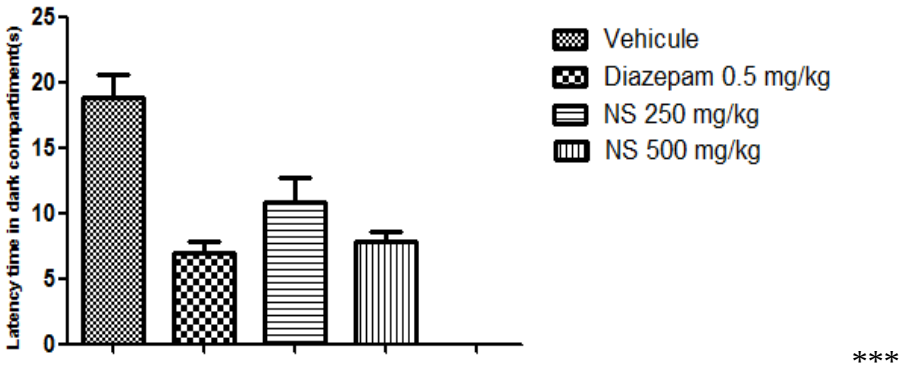

Fig. 1: Effect produced by the oral administration of different doses of the aqueous extract from *Nigella sativa* on the latency time in dark compartment by mice in dark compartment on the light–dark test.**p<0.005, ***p<0.0005 with ANOVA (mean±S.D.). Diazepam used as positive control.

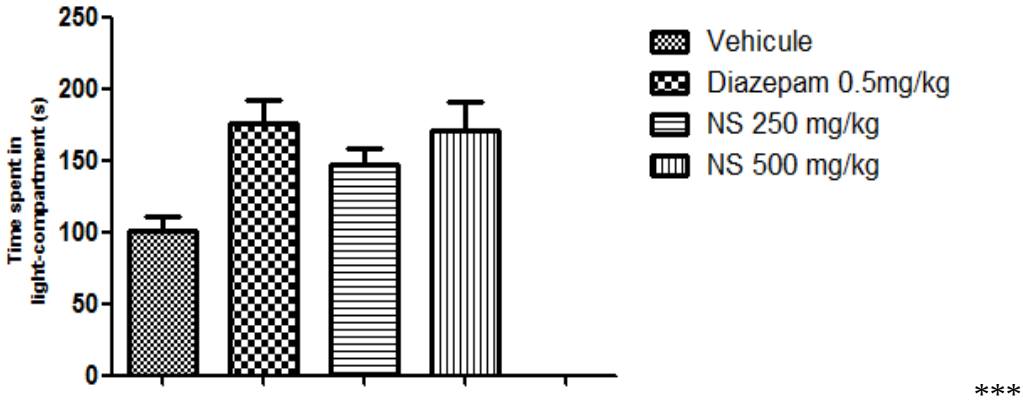

Fig. 2: Effect produced by the oral administration of different doses of the aqueous extract from *Nigella sativa* on the time spent by mice in light compartment on the light–dark test.*p < 0.05, **p<0.005, ***p<0.0005 with ANOVA (mean±S.D.). Diazepam used as positive control.

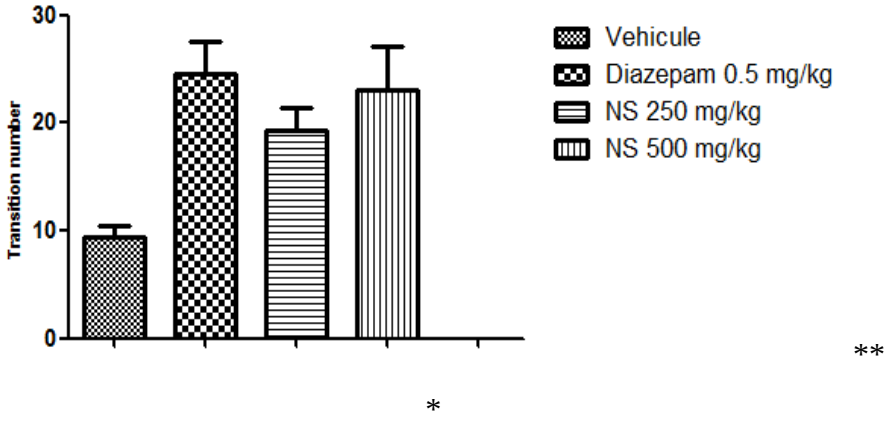

Fig. 3: Effect produced by the oral administration of different doses of the aqueous extract from *Nigella sativa* on the transition number between the two compartment by mice on the light–dark test.*p < 0.05, **p<0.005 with ANOVA (mean±S.D.). Diazepam used as positive control.

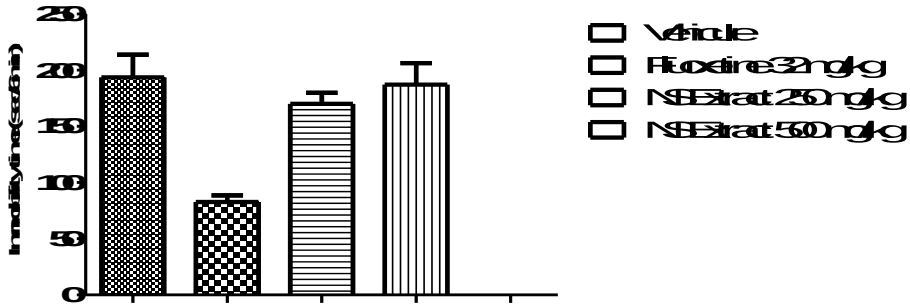

Fig. 4: Effect produced by the oral administration of different doses of the aqueous extract from *Nigella sativa* on the immobility time on the Forced swimming test. *p<0.05 with ANOVA (mean ± S.D.). Fluoxetin used as positive control.

CONCLUSION

The results from the present study show that aqueous extract seeds of N. Sativa possess antipsychotic effect that we will complete by the psychopharmacological investigation like the elevated plus-maze test and the Open Field test. However, further research is necessary to determine the components involved and their mechanism of action in bringing about the observed pharmacological effect.

CONFLICT OF INTEREST

All the authors declared no conflict of interest.

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