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Antidepressant Activity of Aqueous Extract of *Asparagus officinalis* In Mice and Role of Combination of Extract on the Side Effects of Imipramine.

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ABSTRACT

The present study is to evaluate the antidepressant activity of aqueous stems of *Asparagus officinalis L.* and to evaluate the role of combination of extract on the side effects of Imipramine. The study was carried out to assess the antidepressant activity of *Asparagus officinalis* in two animal models of depression. The forced swim and tail suspension tests were performed in Swiss albino mice of either sex after grouping the animals into different groups. Six animals per group were used for the study. Doses of *Asparagus officinalis* used were 0.6 g/kg, 1.5 g/kg and 3.0 g/kg. Distilled water was used as Normal control and imipramine (10 mg/kg) was used as standard. The duration of immobility in seconds was recorded for assessing antidepressant like activity in mice. The study was also conducted to find the role of combination on the side effects of imipramine. Medium dose of *Asparagus officinalis* (1.5 g/kg) was given in combination together with imipramine treatment for fifteen days and the tests for the side effects like anxiety (elevated plus maze and staircase test), hypotension, urine output, weight gain and libido were carried out. The results of the present study indicate that *Asparagus officinalis* has significant antidepressant activity. The literature suggests that the antidepressant effects seen was due to the presence of sarsapogenin; a steroidal saponin present in *Asparagus officinalis*, which may help in increasing the neurotransmitter levels in the brain. It was also observed that medium dose (1.5 g/kg) when given in combination with imipramine improved the side effects. The result of the present study indicates that *Asparagus officinalis* has significant antidepressant activity. It was also observed that medium dose (1.5 g/kg) when given in combination with imipramine improved the side effects.

Keywords: Depression, *Asparagus officinalis*, Imipramine, Sarsapogenin.

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INTRODUCTION

Depression is a psychiatric disorder clinically characterized by a low mood, loss of interest, or pleasure in daily activities, and low self-esteem with a high suicidal tendency [1]. It is the most common of the affective disorders and is a major cause of disability and premature death worldwide [2] and is ranked by the World Health Organization as among the most burdensome diseases of society [3]. An important theory to explain the cause of depression is the monoamine hypothesis which suggests that there is impairment of monoaminergic functions and the decrease of serotonin, norepinephrine and dopamine levels [4, 5].

Antidepressants used for the treatment primarily work on brain chemicals called neurotransmitters, especially serotonin and norepinephrine. These drugs have a success rate of about 60%. Moreover, most therapies require several weeks of treatment before improvement of signs and symptoms are observed [6]. Imipramine is a commonly prescribed tricyclic antidepressant for mental depression. Some of the known side effects of this drug include urinary hesitancy/retention, agitation, anxiety, nervousness, daytime somnolence, orthostatic hypotension and sexual dysfunction [7]. Due to this in many instances the patient either shifts to another mode or discontinue the therapy resulting in precipitation of the disease.

A number of single and compound drug formulations of plant origin are mentioned in Ayurveda for the treatment of psychiatric disorders [8]. Herbal medicines such as St. John's wort have been used as alternative therapies for depression [9]. One of the major advantages of herbal medicine is that they are reported to be devoid of many complications in spite being therapeutically active. Some of the herbs reported to possess anti-depressant activity include *Hypericum perforatum, Centella asiatica, Areca catechu, Clitoria ternatea, Curcuma longa, Mimosa pudica* [10].

Asparagus officinalis L., a well-known nutritious and healthy vegetable, has been reported to exhibit strong hepatoprotective and hypolipidemic activity [11]. The crude saponins from the shoots of asparagus have antitumor activity [12]. Its marked and immediate action on the urinary secretion is well known. In another study anxiolytic-like activity through the secretion of cortisol and 5-HT has been reported for *Asparagus officinalis* [13].

Hence, we planned to evaluate the antidepressant effect of aqueous extract of *Asparagus officinalis* in mice and study the role of the extract on some of the major side effects of Imipramine when used in combination.

