International Food Research Journal 21(6): 2273-2277 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antidepressant activity of a polyherbal mixture in mice

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Article history

Received: 8 December 2013
Received in revised form: 6 March 2014
Accepted: 12 March 2014

Keywords

Forced swim test
Tail suspension test
Depression
Open-field test
Polyherbal mixture
Mice

Abstract

A polyherbal product composed of natural royal jelly, *Panax ginseng* root Extract, Clove powder, Clove oil, Cinnamon oil, Ginger oil, *Nigella sativa* oil and Capsicum powder have been shown to have antioxidant, antistressor, antianxiety and antidiabetic effects. The present study was undertaken to investigate the effect of this polyherbal mixture on depression. Swiss albino mice of either sex (20 – 30 g) were used in this study. Five groups of mice were treated with different treatments (corn oil 10 ml/kg, Xtend® 70, 140 & 280 mg/kg, sertraline 5 mg/kg per os) for 30 consecutive days. Experiments were done on days 1, 15 and 31 by employing two experimental models , the Forced Swim Test (FST) and Tail Suspension Test (TST) to determine the effects of Xtend® on duration of immobility. The results showed that Xtend® in a dose dependent manner significantly reduces the duration of immobility ($p < 0.05$) compared to control in FST and TST. However, these treatments did not affect the number of crossing and rearing in the open-field test. There were no signs of acute toxicity observed for Xtend® in ATS. Results obtained from the present study suggested that, the polyherbal mixture Xtend® exhibited antidepressant – like effects and indicated the potential use of this polyherbal formulation as an adjuvant in the treatment of depression.

Introduction

Depression is considered as an effective disorder characterized by change in mood, lack interest in the surroundings, psychomotor retardation and melancholia. It is a public health problem that can occur to anyone and are likely to occur on adults in between the age of 20 – 50 years old with no relations to race, education status or income (WHO ,1998; Khandelwal *et al*., 2001). According to World Health Organization, depression affects about 121 million people worldwide and it is among the leading cause of disability (YLDs). Depressions are often results from a combination of factors such as genetics, biochemical, environmental and psychological factors and sometimes it can appear without apparent reason or triggers (NIMH, 2008).

Several classes of antidepressants are used to treat depressions but they have side effects such as blurred vision, restlessness, sexual problems agitation and suicidal thoughts (Kahn *et al.*, 2001; NIMH, 2008). To reduce the impact of depression; there is an urge to provide a cost effective treatment to the public. With the increased incidence of depression recently, natural herbs that have antidepressant effect have again more attention as alternative treatment for depression (Tripathi, 2008; Sudhakar *et al*., 2010). The present study, was carried out to investigate the effect of a polyherbal mixture Xtend® in treating depressed mice by employing Forced Swim Test (FST) and Tail Suspension Test (TST). The standard drug, sertraline which is a selective serotonin reuptake inhibitor was used as a positive control to compare the efficacy of a polyherbal Xtend® as antidepressant.

Materials and Methods

Experimental animals

Swiss albino mice of either sex (20 – 30 g), obtained from the Animal House, Faculty of Veterinary Medicine, University of Alexandria, Egypt, were used in this study. Food and water were provided ad libitum and animals observed daily for signs of toxicity and behavioral changes.

Test drugs and chemicals

Xtend®, a commercial polyherbal mixture composed from the following ingredients: Natural royal jelly 70 mg, *Panax ginseng* root extract 50 mg, Clove powder 63 mg, Clove oil 37 mg, Cinnamon oil 50 mg, Ginger oil 50 mg, *Nigella sativa* oil 25 mg and Capsicum powder 10 mg and sertraline (Sertral®) was used as a reference drug , were obtained from SAFE Pharma (a branch of PHARCO Corporation, Alexandria,

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Egypt).

Experimental procedures

The protocol of this study was approved by Ethics Committee of Faculty of Veterinary Medicine, University of Alexandria, Egypt. All experiments were performed in accordance with GCP, as revealed by Helsinki Declaration and approved by World Health Association (WHA).

