ANTIDEPRESSANT ACTIVITY OF POLYPHENOL FRACTION OF *ARTEMISIA ABSINTHIUM* L.

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Summary

Artemisia absinthium (*Asteraceae*) is widely used in traditional medicine. Because of high polyphenol and flavonoids contents of *A. absinthium*, its polyphenol fraction nominated for assay of antidepressant activity. Polyphenol fraction of *A. absinthium* aerial part at flowering stage was screened for antidepressant activity. The activity was determined by forced swimming (FST) and tail suspension tests (TST). Polyphenol fraction showed weak antidepressant activity in FST. The extract shortened remarkably the immobility period during the FST and TST and exhibited a dose dependent activity. But test groups weren't significantly different form control group ($P > 0.05$). Extract at 500 mg kg-1 showed similar activity as imipramine 10 mg kg-1 ($p > 0.05$) in TST. In both experimental models, total extract showed better antidepressant activity than polyphenol fraction. This observation can be due to the possible additive effects of other compounds (except polyphenolic compounds) present in extract.

Key words: Antidepressant, *Artemisia absinthium*, Polyphenol fraction, Forced swimming test, Tail suspension test.

Introduction

Depression is the most common of the affective disorders; it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions [1,2]. Depression constitutes the second most common chronic condition in clinical practice [3]. The causes of depression vary. Psychosocial factors, such as adverse living conditions, can influence the onset and persistence of depressive episodes. Genetic and biological factors also play a part. It is estimated that 5.8% of men and 9.5% of women will experience a depressive episode in any given year. These prevalence figures can, however, vary across different populations. It is estimated that 121 million people currently suffer from depression and it will become the second leading cause of premature death or disability worldwide by the year 2020 [4]. Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing [5].

Although there are many effective antidepressants available today, the current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated. This necessitates the development of newer and more effective antidepressants from traditional medicinal plants whose psychotherapeutic potential has been assessed in a variety of animal models [6]. Many plant extracts and different classes of phytochemicals have been shown to have useful activity [7-9]. So, search for discovering of natural originated drugs with fewer side effect, has been increasing ever since. Today many people prefer to use medicinal plants rather than chemical drugs.

Artemisia (*Asteraceae*) is one of the largest and most widely distributed genuses of the approximately 60 genera in the Anthemideae tribe. This genus comprises more than 400 species, and is predominantly distributed in the northern temperate region of the world. Thirty-four of them have been reported in Iran and some are endemic [10,11]. Previously, antimalarial, antiviral, antitumoral, antipyretic, antihemorrhagic, anticoagulant, antianginat, antioxidant, antihepatitis, antiulcerogenic, antispasmodic, antidepressant, anticomplementary and interferoninducing activities of some substances from this genus have been reported [12-14]. *A. austriaca* and *A. spicigera* are odorous herbs used as antiseptics and stomachics in folk medicine [15]. *A. vestita* is an herb that has been widely used in traditional Tibetan and Chinese medicine for treating inflammatory diseases such as rheumatoid arthritis and contact dermatitis anepsis [16]. *A. dracunculus* has been used orally as an antiepileptic in whom its anticonvulsant potential has been assessed [17]. Studies on *Artemisia* have ascertained the presence of coumarin, acetylenic compounds and sesquiterpene lactones [12]. Previous study showed acetylcholinesterase activity of *A. asiatica* alkaloids [18]. *A. tschernieviana* is rich in monoterpenes and ρ-cymene, β-pinene, α-pinene, γ-terpinene, (Z)-cis-ocimene and α-cadinol [12]. Also previously, its antimicrobial activity has been reported [12]. *A. absinthium* L. (wormwood) is an aromatic-bitter herb, used as traditionally in Iran. This species known to possess many ethnomedical and biological properties [14]. Its antioxidant activity have been reported recently [14, 19,20]. There is published scientific data available for antidepressant activity of this plant. Our previous study showed that *A. absinthium* (total crude extract) has good antidepressant activity [14]. Because of high amount of polyphenolic compounds in this plant [14], polyphenolic fraction of *A. absinthium* was chosen for evaluation of the antidepressant activity. The antidepressant activity was determined by forced swimming test (FST) and tail suspension test (TST) in order to understand the importance of this fraction.

Materials and methods

Animals

Swiss albino mice (20 ± 2 g) of either sex were randomly housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1°C and 45–55% relative humidity, with a 12 h light: 12 h dark cycle (lights on at 7 a.m.). The animals had free access to standard pellet and water *ad libitum*. Each animal was tested once. All of the experiment conducted between 8:00 and 14:00 h. Mice were divided into five different groups (n = 5 per group) and tested in FST and TST. The experiments were conducted in accordance to the ethical guidelines regarding investigation with laboratory animals (NIH guidelines of the Care and Use of Laboratory animals) and were also approved by the Ethical Committee for Animal Experimentation of Mazandaran University of Medical Sciences.

