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Original Article

The Antidepressant-like Activity of Asiatic Acid in Albino Mice Involves the Monoaminergic System

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Abstract

Background: Major depression is a chronic recurring illness that affects up to 20% of the world population. Plant derived compounds have become an attractive source for potentially antidepressant compounds.

Objective: The present study investigated the antidepressant-like activity of asiatic acid (dihydroxyursolic acid) in the mouse by forced swimming test (FST) and tail suspension test (TST), and investigated potential mechanisms of action.

Method: Asiatic acid (5, 10 and 20 mg/kg) or normal saline was orally administered to male Swiss albino mice (25-35 g) and compared against fluoxetine (20 mg/kg), a known antidepressant compound. The effect of asiatic acid on the locomotion was assessed by performance in an actophotometer. The mechanism of action was also studied using a variety of different receptor antagonists.

Results: Asiatic acid produced a statistically significant reduction in the time spent immobile when tested by both FST and TST, without altering normal locomotor performance. Pretreatment with WAY 100635 (0.1 mg/kg, $5HT_{1A/1B}$ receptor antagonist, ketanserin (5 mg/kg, non-selective $5HT_2$ receptor antagonist), ondansetron (1 mg/kg, $5HT_3$ receptor antagonist), prazosin (1 mg/kg, α_1 adrenoceptor antagonist) and yohimbine (1 mg/kg, α_2 adrenoceptor antagonist) abolished the effect of asiatic acid on time spent immobile. However, naloxone (1 mg/kg) -an opioid receptor antagonist, had no significant effect.

Conclusion: Our findings confirmed the antidepressant-like effect of asiatic acid in the FST and the TST. The locomotor activity remained unchanged indicating the absence of any stimulant effect by asiatic acid on the brain. The antidepressant-like effect seems to be mediated through the serotonergic and noradrenergic systems and not through an opioid system.

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Introduction

Major depression is a psychiatric condition associated with depressed mood, lack of interest in day to day activities, social withdrawal and suicidal ideation (1). Though the etiology of this disease is not well understood, there may be a decreased concentration of neurotransmitters such as serotonin and noradrenaline in the brain (2). Recent reports also suggest the possible involvement of the opioid system in the genesis of depression (3, 4). Modern antidepressant drugs act by modulating these neurotransmitters, and these drugs are successful in managing depression symptoms to a certain extent, albeit not without limitations. About 30% of the patients on tricyclic antidepressants or selective serotonin reuptake inhibitors remain treatment resistant or show only a partial response, and therapeutic benefits often take time to appear after a patient begins pharmaceutical treatment. These limitations and other serious side effects represent major hurdles in treating depression (5). This necessitates search for newer drugs with better efficacy and an improved safety profile.

Phytochemicals, the bioactive compounds isolated from plants, have become an attractive source for novel antidepressant compounds. *Centella asiatica*, a plant native to India, China, Australia, Japan, and many other countries, has been traditionally used as a treatment for gastrointestinal disorders, cognitive impairment, and anxiety in Ayurveda and Chinese medicine (6). Prior studies have reported neuroprotective properties of *Centella asiatica*, suggesting its use in patients with memory loss (7). Asiatic acid, or 2α-23-dihydroxyursolic acid, is a bioactive pentacyclic triterpene compound isolated from *Centella asiatica*. Though the studies using asiatic acid are sparse, it has been reported to possess anxiolytic effect in rats (8). Asiatic acid has close structural similarity with ursolic acid, which has been reported to have antidepressant like activity mediated through the monoaminergic system (9). Based on these findings, we set out to further characterize the potentially antidepressant actions and mechanisms of asiatic acid. Depression-like behavior was assessed using the forced swimming test (FST) and tail suspension test (TST), and the role of the serotonergic, noradrenergic and opioid systems were investigated.

Materials and Methods

Animals

Male Swiss albino mice of 3-4 months, weighing 25-35 g were used. The animals were acclimatized for one week with the standard laboratory condition of 25±2°C room temperature on a 12 h light and dark cycle. The study protocol was approved by the Institute Animal Ethics Committee and all care was taken to minimize the pain and suffering of the animal as per the guidelines of The Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The experiments were performed between 9 am to 4 pm and each animal was used only once in each test.