Drug administration

The contents of several Xtend® Soft Gelatin Capsules was mixed well and diluted with corn oil (1: 2 v/v) and given orally to the mice through orogastric tube (30-G) in accordance with the following general schedules and doses were calculated according to Paget and Barnes (Paget and Barnes, 1964). On the day of the experiment, the animals were divided randomly into control and experimental groups (n = 6). Group 1 received the vehicle, corn oil (10 ml/ kg) and served as the control group, groups 2, 3 and 4 received the test drug (Xtend®) in doses of 70, 140 and 280 mg/kg and group 5 received the standard drug sertraline (5 mg/kg) per os. Drugs / vehicle was administered to the animals 60 minutes prior to the behavioral evaluation in acute study. For subchronic and chronic study a new set of animals were used. They were grouped as in acute study and were administered the drugs / vehicle for a period of 14 and 30 days, respectively. Behavioral evaluation was carried out 60 minutes post drug / vehicle administration on 15th and 31st day, respectively. The antidepressant activity of the test drug was evaluated using the following experimental models of depression TST and FST.

Acute toxicity study

Swiss albino mice were fasted for 3 h prior to the experiment. Acute toxicity study was carried out by giving 4 doses (0.25, 0.50, 0.75 and 1.00 g/kg per os) of the polyherbal to Xtend® different mice groups (n = 5). The mortality and general behavior of the mice was observed for 48 h with special attention to the first 30 min and the first 4 h after the single oral administration then periodically during the 48 h and daily for a total of 2 weeks.

Forced swim test

Forced swim test was proposed as a model to test for antidepressant activity by Porsolt *et al.* (1977). Mice were forced to swim individually in a glass jar (25 x 72 x 25 Cm^3) containing fresh water of 15 cm height and maintained at 25°C (± 3°C). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility periods were studied after administering drugs in separate groups of animals. Each animal was used only once.

Tail suspension test

The total duration of immobility induced by tail suspension was measured according to the method described earlier (Steru *et al*., 1985; Dhingra and Sharma, 2006), as a means of evaluating potential antidepressants. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility was recorded during a 6 min period to be immobile when it did not show any movement of body and hanged passively.

Open-field test (OFT)

To assess the effects of the test materials on locomotor activity, the mice divided equally into 4 groups, 6 mice in each group and treated 30 days as follow, the first group given a vehicle 10 ml/ kg and served as a control, the second and third groups given a polyherbal mixture 140, 280 mg/kg, respectively and the fourth group given sertraline as previously mentioned. After the last dose, the mice were individually housed in a rectangular container made of dark polyethylene (40-40-25 cm^2) in a dimly lit room equipped with a video camera above the center of the floor, as described previously (Kim *et al*., 2007) with slight modification, and locomotor activity was measured. The animals were allowed to adapt for 1 h in the container, and the distance they traveled was recorded during the last 10 min of a total 20 min test. The locomotor activity was measured in centimeters. The floor surface of each chamber was thoroughly cleaned with 70% ethanol between tests.

Statistical analysis

The means ± S.E.M. values were calculated for each group. The data were analysed using one-way ANOVA followed by Dunnet's multiple comparison test. $P < 0.05$ was considered to be statistically significant (Steel and Torrie, 1960).

Results

Acute toxicity study

Non of the mice used in this study showed signs of acute toxicity during the 48 h observation. The general behavior of the mice was normal. Mice did not show any problem in locomotion, reacted normally

Table 1. Effect of oral administration of a polyherbal product Xtend® on immobility time in the forced swim test using mice

Group (Drug Treatment)	Duration of Immobility (Sec.) Acute Study	Sub chronic Study	Chronic Study
Group 1 (vehicle 10 ml/kg)	117.83 ± 1.74	118.00± 1.21	121.50 ± 1.62
Group 2 (Xtend® 70 mg/kg)	108.17 ± 3.32 (8.20)[a]	86.83 ± 3.70* (26.42)	79.67 ± 3.03* (34.43)
Group 3 (Xtend® 140 mg/kg)	91.33 ± 3.08* (22.49)	76.17 ± 1.85* (35.45)	70.83 ± 32.73* (41.70)
Group 4 (Xtend® 280 mg/kg)	86.67 ± 3.88* (26.44)	73.67 ± 2.40* (37.57)	66.00* ± 5.35* (45.68)
Group 5 (Sertraline 5 mg/kg)	74.5 ± 4.60* (36.77)	61.17 ± 2.82* (48.16)	57.67 ± 1.60* (52.53)

Values are expressed as mean ± S.E. (N = 6).
* Significantly different compared to control (P < 0.05).
[a] In parences , percentage of reduction in immobility.

