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Antidepressant activity of *Asparagus racemosus* in rodent models

Gireesh K. Singh [a], Debapriya Garabadu [a], A.V. Muruganandam [b], Vinod K. Joshi [c], Sairam Krishnamurthy [a,*]

[a] Pharmacology Division, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, India
[b] Research and Development Centre, Indian Herbs Research and Supply Co. Ltd, Saharanpur, U.P., India
[c] Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India

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ABSTRACT

Asparagus racemosus Linn. (AR) is an Ayurvedic rasayana used as an adaptogen. Adaptogenic drugs are those which are useful as anti-stress agents by promoting non-specific resistance of the body. Although, the adaptogenic effect of AR is well documented, its use in psychological disorders like depression is not scientifically evaluated. Hence, the present investigation evaluates the antidepressant effect of methanolic extract of roots of AR (MAR) standardized to saponins (62.2% w/w). Rats were given MAR in the doses of 100, 200 and 400 mg/kg daily for 7 days and then subjected to forced swim test (FST) and learned helplessness test (LH). The results show that MAR decreases immobility in FST and increases avoidance response in LH indicating antidepressant activity. In behavioral experiments, MAR increased the number of head twitches produced by 5-HTP and increased clonidine-induced aggressive behavior indicating facilitatory effect on both serotonergic and adrenergic systems respectively. However, MAR had insignificant effect on L-DOPA-induced aggressive behavior indicating absence of activity on dopaminergic system. MAR also reversed changes to the endogenous antioxidant system induced by FST. Thus, MAR has significant antidepressant activity and this effect is probably mediated through the serotonergic and the noradrenergic systems and augmentation of antioxidant defenses.

1. Introduction

Depression is a common chronic recurrent syndrome, often refractive to drug treatment affecting quality of life and overall productivity. The one-year prevalence rate is about 5% (Paykel et al., 2005) and recurrence rates up to 85% have been reported (Lee and Murray, 1988). Although, several classes of antidepressants are currently being used, due to clinical limitations and adverse effects there is critical interest in development of efficient and safe drugs for treatment of depression (Tran et al., 2003). Plant sources such as *Withania somnifera* (Bhattacharya et al., 2000), *Bacopa monniera* (Sairam et al., 2002) and St. John's wort extract (Kasper et al., 2006) have been reported to have antidepressant activity and can be effective therapeutic alternatives for treatment of depression.

Asparagus racemosus (AR) is a commonly used rasayana in Ayurveda, an ancient system of Indian Medicine. Ayurvedic rasayanas are those drugs, which prevent ageing, increase longevity, impart immunity, improve mental functions and add vigor and vitality to the body (Sharma, 2001). In accordance with its claimed Ayurvedic use, AR has been reported to have potent adaptogenic activity (Rege et al., 1999). Adaptogenic drugs are those which are useful to counteract stressful factors by promoting non-specific resistance of the body (Brekhman and Dardymov, 1969). Adaptogens are presumed to increase the resistance of the body to stress by modulating stress mediators such as corticosteroids, catecholamines, and nitric oxide (Panossian et al., 1999; Rege et al., 1999) and may also act non-specifically as antioxidant or immunomodulator among other activities (Panossian et al., 1999; Rege et al., 1999). It is also important to note that AR has been reported to have teratogenic activity in experimental animals (Goel et al., 2006). However, further studies are required to confirm this effect. In an earlier study, we have shown that the methanolic extract of AR (MAR) standardized to saponins significantly reduced cold-restraint stress induced-gastric ulcers in rats (Sairam et al., 2003). It has been reported by us that apart from anti-stress activity, rasayana drugs like *B. monniera* and *W. somnifera* also possess antidepressant activity (Rao et al., 2000; Bhattacharya et al., 2000; Sairam et al., 2002). Based on this premise, the present study investigates the potential antidepressant activity of MAR in rat models of depression.

Depression is commonly accepted to be a disorder due to disturbances in neurotransmitters function, particularly serotonin, noradrenalin and dopamine (Maes and Meltzer, 1995; Posener et al., 1994). Reduction in brain serotonin (Anguelova et al., 2003a,b; Drevets, 2001) has been reported to be one of the most important etiological factors for genesis of depression and the most widely used antidepressants namely serotonin reuptake inhibitors (SSRIs) increase extracellular availability of serotonin (Schreiber et al., 1995). Further,

* Corresponding author. Reader in Pharmacology, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, India.
E-mail addresses: saibliss@hotmail.com, ksairam.phe@itbhu.ac.in (S. Krishnamurthy).

