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Original Article

ANTIDEPRESSANT-LIKE ACTIVITY OF METHANOLIC EXTRACT OF *WITHANIA QARAITICA* IN MICE

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ABSTRACT

Objective: The goal of the present study was to assess the antidepressant-like action of *Withania qaraitica* in two behavioral animal models, the forced-swimming test (FST) and tail suspension test (TST) in mice.

Methods: *Withania qaraitica* methanolic extract was prepared by the maceration. The antidepressant activity was measured by the forced-swimming test (FST) using C57BL/6 mice and the tail suspension test (TST) using BALB/c mice. Mice were divided into three groups: control (DMSO), standard (citalopram and desipramine), and Withania qaraitica methanolic extract (n = 6 per group). Drugs were injected (1 ml/100 g) intraperitoneally (i. p.). Data were evaluated using analysis of variance, followed by LSD post-hoc tests, where *p<0.001 was considered significantly different from the vehicle control. The data are expressed as mean±SEM.

Results: In both the FST and the TST, antidepressant-positive controls citalopram and desipramine significantly reduced the time of immobility compared to vehicle control (p<0.001). The methanolic extract of Withania qaraitica at the dose of 40 mg/kg significantly reduced the immobility times with respect to vehicle control as well as lower doses of the same extract (10 and 20 mg/kg) in FST (p<0.001). In a similar fashion, the methanolic extract of Withania qaraitica at the dose of 40 mg/kg significantly decreased the duration of immobility in TST (p<0.005).

Conclusion: The current results show the antidepressant-like activity of *Withania qaraitica* in mice. This observation warrants additional studies to identify the underlining mechanism by which *Withania qaraitica* produces antidepressant-like effects.

Keywords: *Withania qaraitica*, Citalopram, Desipramine, Antidepressant-like effect, Forced-swimming test, Tail suspension test

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INTRODUCTION

Depression is one of the leading psychiatric disorders, characterized by fatigue, diminishing self-confidence and dignity, insomnia, a change in appetite, and poor concentration [1]. It is projected that about 5% of the adult population of the world suffer from this disorder [2]. Individuals in any age range can be affected by depression. However, the age range of 18-29 is experiencing symptoms of depression more than any other age group [3]. The cause of this disorder is the deficiency of monoamines like norepinephrine (NE) and serotonin (5-HT) in the brain.

To treat depression, a number of chemical antidepressants, like selective serotonin reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors, have been widely used [4]. However, all of these drugs used for the management of depression interrupt the method of treatment due to various adverse effects like anxiety, sleep disturbance, and dizziness [5]. Nature is one of the finest and most harmless sources of various medicines. From the available data, it is now believable that nature can provide a remedy for nearly all ailments through herbal medication. In light of that, a lot of importance has been given to the documentation, assessment, and classification of various medicinal herbs and their chemical components in relation to a number of ailments like anxiety, analgesia, and depression [6].

As therapies from plant sources are commonly linked with satisfactory protection without side effects, there are obvious potential benefits of them for being an effective alternative to presently accessible chemical antidepressants. Currently, many studies have been conducted to characterize the therapeutic effects of herbal sources as well as the antidepressant potential of some of the studied natural products [7-9].

It is obvious that plants will continue to be an excellent source of leads for drug development, as there are more than 370,000 species of higher plants existing on this planet, and only a small portion of these have been studied [10, 11]. Investigations aimed at unraveling natural products from herbal sources can accelerate the drug discovery process by identifying new chemical entities for drug development. Although there has been huge effort put into searching for botanical drugs for other diseases, little has so far been directed to the search for antidepressant agents.

