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R E S E A R C H A R T I C L E

Pharmacological Evaluation of Antidepressant Activity of Ethanolic Extract of Magnolia Officinalis in Mice

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A B S T R A C T

Depression is the most common of the affective disorders (disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. There are two types and they are Unipolar and Bipolar Aim of the present study was to evaluate the antidepressant activity of *Magnolia officinalis* (*MO*) in experimental models of depression using mice. To study the effect of *MO* on behavior models of depression like forced swim test, tail suspension test. To study the effect of *MO* on mechanism based models of depression like 5-HTP induced head twitches, clonidine induced aggression and L-Dopa induced hyperactivity and aggressive behavior. To study the effect of *MO* on anti-oxidant levels of brain. The results from the present study confirm the antidepressant activity of magnolia officinalis, since it reduced the immobility in both FST and TST. In the present study, magnolia officinalis significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with magnolia officinalis, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in mice brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of magnolia officinalis might be a part of the mechanism for its antidepressant activity. Results from behavioral experiments indicate that the antidepressant activity of magnolia officinalis, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

Keywords: Depression, *Magnolia officinalis* (*MO*) etc.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Depression is the most common of the affective disorders (disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. There are two types and they are Unipolar and Bipolar. The pharmacological evidence does not enable a clear distinction to be drawn between the noradrenaline and 5-HT theories of depression. Clinically, it seems that inhibitors of noradrenaline reuptake and of 5-HT reuptake are equally effective as antidepressants though individual patients may respond better to one or the other.

Drugs that inhibit 5-HT uptake (termed SSRI) include fluoxetine, fluvoxamine, paroxetine, citalopram and sertraline. Fluoxetine is currently the most prescribed antidepressant. As well as showing selectivity with respect to 5-HT over noradrenaline uptake, they are less likely than TCA to cause anticholinergic side-effects and are less dangerous in overdose. In contrast to MAOI (see below), they do not cause 'cheese reactions'. They are as effective as TCA and MAOI in treating depression of moderate degree but probably less effective than TCA in treating severe depression.

Magnolia officinalis L. (Magnoliaceae) also referred to as touch me not, live and die, shame plant and humble plant is a prostrate or semi-erect subshrub of tropical America and Australia, also found in India heavily armed with recurved thorns and having sensitive soft grey green leaflets that fold and droop at night or when touched and cooled. These unique bending movements have earned it a status of ‘curiosity plant’. It appears to be a promising herbal candidate to undergo further exploration as evident from its pharmacological profile. It majorly possesses antibacterial, antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac, and various other pharmacological activities. The herb has been used traditionally for ages, in the treatment of urogenital disorders, piles, dysentery, sinus, and also applied on wounds. This work is an attempt to explore and compile the different pharmacognostic aspects of the action plant Magnolia officinalis reported till date.

Tricyclic Antidepressants

The main effect of TCA is to block the uptake of amines by nerve terminals by competition for the binding site of the transport protein. Synthesis of amines, storage in synaptic vesicles and release are not directly affected, though some TCA appear to increase transmitter release indirectly by blocking presynaptic α_2-adrenoceptors. Most TCA inhibit noradrenaline and 5-HT uptake by brain synaptosomes to a similar degree but have much less effect on dopamine uptake. It has been suggested that improvement of emotional symptoms reflects mainly an enhancement of 5-HT-mediated transmission, whereas relief of biological symptoms results from facilitation of noradrenergic transmission.[14]

Selective 5-HT Uptake Inhibitors

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Selective 5-Ht Uptake Inhibitors

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Monoamine Oxidase Inhibitors

Drugs of the MAOI type were among the first to be introduced clinically as antidepressants but were largely superseded by TCA and other types of antidepressant with clinical efficacies that were considered better and with generally less side-effects. The main examples are phenelzine, tranylcypromine and iproniazid.