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noradrenergic and dopaminergic systems are reported to be involved and act in tandem with the serotonergic system (Millan et al., 2000; Koch et al., 2002). As AR is reported to have adaptogenic property and as stress and depression share common anatomical and neurochemical substrates, we hypothesized that MAR may have antidepressant potential (Duncan et al., 1986; Dremencov et al., 2003; Deakin et al., 1992; Vertes et al., 1999; Loy et al., 1980; Samson et al., 1990).

Oxidative stress has been reported to play an important role in the genesis of depression (Bilici et al., 2001). Clinical studies show that patients with major depression had elevated antioxidant enzyme activity and increased lipid peroxidation (LPO) (Bilici et al., 2001; Khanzode et al., 2003). Superoxide dismutase (SOD), an antioxidant enzyme, causes the dismutation of superoxide anion radicals into H_2O_2, which is generated in the process of catecholamine deamination by mitochondrial enzyme MAO (Cohen, 1985). Treatment with antidepressant drugs significantly decreased the levels of antioxidant enzymes and decreased lipid peroxidation in patients (Abdalla and Bechara, 1994; Bilici et al., 2001; Khanzode et al., 2003). Thus, lipid peroxidation and the antioxidant enzyme response appear to be synchronized with development of major depression. As oxidative stress appears to be play major role in depression, antioxidant activity of MAR was evaluated in rats exposed to FST.

The major goal of the present study is to evaluate the potential antidepressant activity of MAR in rodent models of depression. Further, behavioral experiments were performed to ascertain the role of neurotransmitters in the antidepressant effect of MAR. The antioxidant effect of MAR was evaluated in the hippocampus and striatum using the FST model.

2. Materials and methods

2.1. Plant material

The cultivated variety of AR was collected in the month of December from the Ayurvedic garden of our Institute and was identified with the standard sample preserved in the Department of Dravyaguna, Institute of Medical Sciences, Varanasi. The fresh roots of AR were size reduced and macerated with methanol for 7 days. The methanolic extract of AR (MAR) was filtered, vacuum dried and stored in a refrigerator until further use. The yield was 10%. MAR was standardized for the total saponin content.

2.2. Standardization of extract

MAR (1 g) was defatted with petroleum ether (60–80 °C) and successively extracted with chloroform and ethyl acetate. Chloroform and ethyl acetate extracts were discarded. Residue was dissolved in methanol (15 ml), filtered and concentrated to 5 ml. The 5 ml concentrate was added drop by drop with constant stirring to 25 ml of acetone in order to precipitate the saponins. The precipitate was filtered, collected and dried to constant weight at 105 °C (0.622 g). Total saponin was found to be 62.2%.

2.3. Animals

Adult Charles Foster strain albino rats (180–220 g) and mice (20–30 g) of either sex were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University. The male and female animals were equally distributed in all the experiments. The animals were housed in polypropylene cages at an ambient temperature of 25 °C± 1 °C and 45–55% RH, with a 12:12 h light/dark cycle. The animals had free access to commercial food pellets (Doodh dhara Pashu Ahar, India) and water *ad libitum* unless stated otherwise. Experiments were conducted between 09:00 and 14:00 h. Animals were acclimatized for at least one week before using them for experiments and exposed only once to every experiment. The experimental procedures were in compliance with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4. Drug treatment

The animals were pretreated orally with 0.3% carboxymethylcellulose (CMC) suspension of MAR for 7 days daily at the doses of 100, 200 or 400 mg/kg/day. All the experimental procedures were started on day 7, 1 h after the drug administration. In case of FST (8 days) and LH (12 days) the drug administration was continued till the end of the experimental schedule. The dose range was selected based on the dose of MAR which showed significant antiulcer effect in the cold-restraint stress model in our earlier study (Sairam et al., 2003). Control rats received the vehicle (0.3% CMC suspension). Imipramine (Torrent Pharma, India; 15 mg/kg) and lorazepam (Ranbaxy, India; 2.5 mg/kg), the reference drugs were administered intraperitoneally 30 min before the experimentation. 5-HTP (100 mg/kg i.p.), clonidine (50 mg/kg i.p.) and L-DOPA (100 mg/kg i.p.) were procured from Sigma, St. Louis, MO.