Drugs and administration

All drugs used were obtained from standard commercial suppliers. Asiatic acid, fluoxetine, WAY 100635, ketanserin, ondansetron, prazosin, yohimbine and naloxone were obtained from Sigma chemicals Co, USA. Asiatic acid was made into a suspension with carboxymethyl cellulose and given to mice as oral gavage. All receptor antagonists were dissolved in saline or 1% Tween 80 and administered to mice by injection, at 10 ml/kg body weight. Appropriate vehicle treated control groups were also assessed simultaneously.

Experimental procedures

Evaluation of the antidepressant-like effect

To evaluate the antidepressant-like activity, mice were treated with a single oral dose of vehicle, fluoxetine or asiatic acid and underwent TST and FST after 45 min. Asiatic acid was used in three dose ranges of 5, 10 and 20 mg/kg to explore any dose dependent effect. Fluoxetine, a well-known selective serotonin reuptake inhibitor, was used as a positive control (20 mg/kg, p.o., single dose). There were no similar studies on asiatic acid, so the dose was selected based on the available *in vivo* literature.

Prior studies reported an anxiolytic effect of asiatic acid when administered at 30 mg/kg, though the authors have not tested it at any higher or lower doses (8). Considering this report and lack of any other data, we decided to test three lower doses starting from 5 mg/kg and increase in a logarithmic fashion to 10 and 20 mg/kg.

Evaluation of the possible mechanisms of action

To determine a potential mechanism of action, a series of receptor antagonists were used to examine the involvement of serotonergic, noradrenergic and opioid receptors. In all instances below, mice were pretreated with receptor antagonist or the respective vehicle, 30 min before asiatic acid (20 mg/kg) administration. Forty-five min post-administration, depression like behavior was assessed by FST. The dose and duration of all the receptor antagonists were determined by previous reports (4, 10-12).

Role of the serotonergic system

The mice were pretreated with WAY 100635 ($5HT_{1A/1B}$ receptor antagonist, 0.1 mg/kg, subcutaneous), ketanserin (non-selective $5HT_2$ receptor antagonist, 5 mg/kg,i.p.) or ondansetron ($5HT_3$ receptor antagonist, 1 mg/kg, i.p.) as an injection, 30 min before asiatic acid (20 mg/kg) or vehicle administration. The animals were evaluated in FST after 45 min.

Role of the noradrenergic system

The mice were pretreated with prazosin (α_1 adrenoceptor antagonist, 1 mg/kg, i.p.) or yohimbine (α_2 adrenoceptor antagonist, 1 mg/kg, i.p.) 30 min before asiatic acid (20 mg/kg) or vehicle administration. The animals were evaluated in FST after 45 min.

Role of the opioid system

The mice were pretreated with naloxone (nonselective opioid receptor antagonist, 1 mg/kg, i.p.), 30 min before asiatic acid (20 mg/kg) or vehicle administration. The animals were evaluated in FST after 45 min.

Behavioral analysis

Tail suspension test: This test was performed in an acoustically and visually isolated room. Mice were submitted to an inescapable stressor by suspending them by their tail from a thin horizontal steel rod 50 cm above the floor by using adhesive tape placed 1 cm from the tip of the tail. Animals exhibiting depression-like behavior fail to exhibit normal escape behavior and remain in an immobile state for an increased duration. Animals treated with antidepressant drugs show reduced time spent immobile. The animal was considered immobile when they hung passively without any motion. The total duration of the test was six min and was done by an expert observer blinded to the treatments (13).

Forced swimming test: This test was conducted by using a slightly modified method from that described by Porsolt *et al.,* (1977) (14). Here mice were individually placed in a five liter glass cylinder of 25 cm height, filled with water to the level of 15 cm. The mouse was considered immobile when it stopped struggling and floated motionless on the water. This behavior was assessed for a period of six minutes and immobility time was recorded during the last four minutes. At the end of the procedure, mice were removed from the water, dried with a towel and the water was changed each time.

Locomotor activity test using actophotometer: The method described by Boissier and Simon, (1965) with slight modification was used (15). All mice were assessed in this test to rule out any change in locomotor activity induced by the test drug. The actophotometer contains a square arena (30×30 cm) with walls that are fitted with photocells just above the floor level. These photocells were checked before the beginning of the experiment to ensure that they were working properly. The number of times each animal crossed the light beam was recorded automatically. The drug/vehicle treated mice were then individually placed in the arena. After a two minutes acclimatization period, the digital locomotor scores were recorded for the next four minutes (15, 16).