MAO is found in nearly all tissues and exists in two similar molecular forms, coded by separate genes. MAO-A has a substrate preference for 5-HT and is the main target for the antidepressant MAOI. MAO-B has a substrate preference for phenyl ethylamine, and both enzymes act on noradrenaline and dopamine. Type B is selectively inhibited by selegiline, which is used in the treatment of Parkinsonism. Most antidepressant MAOI act on both forms of MAO, but clinical studies with subtype-specific inhibitors have shown clearly that antidepressant activity, as well as the main side-effects of MAOI, is associated with MAO-A inhibition. MAO is located intracellularly, mostly associated with mitochondria, and has two main functions.

Other Antidepressant Drugs

The main claims made for these newer agents are:

- Fewer side-effects (e.g. sedation and anticholinergic effects)
- Lower acute toxicity in overdose
- Action with less delay

- Efficacy in patients non-responsive to TCA or MAOI.

In practice, the newer drugs, though no more efficacious than TCA, generally have fewer side-effects and less acute toxicity, but only mirtazapine appears to be more rapid in action. Two substances have been touted as 'natural' antidepressants, namely L-tryptophan (TRP) and St John's wort. [25]

2. Materials and Methods

Drugs and Chemicals

Thiobarbituric acid and DTNB reagent (HiMedia Laboratories Ltd., Mumbai), Trichloroacetic acid (Qualigens Fine Chemicals, Mumbai), Riboflavin (Astra IDL, Bangalore), Sodium dihydrogen phosphate and Disodium hydrogen phosphate (S.D. Fine Chemicals, Mumbai), Lorazepam (Ranbaxy, India), 1,1,3,3,-Tetraethoxy propane, O-Dianisidine, Imipramine hydrochloride, 5-Hydroxy Tryptophan (5-HTP), Clonidine and L-DOPA (Sigma, St. Louis, USA) were used in the study. The other chemicals and solvents used were of analytical grade and purchased from commercial suppliers. Imipramine (IMP), 5-HTP, clonidine, L-DOPA, Lorazepam was administered intraperitoneally by dissolving in normal saline.

Plant Description

Magnolia Officinalis

Magnolia officinalis (commonly called houpu magnolia or magnolia-bark) is a species of Magnolia native to the mountains and valleys of China at altitudes of 300–1500 m.

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Magnoliids
Order:	Magnoliales
Family:	Magnoliaceae
Genus:	*Magnolia*
Subgenus:	*Magnolia*
Section:	*Rhytidospermum*
Species:	***M. officinalis***
Binomial name	
Magnolia officinalis	

Methodology

Collection and Authentification of Plant Material

The Aerial Parts of *Magnolia officinalis* were collected and authenticated

Extraction of Plant Material

The plant is grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Ethanol Extraction)[38]

In this work the cold extraction process was done with the help of ethanol. About 45-60gms of powdered material was taken in a clean, flat-bottomed glass container and soaked in 750 ml of ethanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent

The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

% Yield value of Ethanol Extract from Aerial Parts of *Magnolia officinalis* Plant.

Powder taken for extraction = 250gm
Weight of the empty china dish = 50.0gm
Weight of the china dish with extract = 72.3gm
Weight of the extract obtained = (72.3-50.0) gm
= 22.3

% yield of ethanol extract = (weight of extract)/(powder taken for extraction) × 100
= 22.3 /200 ×100 = 11.15%.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *Magnolia officinalis* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids as per the standard methods [40].

1. Detection of Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a). Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b).Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c).Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d).Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of Carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a). Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of

the violet ring at the junction indicates the presence of Carbohydrates.

b).Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c).Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3.Detection of saponins

a). Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b).Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

4. Detection of steroids.

a). Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b).Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

5. Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of Tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was addexd. Formation of white precipitate indicates the presence of tannins.

7. Detection of Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Animals

Healthy Adult Male mice of 5 weeks old with Average weight in the range of 40-60gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies [31]

Acute toxicity studies will be performed for ethanolic extract according to the acute toxic classic method as per OECD guidelines. Male mice were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract will be administered orally at the dose of 300mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 100, 200, & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72h.