2.5. Experimental methods

2.5.1. Forced swimming test (FST)

The procedure as described by Porsolt et al. (1978) was used, except that the water level was deeper (Detke and Lucki, 1996). Swimming sessions were conducted by placing rats in individual glass cylinders (45 cm high×20 cm in diameter) containing (25±2 °C) water 38 cm deep, so rats could not support themselves by touching the bottom with their feet. Two swimming sessions were performed between 12:00 h and 19:00 h, an initial 15 min pretest followed 24 h later by a 5 min test. Drugs were administered 1 h after the pretest and 1 h (MAR) and 30 min (IMP) before the test session. Following both swimming sessions, the rats were removed from the cylinders, dried with paper towels and placed in heated cages for 15 min, and then returned to their home cages. The immobility period in seconds was measured live in each test session by a blind observer.

2.5.2. Learned helplessness test (LH)

This model is based on the assumption that, exposure to uncontrollable stress associated with repeated experiences of failure to escape from the stress produces a "helpless" situation resulting in performance deficits in subsequent learning tasks (Sherman et al., 1979). On day one of experimentation, an inescapable electric foot shock (scrambled randomized inescapable shocks of 15 s duration, 0.8 mA, every minute+15 s to grid floor) was delivered in (20×10×10 cm) plexiglass chambers. Control rats were placed for 1 h in identical chamber without any shock treatment.

In conditioned avoidance training, the number of escape failures was evaluated 48 h later i.e., on day 3 after inescapable shock pretreatment in the Sidman jumping box (Techno, Lucknow, India). Briefly, jumping box consists of two identical chambers (27×29×25 cm) with stainless steel grid floor (1 cm mesh) that are divided by a partition with 14×17 cm gate. Animals were placed individually and were allowed to adapt for 5 min for the first session only and subjected to 30 avoidance trials with inter-trial gap of 30 s. During the first 3 s of each trial, a sound signal was presented as a cue allowing the animals to avoid shocks. If a response did not occur within this period, a 0.8 mA shock (3 s duration) was applied via the grid floor. In case no escape response occurred within this period, shock and sound conditioned stimulus were terminated. Avoidance sessions were performed in the forenoon period on days 3, 4 and 5. The number of escape failures and avoidance responses on those above-mentioned days were recorded live by a blind observer.

2.5.3. 5-hydroxytryptaphan (5-HTP)-induced head twitches in mice

Mice were treated with 5-HTP (100 mg/kg i.p.) and the numbers of head twitch performed by each mice was counted by staggering method using three 2 min periods (19–21 min), (23–25 min), (27–

29 min) after 5-HTP administration. 5-HTP was administered 1 h after 7 day pretreatment with MAR and head twitches were scored live by a blind observer (Schreiber et al., 1995).

2.5.4. Clonidine-induced aggression in mice

The method of Morpurgo (1968) was used. Four pairs of mice, two pairs from each sex (each pair contained same sex of mice), were given the standard dose of clonidine (50 mg/kg i.p.) dissolved in normal saline. In test pretreated group, clonidine was given 1 h after the last dose of MAR. The animals were then caged in bell shaped glass jar with a floor area of approximate 16 cm^2. A blind observer recorded the biting/fighting episodes live over a period of 30 min, in each pair.

2.5.5. L-DOPA-induced hyper activity and aggressive behavior in mice (LHA)

Mice were treated with L-DOPA (100 mg/kg i.p.) and the experiment was performed according to the method of Serra et al., 1990. The mice of either sex were equally distributed into two groups of eight each. Each group had four pairs of mice (each pair contained same sex of mice). Similar drug treatment protocol was followed as in previous behavioral experiments. Stages of activity and aggressive behavior were recorded live every 10 min for 30 min after L-DOPA administration by the blind observer. The different parameters of observation were piloerection, salivation, increase in motor activity, irritability, reactivity, jumping squeaking, and aggressive fighting. The scores were graded in the following manner:

0—No effect; 1—Piloerection, slight salivation, slight increase in motor activity; 2—Piloerection, salivation, marked increase in motor activity and irritability; 3—Piloerection profuse salivation, marked increase in motor activity, reactivity, jumping, squeaking and aggressive fighting.

2.5.6. Spontaneous motor activity (SMA)

Rats of either sex were equally distributed in each group and were treated orally with 100 mg/kg of MAR or vehicle for 7 days. After 50 min, the rats were placed in the photoactometer (Techno, Lucknow, India) and acclimatized for 10 min. The interruptions of 16 photo beams spaced 2.5 cm apart and 2.5 cm above the floor were detected and scored (Boissier and Simon, 1965). Activity counts were recorded live for 10 min after acclimatization by a blind observer.