Statistical analysis

The data were represented as Mean±S.E.M. The

difference between groups was calculated by one-way ANOVA followed by Newman-Keuls test or two-way ANOVA followed by Bonferroni test as *post hoc* comparison when appropriate. Probability values less than 0.05 (*P* value<0.05) were considered statistically significant.

Results

The effect of asiatic acid on the immobility time in TST and FST

The effect of acute treatment with asiatic acid at 5, 10 and 20 mg/kg doses in TST are presented in Fig. 1. Animals treated with asiatic acid showed a statistically significant reduction in the immobility time compared to the vehicle treated control group in TST [F (4,25)= 4.91, P=0.005]. These observations were comparable to the responses produced by the standard drug, fluoxetine. In FST, a statistically significant reduction in immobility time was noted at 10 and 20 mg/kg doses of asiatic acid [F (4,25)= 8.60, P=0.0002], though at 5 mg/kg dose, such an effect was absent (Fig. 2). Fluoxetine also produced a statistically significant decrease in immobility time (p<0.01) as compared to the control group. Based on these findings, the 20 mg/kg dose of asiatic acid was used for further mechanistic studies.

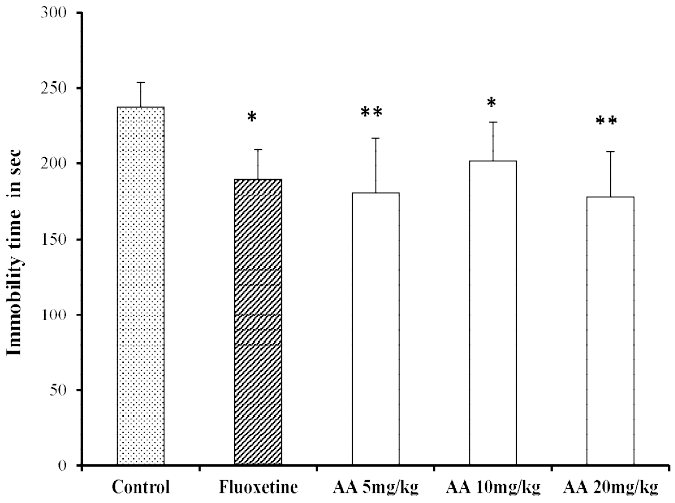

Fig. 1 : Effect of asiatic acid and fluoxetine treatment on mouse tail suspension test. Asiatic acid (5, 10 and 20 mg/kg) and fluoxetine (20 mg/kg) were administered by oral gavage, 45 min before the test. Each column represents Mean±S.E.M. (n=6 animals per group).

*P<0.05, **P<0.01 when compared with the control group. AA- asiatic acid.

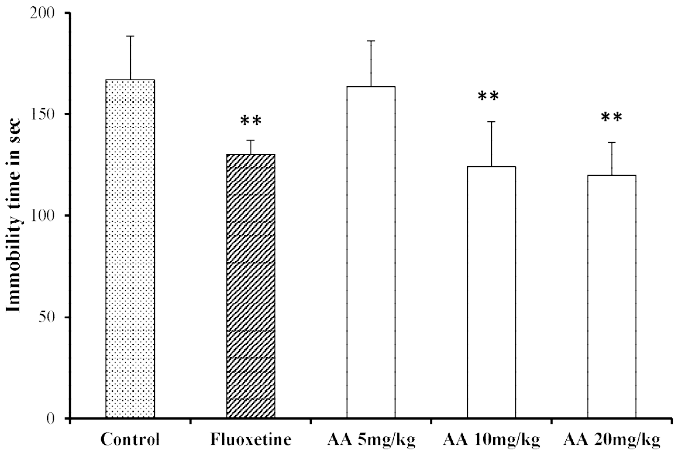

Fig. 2 : Effect of asiatic acid and fluoxetine treatment on mouse forced swimming test. Asiatic acid (5, 10 and 20 mg/kg) and fluoxetine (20 mg/kg) were administered by oral gavage, 45 min before the test. Each column represents Mean±S.E.M. (n=6 animals per group).