Preliminary Phytochemical Screening

Investigation revealed the presence of steroid, Alkaloid, saponins, Tannins, phenols & Flavonoid in Ethanolic Extract of *magnolia officinalis.*

Table.no.1. Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	+
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	+
Flavonoid	+
Saponin	+

(+) Present **(-)** Absent

Acute toxicity studies

As per (OECD) draft guidelines 423 Female albino mice were administered *magnolia officinalis* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as 1/10th and 1/20th i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose anti depressant investigations.

1. Forced Swim Test (FST)

The results (Table. 1) showed that both *Magnolia officinalis* (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the *Magnolia officinalis* (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig. 1).

3. Results and Discussion

Preliminary Phytochemical Screening: Investigation revealed the presence of steroid, Alkaloid, saponins, Tannins, phenols & Flavonoid in Ethanolic Extract of *magnolia officinalis.*

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(+) Present **(-)** Absent

Table. 1. Effect of *magnolia officinalis* and imipramine (IMP) on forced swim test (FST) in mice

Group no.	**Treatment (dose in mg/kg)**	**Immobility period (sec) Mean ± SEM**
I	Control (0.3% CMC) + FST	142.3±2.3
II	*Magnolia officinalis* (100 mg/kg, p.o.) + FST	121.2±4.3
III	*Magnolia officinalis* (200 mg/kg, p.o.) + FST	99.5±5.6*
IV	*Magnolia officinalis* (400 mg/kg, p.o.) + FST	85.0±3.4*
V	Imipramine (15 mg/kg, i.p.) + FST	69.1±3.0*

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. * = $p<0.001$ compared to control

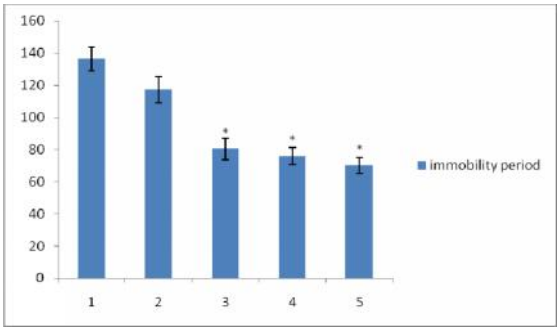

Figure. 1. Effect of *magnolia officinalis* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on forced swim test (FST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. *= $p<0.001$ compared to control

2) Tail Suspension Test (TST)

The results (Table. 2) showed that both *Magnolia officinalis* (100,200,400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the *Magnolia officinalis* (100, 200 and 400 mg/kg) and IMP treated groups were significantly different ($p<0.001$) from the vehicle treated group.

Table.2. Effect of *magnolia officinalis* and Imipramine (IMP) on tail suspension test (TST) in mice

Group no.	**Treatment (dose in mg/kg)**	**Immobility period (sec)**
I	Control (0.3% CMC) + TST	133.2±09.3
II	*Magnolia officinalis* (100 mg/kg, p.o.) + TST	112.6±11.2[a]
III	*Magnolia officinalis* (200 mg/kg, p.o.) + TST	99.3±8.2[a]
IV	*Magnolia officinalis* (400 mg/kg, p.o.) + TST	81.3±7.5[a]
V	Imipramine (15 mg/kg, i.p.) + TST	62.3±4.5[a]

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = $p<0.001$ compared to control

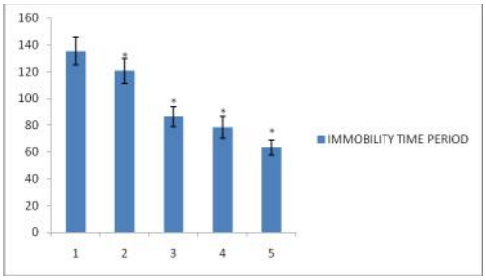

Figure. 2. Effect of *magnolia officinalis* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on tail suspension test (TST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = p<0.001 compared to control

3) 5-HTP induced head twitches in mice

Table.3. illustrates the effect of *magnolia officinalis* and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of *magnolia officinalis* (100, 200 and 400 mg/kg, p<0.01, p<0.001) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase (p<0.001) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *magnolia officinalis* was significantly higher than IMP (p<0.001) (Fig. 3).