MATERIALS AND METHODS

FORCED SWIM TEST (FST) or PORSOLT TEST:

Mice weighing 25 g - 30 g of either sex were used for the study. On the fifteenth day, 30 min after the oral drug administration the experiment was carried out. Mice were individually forced to swim inside a vertical cylinder (height: 25 cm, diameter: 12 cm, containing 15 cm of water maintained at room temperature) and the duration of immobility is recorded during the last 4 minutes of a 6 minute test. A mouse is considered immobile when floating motionless or making only those movements necessary to keep its head above the water surface. The water is changed after each test because urine and the other chemicals released by the first mouse will affect the swimming pattern of the next mouse. Antidepressants decrease the immobility time. The test has been validated by most current types of antidepressants [14].

TAIL SUSPENSION TEST (TST):

Mice weighing 25 g - 30 g of either sex were used. Groups of 6 animals were treated orally with the drugs 30 min prior to the testing. Mice were suspended on the metal rod stand 50-75 cm above the table top by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. The animal was considered to be immobile when it did not show any movement of the body and hanged passively. A decrease in the immobility period is indicative of antidepressant like activity [15].

PROCEDURES TO FIND THE EFFECT OF COMBINATION OF EXTRACT ON THE SIDE EFFECTS OF IMIPRAMINE.

Anxiety: The test for anxiety will be done by plus maze and staircase apparatus.

Elevated plus maze test:

Mice were given access to all of the arms and allowed to move freely between them. The animal was placed on the central platform of the maze facing the open arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. The number of entries into the open arms and the time spent in the open arms were used as indices of open space-induced anxiety in mice [16].

Staircase method:

Each mouse was placed individually at the bottom of the staircase for a 5-minute observation period. The number of steps climbed and the number of readings was recorded as anxiety indexes [17].

Blood pressure:

The process begins with the inflation of the Cuff and ends with its complete disinflation. The Start is operated manually. At the end of the process (25 to 35 secs.) the digital value of Systolic, Diastolic and Mean Pressure were displayed.

Sexual Mating behavior test:

The Male and the female in each group was paired and placed in separate cages to determine the influence of treatment on the libido. Frequency and number of attempts made by the male mice and the response shown by the female mice were recorded during the dark phase (18:00 to 22:00 hours) are noted and compared [18].

Urinary retention:

The urine output was measured using standard metabolic cages for mice after the 24 hour metabolism study. Water sources and urine collection were carefully separated to prevent urine dilution by drinking water. Regular food and water were supplied during the experiments. After different treatments the data were compared to find the effect of imipramine and role of combination and extract with Imipramine on urine output.

Weight gain:

Body weight were recorded on the first day and last day of the experiment. The data were compared with the normal control to find the influence of treatment on body mass. It was also compared with the standard group to see whether the levels of body weight has reduced or not.

Animals

Swiss Albino mice weighing 25-30 g of either sex were selected for the proposed study. These animals were procured from Krupanidhi College of pharmacy. They were housed six per cage at room temperature 22°± 2°C in a well-ventilated animal house under 12 hours light and dark cycle.

The Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animal ethical committee clearance (CPCSEA No. 2015/PCOL/03) was obtained for the procurement of animals.

Experimental design:

Swiss Albino Mice of either sex were divided into 6 major groups. Each of the subgroups consisted of six animals as follows:

Group 1: Normal control treated with distilled water.
Group 2: Low dose of *Asparagus officinalis* extract (0.6 g/kg, per orally).
Group 3: Medium dose of *Asparagus officinalis* extract (1.5 g/kg, per orally).
Group 4: High dose of *Asparagus officinalis* extract (3.0 g/kg, per orally).
Group 5: Imipramine group (Imipramine 10.0 mg/kg, per orally for 10 days).
Group 6: Combination group: *Asparagus officinalis* (1.5 g/kg) [13] and Imipramine (10.0 mg/kg, per orally).