**P<0.01 when compared with the control group. AA- asiatic acid.

The role of the serotonergic system

Asiatic acid (20 mg/kg) was administered to mice which were pretreated with the serotonin receptor antagonists, WAY 100635, ketanserin or ondansetron. After 45 min, mice underwent FST (Fig. 3). Pretreatment with WAY 100635 prevented the reduction in immobility time seen when treated with asiatic acid alone prior to FST. A post hoc analysis showed a significant effect of asiatic acid treatment [F(1,20)=8.81, P=0.007], WAY 100635 pretreatment [F(1,20)=4.92, P=0.038] and asiatic acid x WAY 100635 interaction [F(1,20)= 14.46, P=0.001]. Ketanserin pretreatment also abolished the anti-depressant-like effect of asiatic acid in FST. The two-way ANOVA showed a significant effect of asiatic acid treatment [F(1,20)=16.17, P=0.007], ketanserin pretreatment [F(1,20)=31.21, P=0.001] and asiatic acid x ketanserin interaction [F(1,20)=12.0, P=0.003]. The antidepressant like effect produced by asiatic acid was also abolished by ondansetron pretreatment, a $5HT_3$ receptor blocker. Two-way ANOVA revealed a significant effect of asiatic acid treatment [F(1,20)=20.32, P=0.0002], ondansetron pretreatment [F(1,20)=19.73, P=0.0003] and an interaction between asiatic acid and ondansetron [F(1,20)=