There are more than 1000 terrestrial plants in Oman, including endemic species of medicinal value [12]. *Withania qaraitica* is one of the endemic species in the southern governorate of Oman, called Dhofar Governorate [13]. It is a short-height herb, up to one meter in height, with a stem in the center and branches extending radically from the stem in a star-like design enclosed with woolly hairs. Leaves are alternate and ovate, up to 10 cm in length [14]. *Withania* genus from the Solanaceae family [15] is well-known globally for its therapeutic constituents [16]. Some of the species in the genus *Withania* are *Withania obtusifolia*, *Withania somnifera*, *Withania begonifolia*, *Withania coagulans*, *Withania qaraitica*, and many more. The plants in the genus *Withania* have various chemical constituents that can be used to produce many pharmacological properties, such as anti-inflammatory, antioxidant, cardioprotective, antibiotic, diuretic, narcotic, sedative, and adaptogen [14, 17-19]. In Oman, *Withania qaraitica* is used as a therapeutic agent for the treatment of sedation [20]. However, the neuropharmacological action of this plant has not been explored comprehensively, which motivated us to investigate the antidepressant effect of *Withania qaraitica* in two behavioral antidepressant models, the forced-swimming test (FST) and the tail suspension test (TST).

MATERIALS AND METHODS

Animal

Two strains of mice, each weighing 22-30 g, were used for two different tests. C57BL/6 mice were used for the forced-swimming test (FST). The selection of this strain of mice for the FST was based

on an earlier report on this strain [21]. Male, BALB/c mice, on the other hand, were selected for the tail suspension test (TST) based on an earlier report that demonstrated immobility times between the strains of mice and established the good sensitivity of the BALB/c strain of mice [22]. Animals were housed in groups of six, provided food and water *ad libitum*, and subjected to a 12 h/12 h light/dark cycle. All animal experiments were done after getting approval from the University of Nizwa Animal Ethics Committee (Approval Number: VCGSR/AEC/02/2020 dated June 15, 2020).

Plant

Withania qaraitica was collected from Jarziz (17°02'26.4"N 54°08'15.5"E) in Dhofar Governorate, Sultanate of Oman. Collected leaves from *Withania qaraitica* were identified by botanists at the University of Nizwa. The voucher specimen (UCWS52H) has been deposited at the herbarium of the University of Nizwa. Collected leaves were dried under shade inside the chemical laboratory by placing those in-between two newspapers and used in the present study after grinding. The coarse powder was kept in a secure place to avoid impurities.

Chemicals

Solvents used to prepare crude extracts of *Withania qaraitica* were methanol, absolute ethanol, dimethyl sulfoxide, carbon tetrachloride (all obtained from Fisher Chemical), and sodium dihydrogen phosphate (Merck, Germany). Citalopram and desipramine (Sigma-Aldrich) were used for behavioral studies.

Instruments

Instruments used to prepare crude extracts of *Withania qaraitica* were rotary evaporator (Model RE 801, Yamato Scientific) and centrifuge (Model FC5718R 230V, Ohaus Frontier™ 5000 Multi-Pro Centrifuge).

Preparation of the methanolic extract of *Withania qaraitica*

Four hundred grams of powdered *Withania quaratica* were dissolved in 1.5 L of analytical-grade methanol, and soaked at room temperature for three days. The soaked sample so obtained was filtered through the Buchner apparatus, and centrifuged at a speed of 18,000 rpm for 10 min to remove impurities. Thirty grams of dry extract were taken and dissolved in a 1:1 water-ethanol mixture and successively fractionated with hexane, chloroform, ethyl acetate, and butanol. All solvents were removed under reduced pressure using a rotatory evaporator under a temperature of 42°C and an approximate speed of 250 rpm. The dry extracts were weighed and transferred to vials for further processing [23].

