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Evaluation of the antidepressant activity of aqueous extract of leaves of *Morus alba*

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Abstract
Decrease in the levels of monoamine neurotransmitters and oxidative stress are important factors involved in pathogenesis of depression. *Morus alba* (white mulberry) leaves contain flavonoids which are known to inhibit catechol-o-methyl transferase (COMT) and mono amine oxidase (MAO) and have free radical scavenging property and may prove beneficial for the treatment of depression. The study was done to evaluate the antidepressant potential of aqueous extract of leaves of *Morus alba* and compare it with Imipramine. The study included 48 male swiss albino mice which were divided into four treatment groups of 6 animals each. Antidepressant property was evaluated by using two different models of depression viz. Tail suspension test and Forced swimming test. Mice were injected with normal saline (control), Imipramine (standard), *Morus alba* 100 mg/kg and 200 mg/kg (test drugs) intraperitoneally in both the test models. Duration of immobility was observed for 6 minutes in tail suspension test and for 4 minutes in forced swimming test on separate set of animals. Results were analyzed by ANOVA followed by post hoc tukey's test. Aqueous extract of *Morus alba*, at the above doses significantly reduced the immobility time in both the tests compared to control ($p< 0.001$). The reduction in duration of immobility at the dose of 200 mg/kg was comparable to Imipramine. The study showed that aqueous extract of leaves of *Morus alba* has significant antidepressant activity in acute models of depression.

Keywords: antidepressant, *Morus alba*, Catechol-o-methyl transferase (COMT), Mono amine oxidase (MAO), Imipramine.

1. Introduction
Major depressive disorder (MDD) is a mental disorder common in psychiatric practice, wherein a patient presents with at least one of two major symptoms like constant sadness or anhedonia, accompanied by at least five secondary symptoms for at least two weeks [1]. The secondary symptoms include feelings of worthlessness, difficulty in concentrating, changes in diet & weight, psycomotor agitation/ slowing, fatigue /loss of energy and changes in sleep patterns. This relapsing, remitting illness has recurrence rate more than 40 % over two-year period [2]. It must be distinguished from normal grief, sadness, disappointment, and the dysphoria or demoralization associated with medical illness and from bipolar disorder in which depression alters with hypomania or mania. The condition is often under diagnosed and frequently undertreated [3].

Various drugs are available for the treatment of depression which include monoamine oxidase inhibitors, selective and non-selective monoamine reuptake inhibitors and selective serotonin reuptake inhibitors. These medications work by normalizing the levels of neurotransmitters, notably serotonin and nor-epinephrine. Approximately two-thirds of the depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing [4]. More over these drugs have unusual side effects. The medical need for newer, better-tolerated and more efficacious treatments remains high.

People from different regions of the world have used herbal medicines to alleviate affective disorders for many years. One classical example is the use of St. John's Wort. An increasing number of herbal products have been introduced into psychiatric practice, as alternative or complementary medicines. Ayurveda, the traditional system of Indian medicine mentions a number of plant products which can be used in the treatment of psychiatric disorders. It is perceived that the drugs obtained from plant sources have low side effect profile.

As per literature on the traditional medicine, *Morus alba* possesses various pharmacological properties. The leaves and the roots have been used as a cathartic, analgesic, diuretic, antitussive, sedative, hypotensive for the treatment of edema [5].

It is consumed in some parts of the world as green tea which is supposed to be a health drink.

Decrease in the levels of monoamine neurotransmitters and oxidative stress are important factors in the pathogenesis of neurological and neuropsychiatric disorders. From many experiments, the antioxidant properties of natural products are suggested to have a role in reversing the effects of aging on neuronal communication and behavior [6]. Many flavonoids from St John's Wort could inhibit nitric oxide synthase in blood and cerebral homogenate[7] and are believed to be involved in the antidepressant effects of the plant [8]. Similarly the leaves of *Morus alba* known to contain flavonoids like quercetin which may possibility render the leaves of *Morus alba* the antidepressant property. Quercetin has also been shown to inhibit catechol-o-methyl transferase (COMT) and mono amine oxidase (MAO) in brain [9]. The anxiolytic effect of leaves of *Morus alba* has also been shown in rat models of anxiety [10].