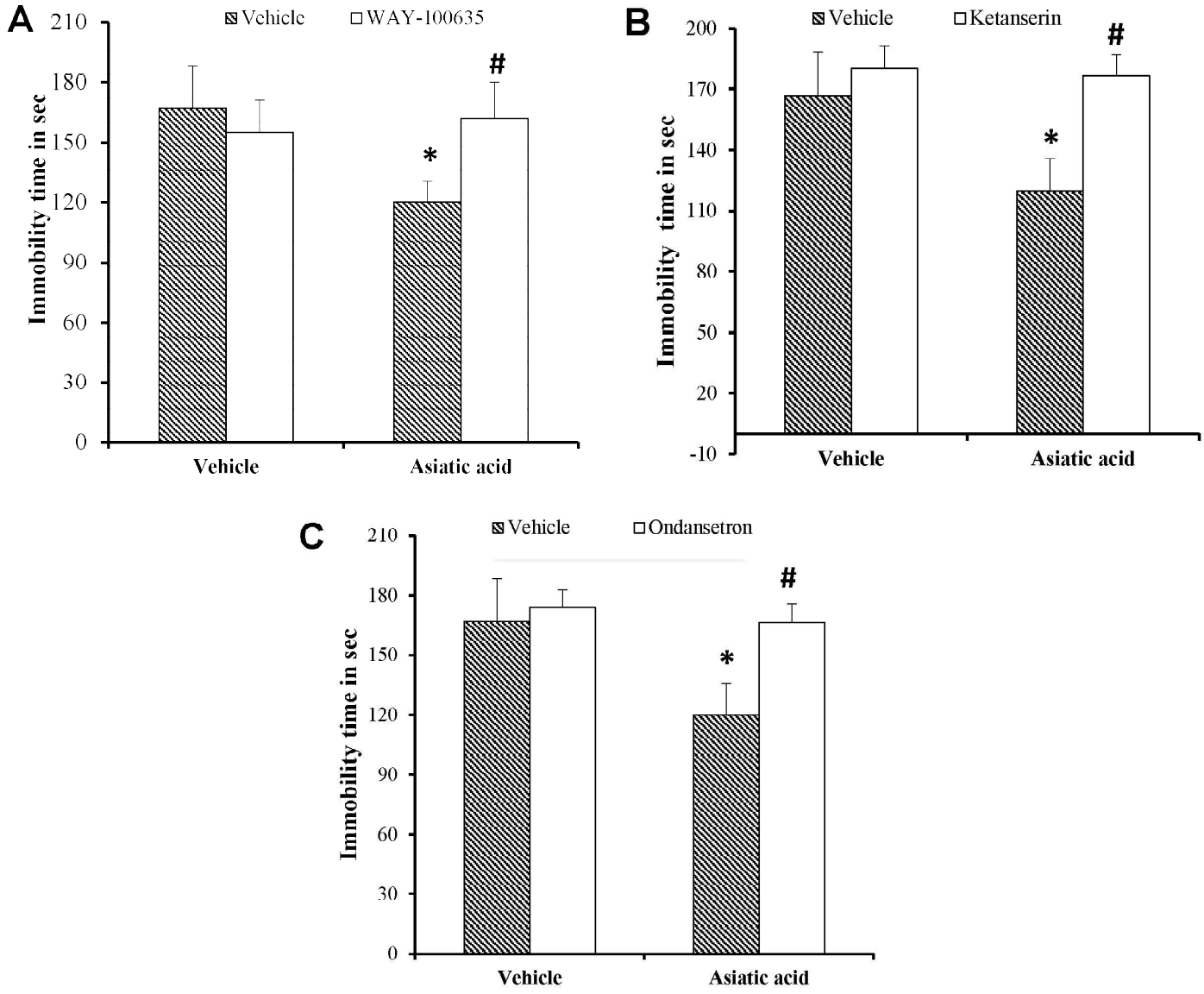

Fig. 3 : Effect of pretreatment of mice with WAY 100635 (0.1 mg/kg, s.c., panel A), ketanserin (5 mg/kg i.p., panel B) and ondansetron (1 mg/kg i.p., panel C) on the immobility time of asiatic acid (20 mg/kg p.o.) in the forced swimming test. Each column represents the Mean±S.E.M. of 6 animals. *P<0.01 when compared with the vehicle treated control. #P<0.01 as compared with asiatic acid alone.

10.77, P=0.004].

The role of the noradrenergic system

Mice which pretreated with prazosin or yohimbine, prior to asiatic acid administration (20 mg/kg). After 45 min, animals were subjected to FST (Fig. 4). Prazosin pretreatment inhibited the effect of asiatic acid on immobility time in a statistically significant manner. Two-way ANOVA showed a significant effect of asiatic acid treatment [F(1,20)=7.09, P=0.01], prazosin pretreatment [F(1,20)=23.27, P=0.001] and asiatic acid and prazosin interaction [F(1,20)=15.81, P=0.007]. Yohimbine pretreatment also produced a similar effect as that of prazosin on the duration of immobility (Asiatic acid treatment [F(1,20)=18.33, P=0.004], yohimbine pretreatment [F(1,20)=17.79, P=0.004] and asiatic acid and yohimbine interaction [F(1,20)=9.71, P=0.005).

The role of the opioid system

Naloxone (a nonselective opioid receptor antagonist) was given to the mice and asiatic acid (20 mg/kg)