Statistical analysis:

The results are expressed as mean ± SEM. Comparison between the treatment group and the standard were performed by one way analysis of variance (ANOVA) followed by Dunnett's multiple range tests. In all tests the criterion for statistical significance are $p < 0.05$.

RESULTS

Forced swim test

The effect of aqueous extract of *Asparagus officinalis* on the duration of immobility was tested by forced swim test (Table-1). A significant ($p<0.01$) dose-dependent reduction in duration of immobility was observed when compared with normal control animals. All the three tested doses (0.6, 1.5 and 3.0 g/kg) exhibited a reduction in immobility and the maximum effect was observed at 1.5 g/kg and 3.0 g/kg. Imipramine at 10 mg/kg also significantly ($p<0.01$) decreased the duration of immobility compared with the control group. When the combination of imipramine (10 mg/kg) and *Asparagus officinalis* (1.5 g/kg) was tested, we observed a further reduction in duration of immobility when compared with normal animals. The combination did not produce any significant alteration when compared with imipramine group.

Table 1: Effect of aqueous extract of *Asparagus officinalis* on duration of immobility in forced swim test

Sl. No.	EXPERIMENTAL GROUPS	DURATION OF IMMOBILITY (Sec)
1.	Normal control	219.48 ± 6.89
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	$166.41 \pm 1.60^{**b}$
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	$120.39 \pm 1.39^{**b}$
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	$116.50 \pm 0.93^{**b}$
5.	Imipramine (10 mg/kg, p.o)	$95.59 \pm 0.84^{**}$
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	$90.34 \pm 0.82^{**}$

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to standard group.

Tail suspension test

The effect of aqueous extract of *Asparagus officinalis* on the duration of immobility was tested by tail suspension test (Table-2). A significant ($p<0.01$) dose-dependent reduction in duration of immobility was observed when compared with normal control animals. All the three tested doses (0.6, 1.5 and 3.0 g/kg) exhibited a reduction in immobility and the maximum effect was observed at 1.5 g/kg and 3.0 g/kg. Imipramine at 10 mg/kg also significantly ($p<0.01$) decreased the duration of immobility compared with the control group. When the combination of imipramine (10 mg/kg) and *Asparagus officinalis* (1.5 g/kg) was tested, we observed a further reduction in duration of immobility when compared with normal animals. The combination did not produce any significant changes when compared to imipramine group.

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Table 2: Effect of aqueous extract of *Asparagus officinalis* on duration of immobility in tail suspension test

Sl. No.	EXPERIMENTAL GROUPS	DURATION OF IMMOBILITY (sec)
1.	Normal control	208.58 ± 1.84
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	170.05 ± 1.20^{**b}
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	130.29 ± 1.37^{**b}
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	124.31 ± 1.34^{**b}
5.	Imipramine (10 mg/kg, p.o)	98.62 ± 1.11**
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	94.59 ± 9.57**

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. *p<0.05, **p<0.01, ***p <0.001 when compared to normal control group. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 when compared to standard group.

Elevated plus maze test

The one-way ANOVA revealed a significant (p<0.01) increase in the time spent in open arms with all the treatment compared with the control. All the three doses of *Asparagus officinalis* increased the number of entries in open arm. 0.6 g/kg of *Asparagus officinalis* and the combination group significantly (p<0.05) increased the number of entries in open arm. Furthermore significance (p<0.01) was shown by medium and high dose of *Asparagus officinalis* .The decreased number of entries and time spent in open arm with imipramine was significantly (p<0.01) increased when imipramine was given in combination with *Asparagus officinalis* (1.5 g/kg) (Table -3). But these changes were not statistically significant for imipramine group.