Certain resistant cases of depressiom which do not respond well to modern medicines, may respond when treated with herbal preparations. Adverse effects of antidepressants like blurring of vision, dry mouth, drowsiness and increased sweating can be overcome if alternate medicines prove beneficial in treatment of depression. Hence this study was planned to evaluate the antidepressant activity of its aqueous extract on two different models of depression viz. Tail suspension test and Forced swimming test in Swiss albino mice.

Imipramine is a tricyclic antidepressant. It is a white or slightly yellow crystalline powder freely soluble in water and in alcohol. Since imipramine has a prolonged half-life, once-daily dosage regimens may also be suitable, usually given at night. Imipramine, as the hydrochloride, has also been given by intramuscular injection in the treatment of depression.

Materials and Method

Male swiss albino mice weighing 20-30g were used for the study. The mice were inbred in the central animal house of the Department of Pharmacology, J.J.M Medical College, Davanagere, under suitable conditions of housing, temperature, ventilation and nutrition.

Equipments: Syringes, 2 Stands with a connecting rod at height of 55 cm from the ground, adhesive tape, cylinder measuring 30 X 30 cm, 60 W bulb and stopwatch.

Drugs

Normal saline: Used as a control in the dose of 0.05ml/10g, administered by intraperitoneal route.

Imipramine hydrochloride: As a standard drug in the dose of 15mg/kg, administered by intraperitoneal route.

***Morus alba*:** The aqueous extract of leaves was procured from Natural remedies, Bangalore. The extract was used at doses of 100 mg/kg and 200 mg/kg. The solution was freshly prepared by dissolving the aqueous extract in normal saline on the day of experiment and administered by intraperitoneal route.

A total of 48 animals (n=48) were used. They were divided into 8 groups of 6 animals each. They were housed in cages containing wooden shavings with 6 animals per cage. Animals were randomly housed at a controlled temperature of 21°± 3°C, with a 12 hour light: 12 hour dark cycle. The animals had free access to standard pellet and water.

They were evaluated for antidepressant activity using two models – Tail suspension test (TST) and Forced swimming test (FST). The experiment was conducted in Post Graduate Experiment Laboratory of the Department of Pharmacology, J.J.M. Medical College between 8:00 A.M. to 2:00 P.M. The laboratory was equipped with standard fluorescent lighting. Animals were brought to the experiment room 1 hr before beginning the experiment and identification mark was placed on the animal tail with indelible ink. Animals were weighed and appropriate dose of drugs were injected intraperitoneally to different groups. The experiment was conducted 30 minutes after injecting the drug.

Tail suspension test – The animals were divided as follows.

Group I: Received 0.05ml/10g of Normal saline intra peritoneally.

Group II: Recieved 15 mg/kg Imipramine intra peritoneally.

Group III: Received 100 mg/kg of aqueous extract of leaves of *Morus alba* intra peritoneally.

Group IV: Received 200 mg/kg of aqueous extract of leaves of *Morus alba* intra peritoneally.

The method was similar to that described by Steru *et al* [11]. Animals were suspended upside down on a metal rod at a height of 55 cm from the ground with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Initially the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The immobility displayed by rodents when subjected to this kind of unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The total duration of immobility was noted during 6 minute period. Each animal was used only once. Forced swimming test

Group V: Received 0.05ml/10g of Normal saline intra peritoneally.

Group VI: Recieved 15 mg/kg Imipramine intra peritoneally.

Group VII: Received 100 mg/kg of aqueous extract of leaves of *Morus alba* intra peritoneally.