Experimental protocol for the forced-swimming test (FST)

Thirty minutes prior to FST, drugs (citalopram: 10 mg/kg, n=6; desipramine: 20 mg/kg, n=6), vehicle control (10% DMSO, n=6) and the herbal extract of *Withania qaraitica* (10, 20, 40 and mg/kg, n=18) were administered intraperitoneally (i. p.) to mice. After treatment, the animals were separately placed into a transparent glass cylinder (25 cm in height, 10 cm in internal diameter), covered with 15 cm of fresh water at room temperature. Each mouse was video tapped for 6 min by placing the camera approximately 30 cm in height from the cylinder. Within this period, the first 2 min were considered acclimation time, and measurement of immobility time was based on the 4 min at the end of each swimming session [24]. The quantification of immobility time was determined by three separate individuals. The time when animals had no movement was considered immobility, except that it was essential to retain their head/nostrils in the air [25]. All the mice used in FSTs were first-time swimmers, and none were used more than once.

Experimental protocol of the tail suspension test (TST)

Thirty minutes prior to TST, drugs (citalopram: 10 mg/kg, n=6; desipramine: 20 mg/kg, n=6), vehicle control (10% DMSO, n=6) and the herbal extract of *Withania qaraitica* (10, 20, 40 and mg/kg, n=18) were administered intraperitoneally (i. p.) to mice. Each mouse was separately suspended upturned and buckled the tail with adhesive tape to a metal bar. The animals were hung 35 cm above a protective surface made of 5 cm thick sponge material. Each mouse was hung for 6 min and video recorded; the location of the camera was at the same level as the animal. Within this period, the first 2 min were considered acclimation time, and measurement of immobility time was based on the 4 min at the end of each suspension session. The quantification of immobility time in each session was determined by three separate individuals. The motionless period of the mouse was considered as immobility [26]. All mice were used once in TST, and none were used more than once. Compared to FST, TST was not associated with the risk of producing hypothermia in the tested animals.

Data analysis

All the data were stated as a mean±SEM. Statistical analysis of the data was accomplished by one-way analysis of variance (ANOVA). Whenever the ANOVA was significant, additional multiple comparisons were done using the LSD test as the post hoc test. All evaluations were completed with the SPSS software (version 21). Degrees of statistical significance were between $p<0.005$ and $p<0.001$.

RESULTS

In FST, the SSRI antidepressant positive control, citalopram, decreased the time of immobility in a dose-dependent fashion (fig. 1). LSD post-hoc analysis indicated a reduction of 29% and 39% immobility was observed with citalopram at a dose of 5 and 10 mg/kg with respect to vehicle control ($p<0.001$). The TCA positive control, desipramine, also significantly decreased the time of immobility in FST (fig. 2). LSD post-hoc analysis showed a reduction of 29% immobility was observed with desipramine at a dose of 20 mg/kg with respect to vehicle control ($p<0.001$). The dose-dependent reduction of immobility in FST was observed with a methanolic extract of *Withania qaraitica* (fig. 3). Moreover, LSD post hoc analysis demonstrated a 32% reduction in immobility with the extract of *Withania qaraitica* at a dose of 40 mg/kg ($p<0.001$) in comparison to vehicle control.

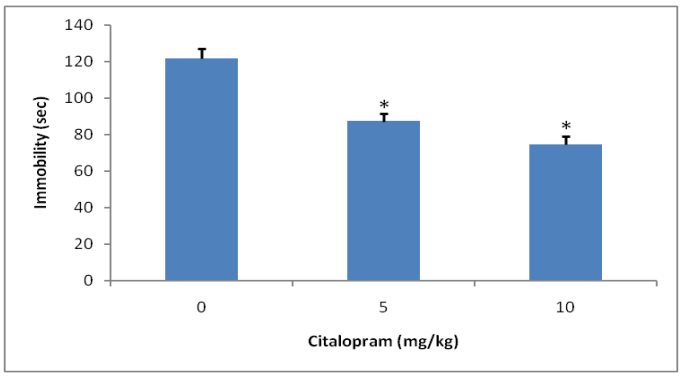

Fig. 1: Dose response of the SSRI positive control, citalopram, in the forced-swimming test. DMSO (10%) served as the vehicle control (0 mg/kg). Vehicle control or citalopram (n=6 per group) was injected 30 min before the test. Data were evaluated using analysis of variance, followed by LSD post-hoc tests, where * $p<0.001$ was considered significantly different from the vehicle control. The data are expressed as mean±SEM