Table 3: Effect of aqueous extract of *Asparagus officinalis* on anxiety studies in mice using elevated plus maze test

Sl. No.	EXPERIMENTAL GROUPS	NUMBER OF ENTRIES IN OPEN ARM	TIME SPENT IN OPEN ARM (sec)
1.	Normal control	4.33 ± 1.02	46.8 ± 0.089
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	8.5 ± 1.12*	121.35 ± 0.31^{**b}
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	12.83 ± 1.01^{**b}	159.78 ± 0.34^{**b}
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	10.16 ± 0.65^{**b}	157.1 ± 0.34^{**b}
5.	Imipramine (10 mg/kg, p.o)	5.5 ± 0.22	97.68 ± 0.12**
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	8.5 ± 1.12*	119.4 ± 0.65^{**b}

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. *p<0.05, **p<0.01, ***p <0.001 when compared to normal control group. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 when compared to standard group.

Staircase test

Table 4: Effect of aqueous extract of *Asparagus officinalis* on anxiety studies in mice using staircase test

Sl. No	EXPERIMENTAL GROUPS	NUMBER OF STEPS CLIMBED	NUMBER OF REARINGS
1.	Normal control	7.78 ± 0.16	7.5 ± 0.22
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	14.31 ± 0.10**	13.0 ± 0.52^{**b}
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	23.31 ± 0.18^{**b}	16.0 ± 0.58^{**b}
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	20.5 ± 0.30^{**b}	16.66 ± 0.33^{**b}
5.	Imipramine (10 mg/kg, p.o)	15.0 ± 0.19**	9.83 ± 0.60*
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	17.8 ± 0.20^{**b}	12.66 ± 0.61^{**b}

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. *p<0.05, **p<0.01, ***p <0.001 when compared to normal control group. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 when compared to standard group.

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All the treatment groups show that the number of rearings and the number of steps climbed were significantly ($p<0.01$) increased when compared to normal control. Imipramine group showed significantly ($p<0.05$) lower value in the number of rearings amongst all the other treatment groups. The decreased the number of rearings and the number of steps climbed with imipramine was significantly increased when imipramine was given in combination with *Asparagus officinalis* (1.5 g/kg) (Table-4).

Body weight

When we consider the values obtained from the body weight, imipramine group and the combination group has significantly ($p<0.01$) increased the body weight when compared with normal control. Other treatment groups do not show any significant increase when compared with normal control. The increase in body weight with imipramine was significantly decreased when imipramine was given in combination with *Asparagus officinalis* (1.5 g/kg) (Table-5).

Table 5: Effect of aqueous extract of *Asparagus officinalis* on body weight

Sl. No.	EXPERIMENTAL GROUPS	BODY WEIGHT (gm)
1.	Normal control	28.0 ± 0.31
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	28.5 ± 0.17^{b}
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	27.41 ± 0.09^{b}
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	27.96 ± 0.14^{b}
5.	Imipramine (10 mg/kg, p.o)	$30.0 \pm 0.17^{**}$
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	$29.33 \pm 0.30^{**}$

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to standard group.

Blood pressure

The result of the study of the effects of imipramine on blood pressure revealed significant ($p<0.01$) decrease in both systolic and diastolic blood pressure when compared with the normal control. All the other treatment groups have shown a significant ($p<0.01$) increase in the systolic and diastolic blood pressure when compared to the imipramine group. There was no significant rise or fall in systolic blood pressure for the other treatment groups when compared to normal control (Table-6).

Table 6: Effect of aqueous extract of *Asparagus officinalis* on blood pressure

Sl. No.	EXPERIMENTAL GROUPS	BLOOD PRESSURE	
		SYSTOLIC (mm Hg)	DIASTOLIC (mm Hg)
1.	Normal control	121.58 ± 0.23	73.2 ± 0.25
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	123.03 ± 0.62^{b}	$74.51 \pm 0.18^{**b}$
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	120.26 ± 0.32^{b}	$71.5 \pm 0.23^{**b}$
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	122.63 ± 0.20^{b}	72.81 ± 0.29^{b}
5.	Imipramine (10 mg/kg, p.o)	$114.38 \pm 0.56^{**}$	$68.53 \pm 0.25^{**}$
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	120.66 ± 0.49^{b}	72.51 ± 0.17^{b}

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to standard group.