Group VIII: Received 200 mg/kg of aqueous extract of leaves of *Morus alba* intra peritoneally.

The forced swimming model to test for antidepressant activity was developed by Porsolt *et al* [12]. As per the original method animals were forced to swim in a plastic cylinder measuring 30 X 30 cm containing water at room temperature to a depth of 20 cm. After an initial 2 minute period of vigorous activity, each animal assumed a typical immobile posture. The mouse was considered 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 next 4 minutes of total 6 minute test. The changes in immobility duration were studied after administering drugs

Following swimming sessions, the mice were dried with towel and placed in a cylinder heated under 60 W bulb. The animals were dried under heated cylinder for 15 minutes before returning to the home cages.

Statistical Analysis

Results are presented as Mean ± SEM. One way ANOVA was used for multiple comparisons followed by Tukey's post hoc test for comparison between groups. For all the tests a 'P' value of ≤ 0.05 considered for statistical significance.

ANOVA (Analysis of variance): In its simplest form ANOVA gives a statistical test of whether the means of several groups are all equal and therefore generalizes Student's two sample t-test to more than two groups.

Post-hoc test: Post-hoc tests (or post-hoc comparison tests) are used at the second stage of the analysis of variance (ANOVA) if the null hypothesis is rejected. The question of interest at this stage is which groups significantly differ from others in respect to the mean. In the present study Tukey's test was used for post-hoc comparison.

Results

Table 1: Duration of immobility (in seconds) during Tail Suspension Test

Groups	Subgroups Duration of immobility in sec						Mean	SD	SEM
	I	II	III	IV	V	VI			
I Normal Saline (0.05ml/10g)	212	223	181	190	171	185	193.7	19.80	8.08
II Imipramine (15mg/kg)	105	97	75	80	67	92	86.00	14.39	5.88
III *Morus alba* (100mg/kg)	180	173	172	151	141	141	159.7	17.41	7.12
IV *Morus alba* (200mg/kg)	80	113	94	103	108	116	102.3	13.43	5.48

Table 2: Duration of immobility (in seconds) during Forced Swimming Test

Groups	Subgroup Duration of immobility in sec						Mean	SD	SEM
	I	II	III	IV	V	VI			
I Normal Saline (0.05ml/10g)	173	193	175	211	180	204	189.0	16.78	6.85
II Imipramine (15mg/kg)	118	130	123	110	106	88	112.5	14.80	6.04
III *Morus alba* (100mg/kg)	170	134	159	175	136	135	151.5	18.81	7.68
IV *Morus alba* (200mg/kg)	73	90	109	121	105	197	99.17	16.62	6.78

Table 3: Effect of aqueous extract of *Morus alba* on immobility period in Tail Suspension Test

Group No.	Drug treatment	Number of animals	Dose (kg-1)	Immobility Time in (secs) (Mean ± SEM)
1.	Control (Normal Saline)	6	5ml	193.7 ± 8.08
2.	Imipramine	6	15mg	86 ± 5.88*
3.	Moru alba	6	100mg	159.7 ± 7.12*
4.	*Morus alba*	6	200mg	102.3 ± 5.48*

Statistical analysis of data was carried out by one-way ANOVA followed by Tukey's test.

*p< 0.05 as compared to control. F = 55.56 (p< 0.001)

Table 4: Tukey's multiple comparison test showing difference between groups in Tail suspension test

Difference Between Groups		
Groups Compared	**Mean Difference**	**(P<0.05)**
Group 1 & 2	107.7	S
Group 1 & 3	34.00	S
Group 1 & 4	91.33	S
Group 2 & 3	-73.67	S
Group 2 & 4	-16.33	NS
Group 3 & 4	57.33	S

S – Significant, NS – Not significant

Table 5: Effect of aqueous extract of *Morus alba* on immobility period in Forces Swimming Test

Group No.	Drug treatment	No. of animals	Dose (kg-1)	Immobility Time in (sec) (Mean ± SEM)
1.	Control (Normal Saline)	6	5ml	189.0 ± 6.85
2.	Imipramine	6	15mg	112.5 ± 6.04*
3.	Moru alba	6	100mg	151.5 ± 7.68*
4.	*Morus alba*	6	200mg	99.7 ± 6.78*

Statistical analysis of data was carried out by one-way ANOVA followed by Tukey's test.