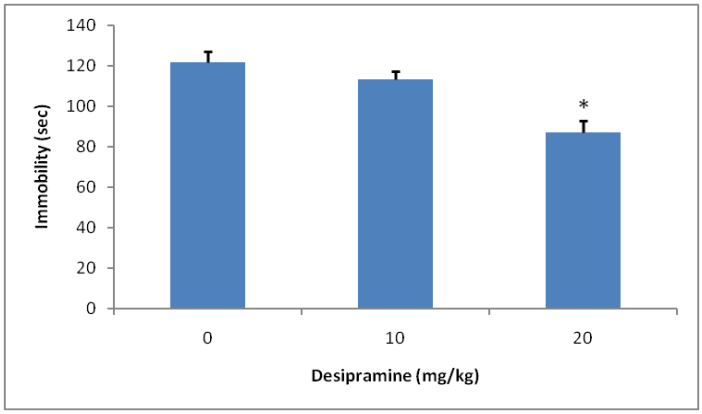

Fig. 2: Dose-response of the tricyclic antidepressant positive control, desipramine, in the forced-swimming test. DMSO (10%) served as the vehicle control (0 mg/kg). Vehicle control or desipramine (n=6 per group) was injected 30 min before the test. Data were evaluated using analysis of variance, followed by LSD post-hoc tests, where * p<0.001 was considered significantly different from the vehicle control. The data are expressed as mean±SEM

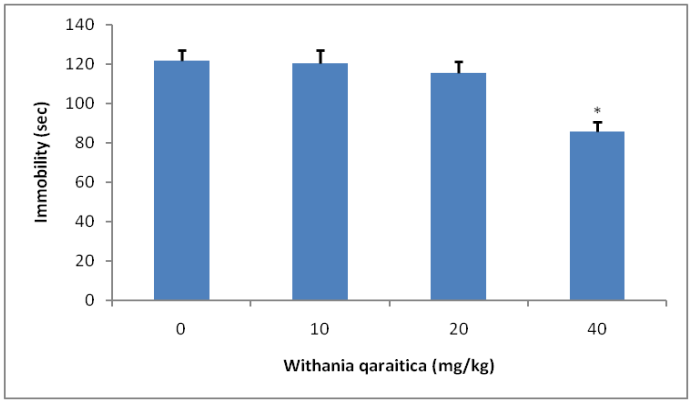

Fig. 3: Dose-response of Withania qaraitica (DMSO as vehicle control) in the forced-swimming test. DMSO (10%) served as the vehicle control (0 mg/kg). Vehicle control or *Withania qaraitica* extract (n=6 per group) was injected 30 min before the test. Data were evaluated using analysis of variance, followed by LSD post-hoc tests, where * p<0.001 was considered significantly different from the vehicle control. The data are expressed as mean±SEM

The SSRI antidepressant positive control, citalopram, reduced immobility time in TST (fig. 4). LSD post-hoc analysis indicated a reduction of 43% immobility was observed with citalopram at a dose of 10 mg/kg with respect to vehicle control (p<0.001). The TCA positive control desipramine also significantly reduced immobility in TST (fig. 4). LSD post-hoc analysis showed a reduction of 56% immobility was observed with desipramine at a dose of 20 mg/kg with respect to vehicle control (p<0.001). A dose-dependent reduction of immobility was observed with the methanolic extract of *Withania qaraitica* in TST (fig. 5). Furthermore, LSD post hoc analysis demonstrated a 34% reduction in immobility with the extract of *Withania qaraitica* at a dose of 40 mg/kg (p<0.005) in comparison to vehicle control.