Plant material and preparation of polyphenol fraction

Aerial parts of *A. absinthium* L. at flowering stage was collected from Golestanak protege area central Elburz, Iran, in summer 2009 and confirmed by Dr Bahman Eslami. The aerial part was dried at room temperature and coarsely ground before extraction. Polyphenols were extracted from fruit, according to method of Sun et al., [21]. The extraction was performed two times at 20 °C in a shaking incubator (115 W, Promax 1020, Heidolph, Germany). Extracting time was 30 min, and extracting solvent was 100 ml of methanol/acetone/water (3.5/ 3.5/ 3) containing 1% formic acid. Extracts were combined and filtered through two layers of cheesecloth. The collected filtrate was centrifuged for 15 min at 7000 g. The supernatant was collected and evaporated under vacuum at 35-40°C to remove methanol and acetone. Lipophilic pigments were then eliminated from the aqueous phase by two successive extractions in a separatory funnel with a twofold volume of petroleum ether. The aqueous phase was collected and further extracted three times by equal volume of ethyl acetate in the separatory funnel. Three ethyl acetate phases were collected and concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Forced swimming test

The mouse was dropped into a glass cylinder (20 cm in height and 12 cm in diameter) containing 8-cm-deep water at 24 - 25°C and left there for 6 min. The duration of mobility was recorded for a period of 5 min [22,23]. Control group was treated with solvent. The other groups of mice received an interperitoneal (i.p.) injection of extracts (300, 400 and 500 mg kg^{-1}) in Tween 80 plus 0.9% (w/v) saline solution and imipramine (15 mg kg^{-1}), 1 h before the experiment. Imipramine was utilized as positive control of the test.

Tail suspension

Male mice weighing 20 -25 g were used preferentially. They were housed in plastic cages for at least 10 days prior to testing in a 12 h light cycle with food and water freely available. Animals were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Groups of 5 animals were treated with the extract (300, 400 and 500 mg kg^{-1}) by i.p. injection 30 min prior to testing. For the test, the mice were suspended on the edge of a shelf, 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of mobility was recorded for a period of 5 min. Mice were considered immobile when they hang passively and completely motionless for at least 1 min. Imipramine (15 mg kg^{-1}) was used as positive control of the test [24,25].

Statistical analysis

Experimental results are expressed as means ± SEM. All measurements were replicated three times. The data were analyzed by an analysis of variance, one way ANOVA with Tukey post test.

Results and Discussion

There are many publish papers that showed polyphenolic compounds such as flavonoids have antidepressant activity [26-30]. Because of high polyphenol and flavonoids contents of *A. absinthium*, its polyphenol fraction nominated for assay of

antidepressant activity. Behavioral despair was proposed as a model to test for antidepressant activity. The forced swim test (FST) was developed by Porsolt and colleagues in the rat and subsequently, in the mouse [31]. This test is the most widely used tool for assessing antidepressant activity pre clinically [32]. The swimming test has been extensively employed to evaluate the effect of various agents on the central nervous system, such as anti-depressants, sedative-hypnotics, psychostimulants, euphorics, nootropics, adaptogens, etc [14]. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Table 1 showed the result of effect of extract on the duration of immobility during forced swimming test. The extract shortened the immobility period during the forced swimming test. Although, the effect was dose dependent but statistical analysis showed that there weren't significantly different between test groups and control group ($P > 0.05$). Imipramine 15 mg/kg, significantly shortened immobility period in comparison with control ($P < 0.001$).

The tail suspension test has been described by Steru et al. [33] as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair which in turn may reflect depressive disorders in humans. The tail suspension test is a facile method evaluating potential antidepressants [14]. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail. Tail suspension test represents the behavioral despair model, claimed to reproduce a condition similar to human depression. The test is based on the observation that animals, following initial escape oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e. behavioral despair) or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli. It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity [34]. TST detects the anti-immobility effects of a wide array of antidepressants, including tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOI), electro-convulsive shock (ECS), and even atypical antidepressants [25].

Extract showed dose dependently decreased the immobility time but there weren't statistical significant between extract and control mice ($p > 0.05$, Table 1). Imipramine 15 mg/kg, significantly shortened immobility period in comparison with control ($P < 0.01$).

It should be noted that in both experimental models the better antidepressant activity observed in the extract can be due to the possible combination of two effects: a direct effect due the active compounds and an indirect (or additive) one due to other compounds present in extract. This latter effect can not account for in polyphenol fraction (single group) and could be one explanation of the surprisingly better antidepressant activity observed for crude plant extract compared to polyphenol fraction isolated from extract.

Table 1. Antidepressant activity of *A. absinthium* extract in FST and TST.

Group	Dose (mg/kg)	Duration of mobility (s), FST	Duration of mobility (s), TST
Control	-	90.00 ± 17.07	94.200 ± 11.98
Extract	300	108.60 ± 17.72	142.40 ± 25.29
	400	119.60 ± 28.20	151.40 ± 21.19
	500	179.00 ± 18.32^{ns}	147.60 ± 24.57 ^{ns}
Imipramine	15	236.00 ± 23.91 ***	225.40 ± 22.00 **

[a] Data are expressed as mean ± SEM (n = 5).
Groups are different from control group (^{ns} P>0.05 ,**P<0.01, ***P<0.001)

Conclusion

The finding of the present investigation suggests the weak antidepressant activity of *A. absinthium* polyphenol fraction in both FST and TST models of depression. *A. absinthium* reduced the immobility period in both FST and TST. However, further studies and other fractionation are necessary for complete understanding the antidepressant activity of *A. absinthium*. Such identified potential and natural constituents could be exploited as cost effective food additives for human and animal health.

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