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Urine Output

On analyzing data from the urine output, a significant ($p<0.05$) increase was shown on treatment with *Asparagus officinalis* (1.5 g/kg) when compared to normal control. The increase in urine output were not significant in other treatment groups. The side effect urinary retention was prominent in imipramine group. Imipramine group significantly ($p<0.01$) reduced urine output when compared to normal control. All the other treatment groups has significantly ($p<0.01$) increased the urine output when compared with imipramine group (Table-7).

Table 7: Effect of aqueous extract of *Asparagus officinalis* on urine output

Sl. NO.	EXPERIMENTAL GROUPS	URINE OUTPUT (ml)
1.	Normal control	1.04 ± 0.13
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	1.08 ± 0.11^{b}
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	$1.47 \pm 0.06^{*b}$
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	1.20 ± 0.10^{b}
5.	Imipramine (10 mg/kg, p.o)	$0.48 \pm 0.11^{**}$
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	1.23 ± 0.14^{b}

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to standard group.

Libido

Our observation on the sexual mating behavior showed that the side effect libido was prominent in imipramine group. The number of attempts made by male and female has significantly ($p<0.01$) reduced when compared with normal control. All the treatment groups have shown a significant ($p<0.01$) increase in the number of attempts made by the male mice when compared with the normal control and the imipramine group. The number of attempts made by the female mice significantly ($p<0.01$) reduced in the treatment groups when compared with normal control. A significant ($p<0.05$) increase in the number of attempts made by female mice was seen in *Asparagus officinalis* (1.5 g/kg) when compared with imipramine group. The other treatment groups did not show any significant increase in the number of attempts made by female mice (Table-8).

Table 8: Effect of aqueous extract of *Asparagus officinalis* on libido

Sl. No.	EXPERIMENTAL GROUPS	NUMBER OF ATTEMPTS MADE BY MALE MICE	NUMBER OF ATTEMPTS MADE BY FEMALE MICE
1.	Normal control	11.16 ± 0.60	3.0 ± 0.52
2.	*Asparagus officinalis* (0.6 g/kg, p.o)	$21.5 \pm 0.76^{**b}$	$0.6 \pm 0.21^{**}$
3.	*Asparagus officinalis* (1.5 g/kg, p.o)	$22.66 \pm 0.71^{**b}$	$1.3 \pm 0.21^{**c}$
4.	*Asparagus officinalis* (3.0 g/kg, p.o)	$23.5 \pm 0.85^{**b}$	$1.0 \pm 0.26^{**}$
5.	Imipramine (10 mg/kg, p.o)	$4.5 \pm 0.43^{**}$	$0.1 \pm 0.00^{**}$
6.	**Combination group**: Imipramine (10 mg/kg, p.o) + *Asparagus officinalis* (1.5 g/kg, p.o)	$20.66 \pm 0.71^{**b}$	$1.0 \pm 0.26^{**}$

All values are represented as mean ± SEM, n = 6. **Statistics**: One-way anova followed by Dunnett's multiple range test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to standard group.

DISCUSSION

The present study was carried out to evaluate the antidepressant activity of aqueous extract of *Asparagus officinalis* in two different models of depression in animals. An attempt was also made to study the role of combination of extract on the side effects of Imipramine. Both forced swim test and tail suspension