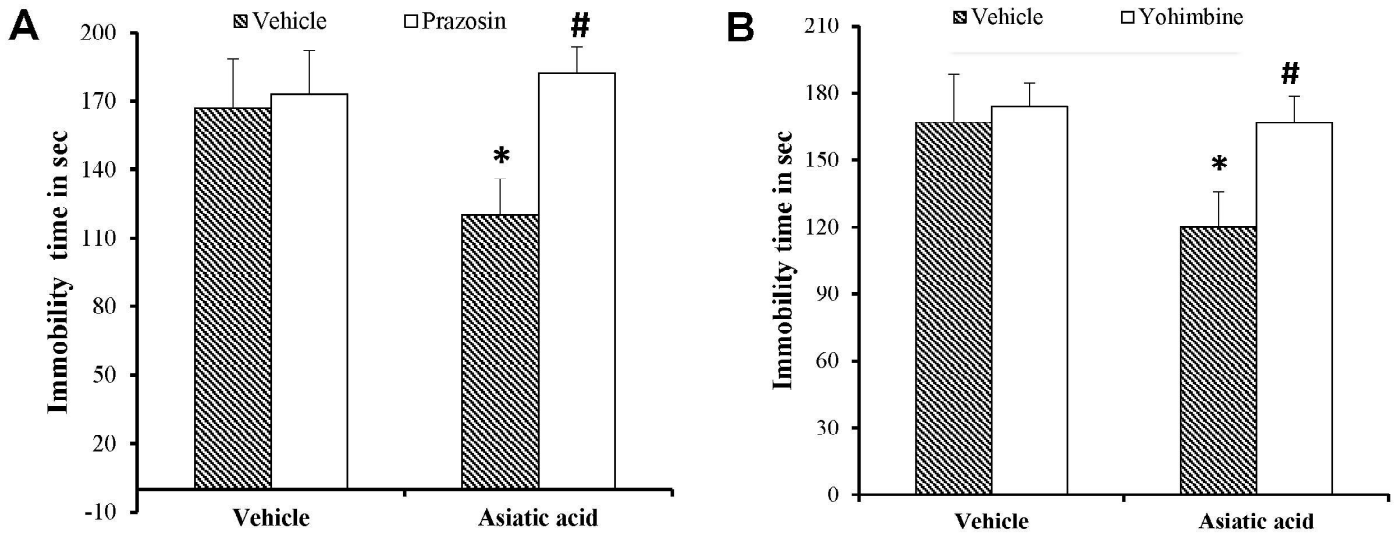

Fig. 4 : Effect of pretreatment of mice with prazosin (1 mg/kg, i.p., panel A) and yohimbine (1 mg/kg i.p., panel B) on the immobility time of asiatic acid (20 mg/kg p.o.) in the forced swimming test. Each column represents the Mean±S.E.M. of 6 animals. *P<0.01 when compared with the vehicle treated control. #P<0.01 as compared with asiatic acid alone.

was administered 30 min later. Animals underwent FST after another 45 min. The results of our study showed that the antidepressant-like activity of asiatic acid was unaltered by naloxone pretreatment in FST (Fig. 5). Two-way ANOVA revealed no significant differences of naloxone pretreatment [F(1,20)=1.20, P=0.29] or asiatic acid x naloxone interaction [F(1,20)= 1.69, P=0.21], but showed significant effect with asiatic acid treatment [F(1,20)=30.90, P=0.0001].

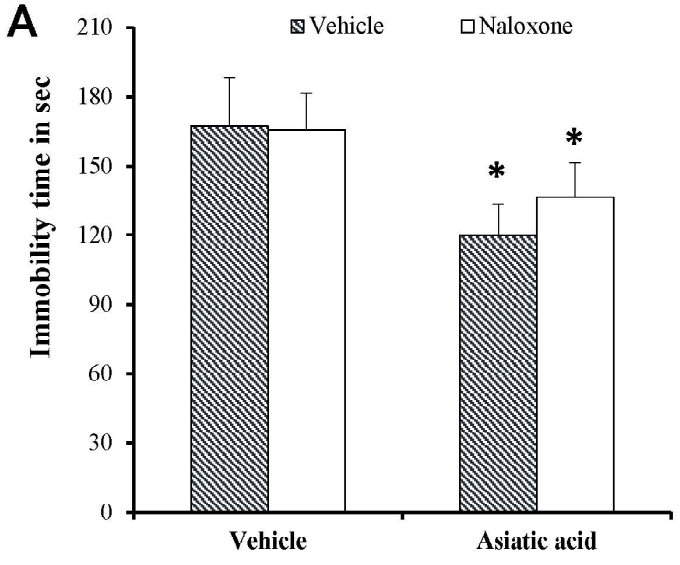

Fig. 5 : Effect of pretreatment of mice with naloxone (1 mg/kg i.p.) in the forced swimming test. Each column represents the Mean±S.E.M. of 6 animals. *P<0.01 when compared with the vehicle treated control.

Effect on locomotor function

The locomotor activity of mice was evaluated based on the actophotometer performance (Table I). Asiatic acid did not alter the locomotor activity of mice in any of the doses tested [F (4,25)=0.650, P=0.119].

TABLE I : Effect of asiatic acid treatment on the locomotor activity as measured by an actophotometer.

Drug	*Number of crossings*
Saline	164.50±7.06
Fluoxetine, 20 mg/kg	170.17±14.60
Asiatic acid, 5 mg/kg	174.26±3.90
Asiatic acid, 10 mg/kg	166.20±13.59
Asiatic acid, 20 mg/kg	155.00±12.85

Results are expressed as Mean±S.E.M of 6 animals. Mice received a single dose of vehicle or one of the above drugs before being tested in an actophotometer.

Discussion

The present study has evaluated the antidepressant-like effect of a triterpenoid compound, asiatic acid, in models of behavioral despair while also exploring potential mechanisms of action. This study

demonstrated the antidepressant-like activity of asiatic acid at 5, 10 and 20 mg/kg doses, in both TST and FST. To our knowledge, this is the first time it has been shown that the antidepressant-like action of asiatic acid was mediated through serotonergic and noradrenergic systems and that opioid receptors play no role in this antidepressant activity. Further research is needed to determine other possible mechanisms by which asiatic acid may exert its effects.