*p< 0.05 as compared to control.

F = 34.96 (p< 0.001)

Table 6: Tukey's multiple comparison test showing difference between groups in Forced swimming test

Difference Between Groups		
Groups Compared	**Mean Difference**	**(P<0.05)**
Group 1 & 2	76.50	S
Group 1 & 3	37.50	S
Group 1 & 4	89.83	S
Group 2 & 3	-39.00	S
Group 2 & 4	13.33	NS
Group 3 & 4	52.33	S

S – Significant, NS – Not significant

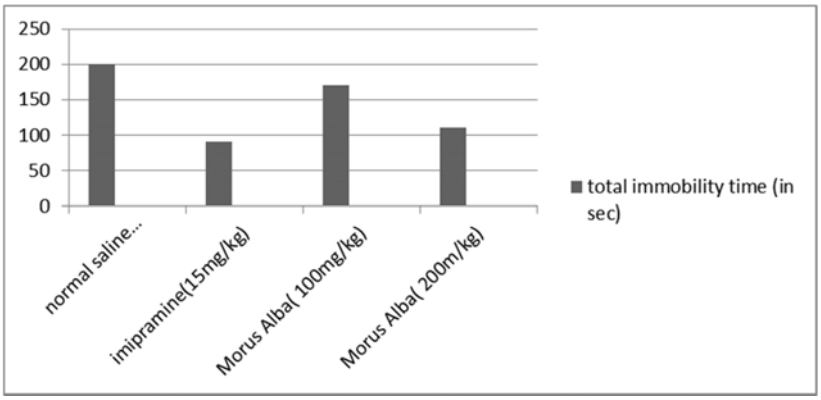

Fig 1: Bar diagram showing duration of immobility in Tail Suspension Test (TST)

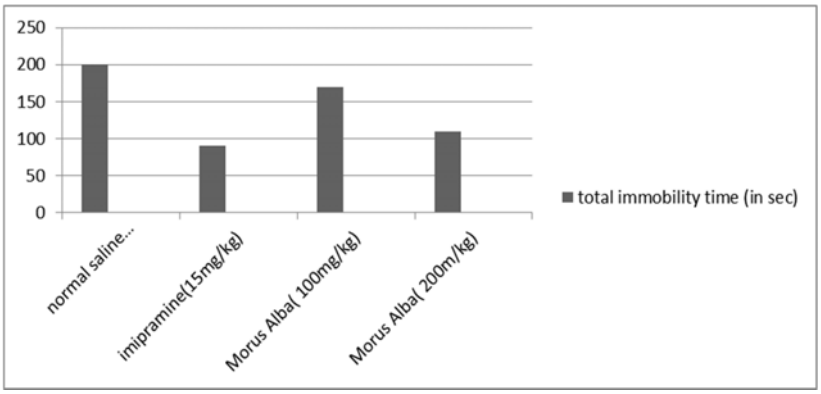

Fig 2: Bar diagram showing duration of immobility in Forced Swimming Test (FST)

Discussion

The present study evaluated the possible antidepressant activity of aqueous extract of leaves of *Morus alba*, by using the conventional experimental models of antidepressant activity.

A study was conducted by Sattayasai *et al* to evaluate the effects of an aqueous extract of *Morus alba* leaves green tea on mouse behaviors (depression, anxiety, climbing activity and thermal response), muscle coordination and muscle strength were studied [13] The model used was forced swimming test. The study showed significant antidepressant activity at the doses of 100 mg/kg and 200 mg/kg. The odor that emanates from the green leaves was shown to have serotonergic system mediated antidepressant activity in forced swimming test in mice by Natakomi *et al* [14] It has been reported to inhibit COMT and MAO enzymes in brain which may be involved in its antidepressant action [9].