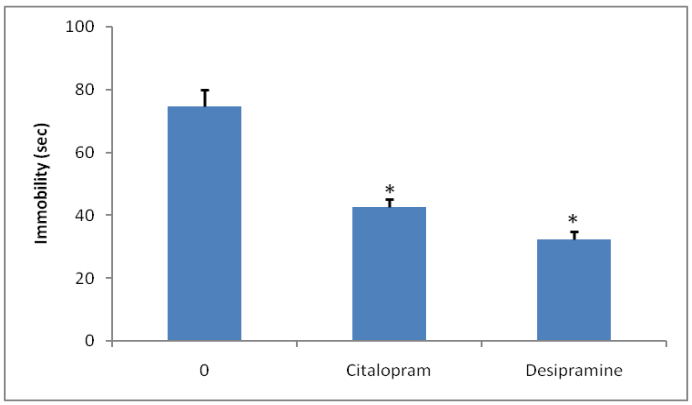

Fig. 4: Effects of citalopram (10 mg/kg) and desipramine (20 mg/kg) in the tail suspension test. DMSO (10%) served as the vehicle control (0 mg/kg). Vehicle control, citalopram or desipramine (n=6 per group) was injected 30 min before the test. Data were evaluated using analysis of variance, followed by LSD post-hoc tests, where *p<0.001 was considered significantly different from the vehicle control. The data are expressed as mean±SEM

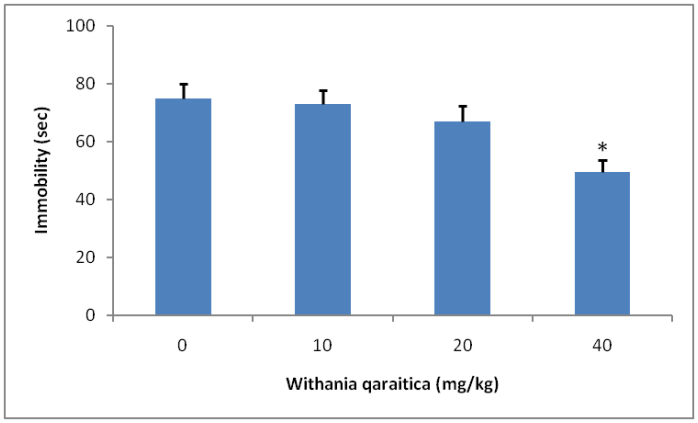

Fig. 5: Dose-response of withania qaraitica (DMSO as vehicle control) in the tail suspension test. DMSO (10%) served as the vehicle control (0 mg/kg). Vehicle control or Withania qaraitica extract (n=6 per group) was injected 30 min before the test. Data were evaluated using analysis of variance, followed by LSD post-hoc tests, where *p<0.005 was considered significantly different from the vehicle control. The data are expressed as mean±SEM

DISCUSSION

The frequency of neurological disorders like depression and anxiety is very high in the world's population nowadays and is accompanied by considerable morbidity. It is necessary for modern times to address these problems and formulate effective medications. Although numerous medicines are available, all have some limitations, and it is essential to go for alternative drugs to treat these ailments. The physiological stages of depression are multifactorial, and one of the possible factors of depression is increased oxidative stress [27]. As soon as the human body fails to manage the oxidative mediators due to an imperfect antioxidant system, oxidative stress is produced. In that situation, various metabolic and other health-related disorders have evolved.

Available data indicates that oxidative stress damages the brain because of its higher oxygen intake and weaker defense system against reactive oxygen species. The production of reactive oxygen species is a primary cause of neurodegeneration, and its contribution to the progression of depression is obvious [28]. It is authenticated that plants from the *Withania* genus have the ability to influence non-enzymatic and enzymatic antioxidants [29]. In view of these properties and antidepressant effects of extracts from *Withania* genus, present investigators were inspired to study the antidepressant effect of *Withania qaraitica*, an indigenous plant available in Dhofar Governorate of Oman.