2.5.7. Antioxidant studies

Rats were divided into four groups of 6 animals each. One served as the control and was administered the vehicle. The rest of the groups were subjected to FST as described earlier. The rats were pretreated for 7 days with MAR orally in the dose of 100 mg/kg and the other group received imipramine (15 mg/kg) on the day of experiment. This dose was selected, as this was the optimum dose as observed from the antidepressant studies. On the 7th day of the test drug treatment, the rats were subjected to FST. The brain after decapitation was microdissected for hippocampus and striatum. The levels of LPO as measure of oxidative damage and the antioxidant enzymes namely; SOD and catalase (CAT) were estimated. Protein was estimated using the method of Lowry et al., 1951.

2.5.7.1. Lipid peroxidase estimation. LPO product malondialdehyde was estimated spectrophotometrically using 1,1,3,3-tetraethoxypropane as the standard following the method of Ohkawa et al., 1979. The results are expressed as n mol/mg of protein.

2.5.7.2. Superoxide dismutase estimation. SOD was estimated by following the method of Kakkar et al., 1984. The inhibition of nitro blue tetrazolium to blue coloured formazan in presence of phenazine methosulphate and NADH was measured at 560 nm using *n*-butanol as blank. The results are expressed as units (U) of SOD activity/mg of protein.

2.5.7.3. Catalase estimation. Decomposition of hydrogen peroxide in presence of CAT was followed at 240 nm (Beers and Sizer, 1952). The results are expressed as units (U) of CAT activity/mg of protein.

2.6. Statistical analysis

The data were analyzed with GraphPad Prism 4 (San Diego, CA). Statistical analysis of data was done by One-way ANOVA, followed by Newman Keuls test except for L-DOPA-induced hyperactivity and aggressive behavior, and spontaneous motor activity for which analysis was done by Student's *t* test. Data are expressed as mean ± S.E.M. A level of $p<0.05$ was accepted as statistically significant.

3. Results

3.1. Forced swimming test

Fig. 1 illustrates the effect of MAR on the duration of immobility time in the FST model. One-way ANOVA revealed that there were significant differences between treatment groups [$F(4,25)=21.702$, $p<0.05$]. Post-hoc analysis showed that the MAR (100, 200 and 400 mg/kg) and IMP treated groups were significantly different ($p<0.05$) from the vehicle treated group. MAR significantly decreased the duration of immobility time indicating antidepressant effect. There was no significant dose-dependent effect of MAR. The effect of MAR was comparable to that of IMP.

3.2. Learned helplessness test

The effect of MAR (100, 200 and 400 mg/kg) on escapes failures and avoidance responses on days-3, 4 and 5 are depicted in Fig. 2A, B and C respectively. One-way ANOVA indicated that there were significant differences in escape failures between experimental groups on day-3 [$F(4,25)=155.62$, $p<0.05$] and day-4 [$F(4,25)=101.9$, $p<0.05$] after shock treatment. However, there were no significant differences in escape failures between experimental groups on day-5 [$F(4,25)=1.083$, $p>0.05$]. Post-hoc analysis showed that MAR significantly decreased escape failures on days-3 and 4 ($p<0.05$) in a dose independent manner and the effect was comparable to that of IMP after 7 days of pretreatment and 5 days of MAR treatment during the experimental schedule. Further, One-way ANOVA revealed that there were significant differences in avoidance response between experimental groups on day-3 [$F(4,25)=143.03$, $p<0.05$] and day-4 [$F(4,25)=108.75$, $p<0.05$] after shock treatment and there were insignificant differences in avoidance response between experimental groups on day-5 [$F(4,25)=1.083$, $p>0.05$]. Post-hoc analysis showed that MAR significantly increased the avoidance response on days-3 and 4

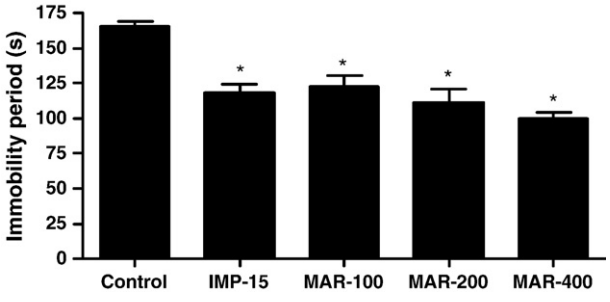

Fig. 1. Effect of methanolic extract of *Asparagus racemosus* (MAR) and imipramine (IMP; 15 mg/kg) on forced swim test (FST) in rats. MAR (100, 200 and 400 mg/kg) pretreatment for 7 days significantly decreased the immobility time in the FST indicating antidepressant effect in this model. Each column represents mean ± S.E.M. of immobility period (s) of 6 animals in each group. *$p<0.05$ compared to control (One-way ANOVA followed by Student–Newman–Keuls test).