Many triterpenoid compounds have been reported to possess antidepressant activities. Triterpenoid saponins from *Fructus akebiae* and several other similar compounds from *Polygala tenuifolia* and *Passiflora edulis*have been reported as having antidepressant activities mediated by monoaminergic mechanisms (18, 19). Ursolic acid is a pentacyclic triterpenoid carboxylic acid which is present in *Rosmarinus officinalis* and *Fugenia brasilienis* and it has been reported to have antidepressant-like activity mediated through the monoaminergic system (9). Our studies explored the antidepressant activity of asiatic acid, another triterpinod compound and derivative of ursolic acid. Asiatic acid is derived from *Centella asiatica,* a plant used in traditional medicine for neuro-psychological disorders (6).

In the present study, we showed convincing evidence that asiatic acid at doses of 5, 10 and 20 mg/kg produced an antidepressant like effect in both TST and FST models. This was evident in all the doses in TST, whereas, in FST the lower dose, 5 mg/kg did not produce a significant reduction in immobility time. Such variations are expected in behavioral models depending on the sensitivity of the test or due to variations in the mode of actions of drugs (20). Cerebuga *et al.,* (2015) examined the anxiolytic and antidepressant effect of asiatic acid at 30 mg/kg, after intraperitoneal administration (8). The authors used rats and evaluated the antidepressant activity using FST. Though a trend of increased mobility time was observed, their results were not statistically significant. This may be due to increased variance reported in their studies as evidenced by the large standard error of mean. Moreover, our study utilized an oral administration route, as this is preferable over intraperitoneal administration, as was used in their studies. Additionally, many CNS drugs have a narrow therapeutic window, where doses above or below the effective dose fail to elicit the desired action (17). This prompted us to start with a lower dose of 5 mg/kg and increase in a logarithmic fashion. At the tested dose levels of asiatic acid (5, 10 and 20 mg/kg), we did not observe any dose dependent effect or U shaped-trend as reported with many conventional antidepressant drugs (17, 21). However, an inverted U-shaped trend was observed in our study, when antidepressant like activity was measured by TST. This has also been reported for other phytochemicals (22). Without a larger sample size, it would be inappropriate for us to make such assumptions.

The studies done using the actophotometer revealed that asiatic acid at the doses of 5, 10 and 20 mg/kg, did not influence the normal locomotor functions of mice. If the test drug has any psychostimulant effect, it could have led to a false positive result (17). Our finding on locomotor activity testing ruled out any such stimulant or depressant effects of asiatic acid and confirmed the reduction in the immobility time in TST and FST were due to its antidepressant-like effect.

The role played by the monoaminergic system in the genesis of depression is well established, and $5HT_{1A}$ receptors play an important role in the antidepressant effect of many drugs. The $5HT_{1A}$ receptors are present presynaptically in the raphae nuclei and postsynaptically in limbic and cortical regions of the brain (4, 24). To determine if asiatic acid may be working through these receptors, WAY 100635, a selective $5HT_{1A/1B}$ receptor blocker was used. It was observed that the antidepressant like action produced by asiatic acid was abolished by WAY 100635, indicating the involvement of these receptors. The $5HT_2$ receptors are also abundant in the brain, in a pattern suggesting that their activation may be implicated in the regulation of mood disorders. Brains of depressed individuals that committed suicide showed a hypersensitivity of $5HT_2$ receptors (11, 23). In the present study, pretreatment with ketanserin, a non-selective $5HT_2$ receptor antagonist reversed the effect of asiatic acid. This suggests a probable participation of $5HT_2$ receptors in this effect. The

role of $5HT_3$ receptors played in depression are also reported by many authors (25). A $5HT_3$ receptor blocker, ondansetron also inhibited the reduction in immobility time produced by asiatic acid. This provides indirect evidence of the participation of $5HT_3$ receptors in the antidepressant effect of this triterpenoid compound.

Noradrenergic system also plays a role in the genesis of depression and many clinically useful drugs can act by increasing the concentration of noradrenaline in the synapse or by direct interaction with noradrenergic receptors (21, 17). In the present study, prazosin (an α_1 adrenoceptor antagonist) and yohimbine (an α_2 adrenoceptor antagonist) pretreatment abolished the antidepressant like effect produced by asiatic acid. This provides evidence that asiatic acid may be modulating the noradrenergic system, which may be partly responsible for the antidepressant activity.