The aim of the current study was to evaluate the antidepressant-like action of a methanolic extract of *Withania qaraitica* using behavioral models in mice. Instituting an accurate animal behavioral model to identify valid antidepressant treatments is not an easy task [30]. In fact, FST and TST in rodents are commonly recognized animal models for the evaluation of antidepressant-like behavior. Immobility has been assessed in these behavioral tests, which are comparable to human behavior. Available reports indicate that antidepressants have the ability to decrease the time of immobility in experimental animals [24]. Immobility seen in mice during FST is a mandatory stress believed to imitate a state of hopelessness that is supposed to mimic depressive ailments in the human population. Additionally, antidepressant drugs are capable of reducing immobility time. A substantial association between the clinical efficiency of antidepressants and their effectiveness in the FST is seen, which is unique to this model [31].

The FST in the current study showed that the methanolic extract of *Withania qaraitica* confirmed the antidepressant prospectives and has the potential to be the drug of choice against depression. The ability of *Withania qaraitica* to demonstrate antidepressant-like activities in the FST may be explained by its continuing decrease in immobility time with increasing dose. Moreover, it was found to retain the antidepressant activity of the methanolic extract of *Withania qaraitica* in both FST and TST, comparable to that of typical antidepressants like citalopram and desipramine. Citalopram demonstrates its actions by inhibiting serotonin (5-HT) reuptake, whereas the mechanism of action of desipramine is to inhibit the reuptake processes of 5-HT and norepinephrine (NE). These two chemical antidepressants have been used as standard drugs in various studies. The favorable outcome of citalopram and desipramine in the FST appears as a consequence of increased accessibility of these neurotransmitters at the postsynapse after the inhibition of the reuptake process [32, 33].

Preliminary postulates of depression have been articulated more than a half-century ago, suggesting that the indicators of depressive symptoms are due to the insufficiency of functional neurotransmitters like 5-HT, dopamine (DA), NE at synaptic cleft [34]. Studies have also shown that plant extracts can enhance the state of non-specific resistance in several stress parameters and reduce the sensitivity to stress via the regulation of monoamines. This may provide an indication that plant extracts can express their potential antidepressant-like activities through the reinstatement of monoaminergic neurotransmitters at a normal level [35].

Withania qaraitica, an indigenous plant in Oman, may share chemical constituents with other species of the *Withania* genus. The probable mechanisms by which these plants produce beneficial effects on stress, anxiety, depression, and insomnia may be due to these constituents. Some of these constituents have the antioxidant potential [36, 37]. Recent investigators have indicated that there is considerable overlap between the antioxidant potential and antidepressant activities. It is also reported that plant species from the *Withania* genus improve oxidative stress and, as a result, increase the level of serotonergic neurotransmitters in the synapses [38].

Additionally, some species of the *Withania* genus, such as *Withania coagulans*, *Withania somnifera,* and *Withania obtusifolia*, all of which have antioxidant activity as well as antidepressant-like actions [38, 39]. Hence, there is a possibility that the methanolic extract from *Withania qaraitica* will demonstrate antidepressant potential by modulating its antioxidant properties. Nevertheless, more research studies warrant explaining the mode of action of *Withania qaraitica* in the central nervous system.

CONCLUSION

This study reported the antidepressant-like action of *Withania qaraitica* in two typical antidepressant models, the forced-swimming test (FST) and the tail suspension test (TST). Results from this study indicated that the methanolic extract of *Withania qaraitica* showed substantial antidepressant action similar to the typical antidepressant drugs citalopram and desipramine. More investigations are needed to explain the mode of action of *Withania qaraitica* in producing beneficial effects against depression. The results of the current research also warrant additional studies to

identify the active constituents in *Withania qaraitica,* that produce antidepressant-like effects.

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AUTHORS CONTRIBUTIONS

Jamaluddin was the principal investigator and was instrumental in bringing out the concept and in designing the study. He also interpreted the results and wrote the manuscript. Reem conducted the studies, interpreted the results, and helped with manuscript writing. Afaf and Sadri helped with study design and manuscript writing.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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