The results have been compared with that of standard antidepressant drug Imipramine. Both these methods are widely used to screen new antidepressant drugs. There is a significant correlation between the potency of antidepressants in both forced-swimming and tail-suspension tests and clinical potency of the drugs [15]. It has been argued that tail suspension is less stressful than forced swimming test and has greater pharmacological sensitivity [16]

Tail suspension test: The results of tail suspension test have been expressed as mean duration of immobility during 6 minute period ± standard error of mean. The antidepressant activity is indicated by reduction in the duration of immobility. From the table 3 mean duration of immobility (in seconds) in four groups were - Normal saline (Group I) - 193.7 ± 8.08; Imipramine (Group II) - 86 ± 5.88; *Morus alba* 100 mg/kg (Group III) - 159.7 ± 7.12; *Morus alba* 200 mg/kg (Group IV) - 102.3 ± 5.48. The groups treated with Imipramine and *Morus alba* 100 mg/kg and 200 mg/kg resulted in significant decrease in the duration of immobility compared to Normal saline. This shows that the extract of leaves of *Morus alba* has significant antidepressant activity as compared to the control.

The inter group comparison (Table 4) of Group I (Normal saline) and II (Imipramine 15 mg/kg), Group I (Normal saline) and III (*Morus alba* 100mg/kg), Group I (Normal saline) and IV (*Morus alba* 200 mg/kg), indicates that 15 mg/kg of Imipramine and 100 mg/kg and 200 mg/kg of aqueous extract of leaves of *Morus alba* was significantly better than control in reduction of duration of immobility ($p< 0.05$). Comparison between Group III and IV shows that *Morus alba* 200 mg/kg was significantly better than 100 mg/kg ($p< 0.05$) in terms of reduction of immobility. Also the reduction in the duration of immobility in *Morus alba* 200 mg/kg (Group III) and Imipramine 15 mg/kg (Group II) was similar ($p> 0.05$) showing that their antidepressant effects are comparable, but *Morus alba* 100 mg/kg (Group III) was inferior to Imipramine in terms of antidepressant activity. Thus the intergroup comparison shows that *Morus alba* at a dose of 100 mg/kg and 200 mg/kg has antidepressant activity.

Forced swimming test: The results of forced swimming test are summarized in table 5. The duration of immobility during last four minutes of six minute test in different groups (in terms of mean ± standard error of mean) were - Normal saline (Group I) - 189.0 ± 6.85; Imipramine (Group II) 112.5 ± 6.04; *Morus alba* 100 mg/kg (Group III) - 151.5 ± 7.68; *Morus alba* 200 mg/kg (Group IV) - 99.7 ± 6.78. In the forced swimming test also the doses of *Morus alba* i.e. 100 mg/kg and 200

mg/kg showed significant antidepressant activity compared to control.

In the forced swimming test also, the inter group comparison showed similar results (Table 6). Comparison between Group I (Normal saline) and II (Imipramine), Group I (Normal saline) and III (*Morus alba* 100mg/kg), Group I (normal saline) and IV (*Morus alba* 200 mg/kg), indicates that 15 mg/kg of Imipramine and 100 mg/kg and 200 mg/kg of aqueous extract of leaves of *Morus alba* were significant than control in reduction of duration of immobility. Comparison between Group III and IV again shows that *Morus alba* 200 mg/kg was significantly better than 100 mg/kg ($p< 0.05$) in terms of reduction of immobility. The reduction in the duration of immobility in *Morus alba* 200 mg/kg (Group IV) and Imipramine 15 mg/kg (Group II) was similar ($p> 0.05$) showing that their antidepressant effects are statistically comparable and *Morus alba* 100 mg/kg (Group III) was inferior to Imipramine in terms of antidepressant activity.