The role played by the opioid system is also implicated in the genesis of depression where the mood changes associated with depression may be attributed to deranged endogenous opioids (4). The clinical trials indicate that opioid compounds such as β-endorphins and buprenorphine have beneficial effects in depressed patients. It has been shown that there is a pronounced reduction in the μ opioid receptor availability in the thalamus and anterior cortex of the patients with major depressive disorders (9). In our study, the nonselective opioid receptor blocker, naloxone could not reverse the antidepressant effect produced by asiatic acid in FST. Even though the present study failed to use any specific receptor blockers for the confirmation, these preliminary results suggest that opioid receptors play no role in the effects seen with asiatic acid.

The cognitive functions such as attention, concentration, memory and information processing are largely regulated by acetylcholine. In depressive patients, cognitive functions will be impaired indicating deficits in cholinergic function. The stress response induces acetylcholine release in the forebrain and activates the septohippocampal pathway. This mediates physiological and emotional responses, in part through acetylcholine action on the HPA-axis (26). A study by Chen *et al.,* (2005) in rats, found that *C. asiatica* may ameliorate the functions of the HPA axis and thereby increase the contents of monoamine neurotransmitters in the brain (27). *Centella asiatica* administration produced a reduction of the corticosterone level in serum and increase of the contents of 5-HT, NE, DA and their metabolites 5-HIAA, MHPG in rat brain.

Chronic stress has been reported to decrease acetylcholinesterase (AChE) activity in the hippocampus and was associated with learning and memory deficits after exposure to chronic stress. Pharmacological augmentation of septohippocampal cholinergic activity enhances learning and memory performance in cognitively impaired animals. Some classical antidepressants also partially target cholinergic neurotransmission. The SSRI, citalopram, has been shown to reverse memory impairment by enhancing acetylcholine release in the hippocampus of laboratory animals. The mood stabilizer, lithium, has also been shown to upregulate hippocampal cholinergic muscarinic receptors (26). Asiatic acid has been reported to inhibit AChE and enhance the cholinergic activity and can correct the deficiency of this neurotransmitter at the synaptic junctions. This may be another possible mechanism of its action apart from the well-known monoaminergic system. Its selective modulatory action on $GABA_b$ receptors also can translate into improved memory and learning without sedative action (28).

Brain-Derived Neurotrophic Factor (BDNF) plays an important role in neurogenesis and neuronal remodeling (29). Studies have shown that hippocampal BDNF levels will be decreased upon chronic stress in animals. *Centralla asiatica* extract, which contains asiaticosides, can prevent the decrease in BDNF levels induced by various stressors in animals. Among various regulators of BDNF expression, cAMP-response element binding protein (CREB) complex, which is activated by sirtuin 1(SIRT1), is considered as a major factor. Rochmah*et al.,* (2019) reported that the SIRT1 and their subsequent targets such as CREB may not be directly involved in the mechanism of *Centralla asiatica* effect on hippocampal BDNF (30).

Taken together, the present study indicate that the antidepressant effect of asiatic acid, at least in part, may be mediated through the interaction with serotonergic system ($5HT_{1A/1B}$, $5HT_2$ and $5HT_3$ receptors) and noradrenergic system (α_1 and α_2 adrenoceptors.) additional studies are necessary to determine if other monoamines like dopaminergic system could play a mechanistic role in the effects of asiatic acid. Future studies should confirm the involvement of the serotonergic and adrenergic systems by directly measuring the neurotransmitter levels within the brain. Further insight into the role played by the cholinergic system and its modulation by $GABA_b$ receptors will be valuable. The influence of asiatic acid on Brain Derived Neurotrophic Factors are reported, which will produce a neurotrophic effect (29). How asiatic acid mediates the changes in hippocampal BDNF expression may be explored in future studies.

Conclusion

The results of the present study show the antidepressant-like effect of asiatic acid on behavioral despair models. The administration of asiatic acid reduced the immobility time during TST and FST, indicating an antidepressant like activity. In addition, the present study provides evidence that the antidepressant like effect of asiatic acid may be mediated through serotonergic and noradrenergic systems, but not through opioid signaling. Additional studies need to be conducted to assess the viability of asiatic acid as a chronic treatment in animal models and clinically depressed patients.

Abbreviations:

BDNF- Brain Derived Neurotropic Factor, AA- asiatic acid, FST- Forced swimming test, TST- Tail suspension test, CREB- cAMP-response element binding protein

Conflicts of interest:

None declared

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