tests are standard animal models, predictive of antidepressant activity. Since their introduction almost 20 years ago, the tail suspension test and forced swim tests have become the most widely used models for assessing antidepressant activity in animals. These models were based on the fact that animals subjected to the short-term, inescapable and immobile posture will develop stress which will have clinical similarities [19]. *Asparagus officinalis* in this study were tested at 0.6 g/kg, 1.5 g/kg and 3.0 g/kg and was given orally for a duration of 15 days. On the fifteenth day, tail suspension and forced swim test were carried out to study the antidepressant activity. Our observation from the forced swim test and tail suspension test indicated that all the tested doses of *Asparagus officinalis* significantly ($p<0.01$) reduced the duration of immobility compared with normal group and maximum effect was observed at 1.5 and 3.0 g/kg (Table-1). Reports suggest that sarsasapogenin from *Anemarrhena asphodeloides* Bunge (Liliaceae) possess antidepressant activity by mediation of the central monoaminergic neurotransmitter systems [20]. The literature survey revealed that the steroidal saponins (sarsapogenin) are the main biologically active constituents of Asparagus. Since, content of sarsapogenin in Asparagus is found to be 17.80%, the possible mechanism of antidepressant action may be due to the increase of serotonin and noradrenaline levels [13]. The administration of tricyclic antidepressant Imipramine (10 mg/kg) for 10 days showed significant ($p<0.01$) antidepressant activity (Table-1 & 2). Imipramine exerts its antidepressant action principally by inhibiting the reuptake of serotonin and, to a lesser extent, norepinephrine, two important neurotransmitters in the central nervous system, thereby boosting neurotransmission [21]. Co-administration of the medium dose of *Asparagus officinalis* (1.5 g/kg) with Imipramine for 15 days reduced the duration of immobility in the forced swimming test and tail suspension test, when compared to normal and Imipramine group. Combinations may have provided synergistic therapeutic effect.

In the second part of the study, role of *Asparagus officinalis* on the side effects of Imipramine was studied. Imipramine is the first tricyclic antidepressant to be synthesized. It is no longer used as a first-line treatment for depression because of its side effects [21]. It is still, however, the most effective medication for treating resistant depressive states. One of the causes for infrequent use of Imipramine is linked to its undesirable side effects, such as urinary hesitancy/retention, agitation, anxiety, nervousness, daytime somnolence, orthostatic hypotension and sexual dysfunction. In the present study, the side effects of Imipramine like anxiety, hypotension, urinary retention, decrease in libido and weight gain were studied. Imipramine (10 mg/kg) and *Asparagus officinalis (*1.5 g/kg) were given orally in combination for a period of fifteen days to study the role of the extract on side effects of Imipramine. The aqueous extract of *Asparagus* stem exhibited a strong anxiolytic-like effect at dose of 1.5 and 3.0 g/kg in mice, hence 1.5 g/kg dose of the extract was selected for combination studies with Imipramine.

The anxiety studies were conducted in two models viz., elevated plus maze and stair case. In the elevated plus maze test, Imipramine showed a decreased number of entries and time spent in the open arm when considered with other groups (Table- 3). While the data obtained from the staircase test also showed increased anxiety parameters such as decreased number of rearing and steps climbed (Table-4). Chronic restraint stress produces differential gene expression profiles in the hippocampus and amygdala. It is also possible that a single or a set of its related genes is induced by chronic antidepressant treatment such as Imipramine commonly in different brain regions, which in turn facilitates suppression of depression-related behaviors while increasing anxiety-related behaviors [22]. The treatment with the aqueous extract of *Asparagus officinalis* had proved in decreasing anxiety which is evident by increasing the number of entries and the time spent in open arm than the imipramine group. The medium dose of aqueous extract of *Asparagus officinalis* (1.5 g/kg) had shown a significant ($p<0.01$) increase in the number of entries and time spent in the open arm (Table-3). The above mentioned mechanism of *Asparagus officinalis* have contributed to the anxiolytic effects. The combination has shown to decrease the anxiety which was produced with Imipramine.