Table 2. Effect of a polyherbal product Xtend® on immobility time in the tail suspension test (TST) using mice

Group (Drug Treatment)	Duration of Immobility (Sec.) Acute Study	Sub chronic Study	Chronic Study
Group 1 (vehicle 10 ml/kg)	210.67±1.45	212.16±2.34	211.17±2.27
Group 2 (Xtend® 70 mg/kg)	199.96±4.58 (5.08)[a]	178.86±5.55* (15.69)	173.00±3.28* (18.07)
Group 3 (Xtend® 140 mg/kg)	164.66 ±4.03* (21.84)	160.50 ±2.36* (24.35)	155.33 ±2.75* (26.44)
Group 4 (Xtend® 280 mg/kg)	142.33±2.48 (32.44)	138.83±3.21* (34.56)	129.00 ±1.39* (38.91)
Group 5 (Sertraline 5 mg/kg)	133.00±5.02 (36.87)	120.33±3.64 (43.28)	111.00 ±2.94* (47.44)

Values are expressed as mean ± S.E. (N = 6).
* Significantly different compared to control (P < 0.05).
[a] In parences , percentage of reduction in immobility.

Table 3. Effect of oral administration of a polyherbal product Xtend® on the open- field test using mice

Group	Dose (mg/kg)	Number of crossing	Number of rearings
Control	vehicle*	51.3±2.4	11.7±0.9
Xtend	140	49.2±1.5	11.9±1.1
Xtend	280	52.5±1.2	12.2±1.0
Sertraline	5	55.1±2.8	11.5±1.2

Values are expressed as mean ± S.E. (N = 6).
* (10 ml/kg)

to noise and pinch, the tail was flexible. There was no mortalities up to the dose 1 g/Kg.b.wt.

Effect of a polyherbal Xtend® on the immobility period in FST and TST

The result of the antidepressant effects of a polyherbal Xtend® was presented in tables 1 and 2. A significant ($p < 0.05$) decrease in the standard drug sertraline and the polyherbal Xtend® (given for one day) in dose-dependent manner, indicating significant antidepressant like activity. Among three doses administered for one day (Acute study), a dose of 280 mg/kg per os of Xtend® showed most a potent antidepressant like activity as indicated by highest decrease in immobility period. On the other hand, low doses (70 mg/ kg per os) did not show significant effect on immobility period when compared to control group. While, all doses studied were significantly decreased the immobility period in a dose-dependent manner in both subchronic and chronic study models compared to control. The efficacy of Xtend® (140 and 280 mg/kg) was found to be comparable to sertraline 5 mg/kg (Tables 1 and 2).These treatments did not affect the number of crossing and rearing in the open-field test (Table 3).

Discussion

Depression is a neurological disorder that is widely prevalent to modern fast paced life. Stressful lifestyle facilitates the evolution of depressive disorder as the stress can influence the function of central nervous system by altering a number of neurotransmitters, endocrine and neuroendocrine systems (Gopalakrishna *et al.*, 2010). The most lethal complication of depression is the suicidal behavior (Khandelwal *et al*., 2001; Sudhaker *et al.*, 2010). Despites the widely use of associated with adverse effects and limitations. Along with the classical theory of decrease in the neurotransmitter levels in the brain leading to the pathogenesis of clinical depression, recent studies have also shown the involvement of oxidative stress in the phenomenon (Sarandol *et al*., 2007). Depression is usually treated with a combination therapy and medications as well as lifestyle changes. Certain foods and ingredients have been linked to lessening depression including antioxidants. Antioxidants neutralize and reduced mental functioning. The search for a natural product with fast onset of action, wide safety margin and less wide side effects has come to attention. The effective components of herbs that have antidepressant-like effect includes flavonoid, oligosaccharide, polysaccharide, alkaloid, organic acid (Velraj *et al*., 2009). The present study was designed to elucidate the effect of a polyherbal mixture in Xtend® treating depression using FST and TST in mice. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs (Porsolt *et al*., 1977; Steru *et al*., 1985; Detke *et al*., 1995). In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly, 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 (Willner, 1984). It has been reported that the TST is less stressful and has higher pharmacological sensitivity than FST (Thierry *et al*., 1986).