The duration of immobility recorded over four minutes in different groups in forced swimming test is almost similar to duration of immobility recorded over six minutes in tail suspension test which substantiate the fact that forced swimming test is more stressful than tail suspension test.

To sum up, the present study has shown that the aqueous extract of leaves of *Morus alba* at 100 mg/kg and 200 mg/kg significantly reduced the duration (time) of immobility of animals as compared to the control in both tail suspension test and forced swimming test of depression, showing that in both the doses, it has significant antidepressant activity.

The exact mechanism by which extract of *Morus alba* leaves exert antidepressant action is not precisely known. Experimental studies have shown that the mulberry tree leaves, bark and root possess various pharmacological properties. The leaves of *Morus alba* contain many flavonoids like quercetin. Quercetin is 3,5,7,3',4'-pentahydroxyflavone widely distributed potent bioflavonoid present in various vegetables and fruits. It has been reported to inhibit COMT and MAO enzymes in brain [9]. Hence it increases the concentration of catecholamines in synaptosomes and thus attenuates depression.

Oxidative stress is one of the mechanisms involved in neurological and neuropsychiatric problems. Many natural products have antioxidant property which is involved in reversing the deleterious effects of neuronal communication and behavior. Nitric oxide (NO) is one of the free radicals suggested, role in stress and depression either by modulating the release of other neurotransmitters, acting as a cellular communicator in plasticity and development, and/or acting as a vasodilator in the regulation of blood flow [17]. The alterations of the neuronal nitric oxide synthase (NOS) activity and the antioxidant systems in patients with affective disorders have been demonstrated [18].

Quercetin and other flavonoids are present in Hypericum perforatum (St. John's wort) which inhibit nitric oxide synthase in blood and cerebral homogenate [7] and are believed to be involved in antidepressant effect of plant [8]. St. John's wort is commonly used in many countries in the treatment of mild to moderate depression. The extract of *Morus alba* contains flavonoids, tannins, triterpenes, anthrocyanin, anthroquinones, sterols, alkaloids and saponins. The aqueous extract of *Morus alba* contains flavonoids like quercetin which has significant radical scavenging property [19]. and decreases the expression of nitric oxide synthase in the hypothalamus of streptozotocin induced diabetic rats which may contribute to its antidepressant activity [20] It has also shown antioxidative and the antiatherogenic protective effects in LDL-receptor-deficient mice [21].

Thus it may be possible that in addition to inhibition of COMT and MAO in brain, antioxidant property of *Morus alba* may also be responsible for the antidepressant-like property observed in mice.

Conclusion

- The potential antidepressant activity of aqueous extract of leaves of *Morus alba* was evaluated in swiss albino mice using two acute models of depression namely tail suspension and forced swimming test.
- Normal saline (5 ml/10g), Imipramine (15 mg/kg) and *Morus alba* 100 mg/kg and 200 mg/kg in normal saline were injected intraperitoneally into mice 30 minutes before each test.
- In tail suspension test total duration of immobility was recorded during six minutes and in forced swimming test the total duration of immobility was recorded during last four minutes of total six minute test.
- With both the doses of *Morus alba* there was a reduction in the duration of immobility as compared to control which shows that the aqueous extract of leaves of *Morus alba* has significant antidepressant activity. The antidepressant activity of *Morus alba* (200 mg/kg) and Imipramine (15 mg/kg) were comparable.
- Both the tests showed consistent results in terms of reduction of duration of immobility.
- Considering the results of two different animal models of depression, it can be concluded that the aqueous extract of leaves of *Morus alba* has significant antidepressant activity at doses of 100 mg/kg and 200 mg/kg with antidepressant effect of 200 mg/kg comparable to Imipramine 15 mg/kg.
- Further studies are required to elucidate the exact mechanism of action of antidepressant activity of aqueous extract of leaves of *Morus alba*.

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