Table. 3. Effect of *magnolia officinalis* on 5-HTP-induced head twitches in mice

Group no.	Treatment (dose in mg/kg)	Head twitches Mean ± SEM
I	Control (0.3% CMC)	11.9±1.4
II	*Magnolia officinalis* (100 mg/kg, p.o.)	21.5±1.1[a]
III	*Magnolia officinalis* (200 mg/kg, p.o.)	28.0±2.1[b]
IV	*Magnolia officinalis* (400 mg/kg, p.o.)	36.8±3.1[b]
V	Imipramine (15 mg/kg, i.p.)	24.2±2.4[b]

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001 compared to control

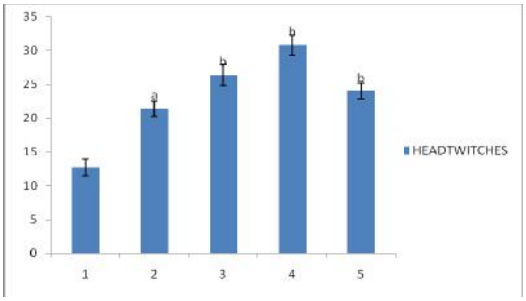

Figure. 3. Effect of *magnolia officinalis* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on 5-HTP-induced head twitches in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001, compared to control

4) L-DOPA induced hyperactivity and aggressive behavior in mice:

The effect of *magnolia officinalis* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 4. Post-hoc analysis revealed that three doses of *magnolia officinalis* (100,200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group.

Table. 4. Effect of *Magnolia officinalis* and Lorazepam on L-DOPA-induced hyperactivity and aggressive behavior in mice.

Group o.	Treatment (dose in mg/kg)	Behavioral score
I	Control (0.3% CMC)	1
II	*Magnolia officinalis* (100 mg/kg, p.o.)	2.0 ± 0.2[a]
III	*Magnolia officinalis* (200 mg/kg, p.o.)	2.0 ± 0.2[a]
IV	*Magnolia officinalis* (400 mg/kg, p.o.)	2.6 ± 0.2[a]
V	Lorazepam (2.5 mg/kg, i.p.)	2.1 ± 0.2[a]

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001

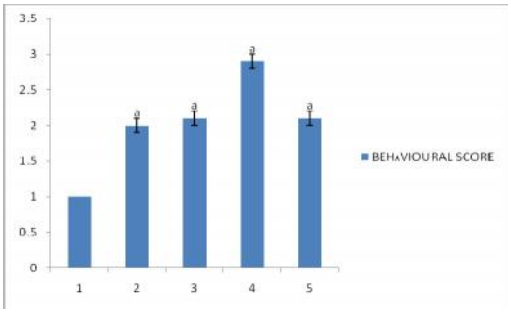

Figure. 4. Effect of *magnolia officinalis* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on L-DOPA-induced hyperactivity and aggressive behavior in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control

5) Clonidine induced aggression in mice

Table. 5. indicates the effect of *magnolia officinalis* (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *magnolia officinalis* (p<0.001) significantly increased the latency to first attack and decrease the no. of bouts compared to control.

Table 5: effect of magnolia officinalis on clonidine induced aggression in mice.