Acute toxicity study

ATS is the first in the toxicological analysis of herbal drugs (Mustaffa *et al*., 2010). In ATS, all of the mice survived during 48 h observation. The general behavior of the mice during the study was found to be normal. The oral lethal dose 50 (LD 50) of Xtend®

was found to be more than 1 g/kg.

Forced swim test

FST is a drug screening test for antidepresents, it is able to detect activity of a broad spectrum of clinically effective antidepressants (Cryan *et al*., 2005). FST has a higher degree of predictive validity to major class of antidepressants such as Tricyclic Antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), a Typical Antidepressants Selective Serotonin Reuptake Inhibitors (SSRIs) and electronoconvulsive therapy (Woode *et al*., 2010). This preclinical test evaluates the behavioral despair, a measure of failure to seek escape from an aversive stimulus in the tested mice. The immobility time is considered as the measure of depression–like behaviors, whereas, the reduction in the duration of immobility is characterized as antidepressant response (Woode *et al*., 2009; Woode *et al*., 2010). Oral administration of Xtend® for 1, 14 and 30 days produced significant effect in mice when compared with control.

Tail suspension test

TST can detect a broad spectrum of antidepressant irrespective of their underlying mechanism (Cryan *et al*., 2005). TST was reported to have greater sensitivity in detecting SSRIs activity compared to FST (Cryan *et al*., 2005; Woode *et al.*, 2010) and less stressful than FST (Dhingra and Sharma, 2006; Velraj *et al*., 2009). Oral administration of Xtend® has significant result; it reduced the immobility time in a dose-dependent manner. The reduction of immobility time at a dose level of 280 mg/kg, for 14 and 30 days was comparable to sertraline 5 mg/kg a SSRIs antidepressant drug prescribed as a first line treatment for depression.

In the forced swim test and tail suspension test, false positive results can be obtained by some drugs, which decrease time by stimulating locomotor activity (Bourin *et al*., 2001). Therefore, the effect of tested materials on locomotor activity was evaluated by open-field test in the present study. The results showed that these treatments did not affect the number of crossing and rearing in the open-field test. This suggested that the decrease in the immobility induced by the tested materials in the forced swim test and tail suspension test was unrelated to the psychostimulant effect of the tested materials. In other words, the antidepressant-like effect of a polyherbal mixture, xtend was confirmed. Exact mechanism underlying the antidepressant action cannot be concluded at the moment due to the presence of large number of phytochemicals in the polyherbal mixture Xtend®. However the antidepressant activity may be attributed to the presence of tannic acid and ascorbic acid in the mixture. Tannic acid has been shown to be a non selective inhibitor of monoamine oxidase, therapy increasing the levels of monoaminergic neurotransmitters in the brain. Chronic use of Gallic acid has been shown to have a neurotropic action on the hypothalamus (Dar and Khatoon, 1997). Another possible mechanism of action is the attenuation of oxidative stress produced during depression, by the polyphenols and tannic acid present in Xtend® (Gad *et al*., 1963; Muthu Kumara and Huat, 2001). Previous study on our lab showed a higher antioxidant activity of Xtend® (El-Ashmawy and A-Mohsen, 2011). It is well known that this herbal mixture contains several flavonoid compounds (Kramer, 1985; Evans, 1989). In one study on *Ginkgo biloba*, it was shown that the flavonoids – containing leaves extract inhibits mono – amine oxidase enzyme (MAO) (White *et al.*, 1996). In another study on Hypericum perforatum, a significant antidepressant effect on the swimming test (Butterweek *et al*., 2000) was reported. Moreover, flavonoid compounds from this plant have similar chemical structures to known MAO inhibitors. It could be concluded that a polyherbal mixture Xtend® can be used to treat depression. Further research are necessary for study and understanding of the actual mechanism of action involved in the antidepressant activating of a polyherbal mixture Xtend®.

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