The changes on the body weight is represented in Table-5, imipramine at the tested dose increased the body weight. Imipramine by blocking H-1 receptors often show greater weight gain potential, possibly through deactivating brain satiety centers. Another mechanism may be related to blockage of anticholinergic sites, which is associated with appetite stimulation [23]. Our observations indicated that *Asparagus officinalis* treated groups showed significant ($p<0.01$) reduction in body weight compared to the normal group. Aqueous extract of *Asparagus officinalis* has strong hypolipidemic and hepatoprotective properties and has significantly reduced the levels of body weight gain in mice [24]. The medium dose of aqueous extract of *Asparagus officinalis* (1.5 g/kg) had shown a significant decrease in the body weight when it was given in combination

with Imipramine. Hence it can be suggested that *Asparagus officinalis* mediated hypolipidemic activity could be responsible for the reduction in body weight when *Asparagus officinalis* was tested in combination with Imipramine.

The effect of *Asparagus officinalis* and Imipramine on the blood pressure is summarized in Table-6. Our data indicated that Imipramine decreased both systolic and diastolic blood pressure in mice. It was also observed that the administration of all the three doses of *Asparagus officinalis* for 2 weeks neither decreased nor increased the systolic and diastolic blood pressure. While comparing with the Imipramine group, combination group normalized the blood pressure, which was decreased by the Imipramine administration. In the literature, it was reported that Imipramine produces hypotension by directly blocking the peripheral α-adrenergic receptor. A precise mechanism for the *Asparagus officinalis* effect on the blood pressure could not be established, the antagonistic effect of *Asparagus officinalis* on the Imipramine mediated changes on B.P cannot be ruled out.

On the urinary output side effects, our data indicated that Imipramine administration for 10 days induced urinary retention. Further, it was observed that the urine output after the administration of *Asparagus officinalis* (0.6 g/kg, 1.5 g/kg and 3.0 g/kg) was increased significantly ($p<0.01$) compared to the Imipramine group. The combination of *Asparagus officinalis* and Imipramine showed significantly ($p<0.01$) increased the urine output compared to Imipramine alone treatment (Table-7). Imipramine has both alpha-agonist and anticholinergic properties and produces urinary retention by decreasing bladder detrusor muscle contraction [25]. *Asparagus officinalis* in the earlier studies was reported to possess diuretic action, the same mechanism could be responsible in the present study, where *Asparagus officinalis* either alone or in combination with Imipramine enhanced the urine output.

When we consider the side effects of Imipramine on sexual dysfunction, decreased libido was observed in both male and female mice treated with Imipramine. Alteration in the monoamine levels could be related to this side effect. On the other hand, *Asparagus officinalis* enhanced the libido significantly ($p<0.01$) in the tested doses (Table-8). Earlier studies reported that *Asparagus officinalis* possess aphrodisiac property. We presume that this activity could be responsible for enhancing the sexual activity when *Asparagus officinalis* was tested alone as well as in combination with Imipramine.

So from the above observations, we found that the medium dose of aqueous extract of *Asparagus officinalis* (1.5 g/kg) and high dose of aqueous extract of *Asparagus officinalis* (3 g/kg) when given orally for 15 days produced antidepressant effects. The more promising antidepressant effect was seen when the medium dose of *Asparagus officinalis* (1.5 g/kg) was given in combination with Imipramine. The side effects of Imipramine like anxiety, urinary retention, blood pressure, weight gain and libido were improved when it was given in combination with a medium dose of *Asparagus officinalis*.

CONCLUSION

Asparagus officinalis is a healthy vegetable which is used worldwide. In the present study, mice were treated with the aqueous extract of the stems of *Asparagus officinalis*. We observed that treatment with *Asparagus officinalis* was significantly effective in reducing the duration of immobility in both Forced swim and Tail suspension animal models of depression. It may be concluded that the antidepressant effects seen was due to the presence of sarsapogenin; a steroidal saponin present in asparagus, which may help in increasing the neurotransmitter levels in the brain. When mice were given the medium dose (1.5 g/kg of *Asparagus officinalis*) of the extract along with imipramine, the side effect profile was improved.

In conclusion, the result of the present study indicates that *Asparagus officinalis* has significant antidepressant like activity. Further studies can be carried out so that the extract can be combined effectively with Imipramine to reduce the side effects produced by the same.

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