Group no.	Treatment (dose in mg/kg)	% Response(MEAN ± SEM)	
		Latency to 1st attack	Fighting response
I	Control (0.3% CMC)	101.1 ± 10.1	100.6 ± 8.3
II	*Magnolia officinalis* (100 mg/kg, p.o.)	123.6 ± 11.1 [a]	121.78 ± 8.6 [a]
III	*Magnolia officinalis* (200 mg/kg, p.o.)	131.5 ± 03.5 [b]	129.7± 6.6 [b]
IV	*Magnolia officinalis* (400 mg/kg, p.o.)	132.7 ± 9.6 [b]	130.2 ± 6.2 [b]
V	Lorazepam (2.5 mg/kg, i.p.)	141.0 ± 8.0 [b]	139.60± 4.8 [b]

Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

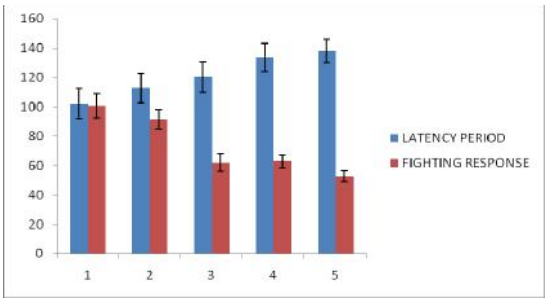

Figure. 5. Effect of *magnolia officinalis* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on clonidine induced aggression in mice. Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

Discussion

In the present study, 7 days pretreatment with *Magnolia officinalis* at the doses of 100, 200 and 400 mg/kg showed antidepressant activity in the forced swim test and tail suspension tests. The FST is the tool most widely used for assessing antidepressant activity preclinical. The widespread use of this model is largely a result of its ease of use, reliability across laboratories, and ability to detect a broad spectrum of antidepressant agents[49]. Most clinically active antidepressants are effective in the FST, while neuroleptics and anxiolytics produce different effects.[34] In the forced swim test, *MO* significantly reduced immobility period suggesting anti-depressant activity and the activity was comparable to the reference drug IMP. Immobility is a state of lowered mood or hopelessness, which the mice experience when they are allowed to swim in a restricted space from which they cannot escape.

Clinical studies show that combined 5-HT and NE reuptake inhibitor is more effective than used alone. Based on these observations, we evaluated the role of NE in the antidepressant effect of *MO*. *MO* significantly increased the clonidine-induced aggressive behavior indicating increased activity of noradrenergic system. Hence, the antidepressant activity of *MO* may involve both serotonergic and noradrenergic systems. Several studies have shown that the antidepressant effect involves augmentation of dopaminergic neurotransmission. Such interference with the dopaminergic system could explain at least in part the acute effects of some of the antidepressants. However, *MO* also alter L-DOPA induced aggressive behavior indicating effect on the dopaminergic system. Hence, it is possible that this may be the reason for significant effect of *MO* on L-DOPA-induced aggression.

The above results indicate that *MO* has antidepressant activity by virtue of its action on serotonergic, noradrenergic and dopaminergic systems based on behavioral experimental evidence. Generally, repeated treatment with antidepressants has been reported to facilitate both serotonergic and/or noradrenergic transmission.[46] The dual action of *MO* may have several advantages over SSRIs. Although, SSRIs are widely used, side effects such as nausea, sexual dysfunction and sleep disorders may limit their potential use. Moreover, clinical studies indicate that they may not offer too many advantages over classical antidepressants. Further, clinical studies have shown that mixed 5-HT and NE reuptake inhibitors (SNRIs) are effective and well-tolerated antidepressants. Although, the specific mechanism of action of *MO* needs to be explored before coming to any conclusions on its mechanism of action, preliminary investigations indicate that *MO* may potentially have the more desirable dual action on 5-HT and NE. The present study establishes the antidepressant activity of *Magnolia officinalis* in mice models of depression. Further, results from behavioral experiments indicate that this activity may be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic system.

4. Conclusion

- The results from the present study confirm the antidepressant activity of *magnolia officinalis*, since it reduced the immobility in both FST and TST.
- In the present study, *magnolia officinalis* significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced

aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression.

- Pretreatment with *magnolia officinalis*, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in mice brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of *magnolia officinalis* might be a part of the mechanism for its antidepressant activity.
- Results from behavioral experiments indicate that the antidepressant activity of *magnolia officinalis*, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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