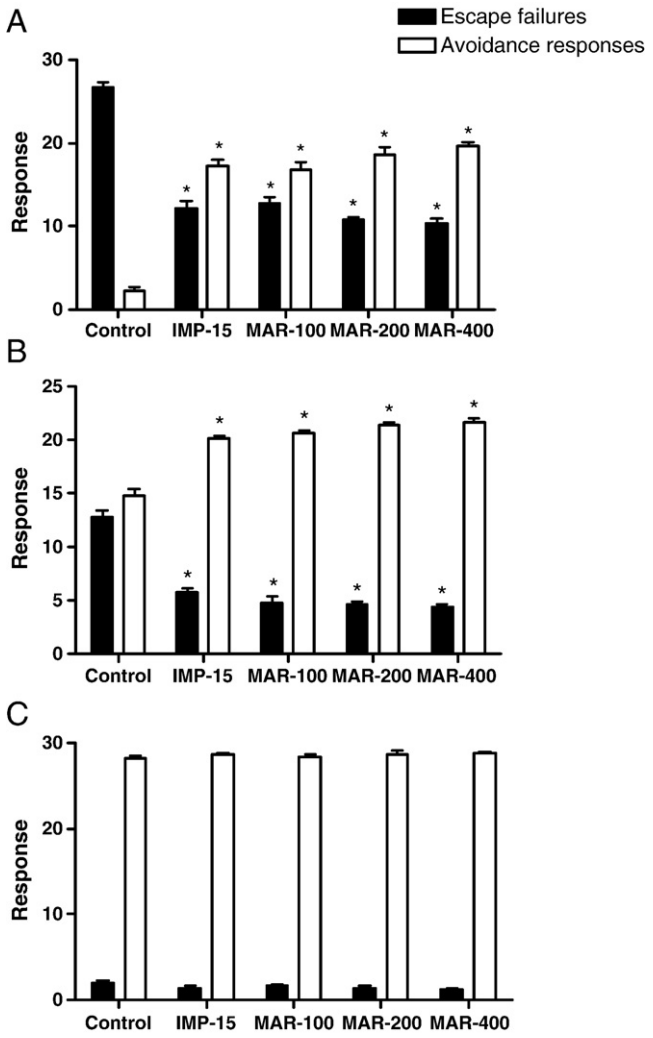

Fig. 2. Effect of MAR and IMP on learned helplessness model of depression in rats. MAR (100, 200 and 400 mg/kg) pretreatment for 7 days significantly decreased escape failures and increased avoidance responses in contrast to control rats gradually from day-3 (A) to day-4 (B). The escape failures and avoidance responses of MAR on day-5 were not significantly different from control (C). Each column represents mean ± S.E.M. of response of 6 animals in each group. *$p<0.05$ compared to control (One-way ANOVA followed by Student–Newman–Keuls test).

($p<0.05$) in a dose-independent manner and the effect was comparable to that of IMP after 7 days of pretreatment and 5 days of MAR treatment during the experimental schedule. Therefore, MAR decreased escape failures and increased avoidance response on days-3 and 4 indicating antidepressant activity in this model. However, MAR did not show any change in escape failures and avoidance response on day-5.

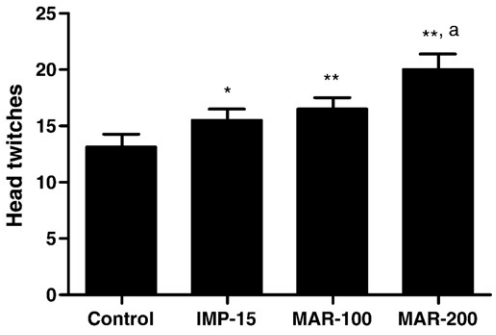

Fig. 3. Effect of MAR on 5-HTP-induced head twitches in mice. MAR (100 mg/kg and 200 mg/kg) pretreatment for 7 days significantly increased the 5-HTP-induced head twitches. Each column represents mean ± S.E.M. of number of head twitches of 8 mice in each group. **$p<0.01$, *$p<0.05$, compared to control and [a]$p<0.05$ compared to 100 mg/kg of MAR (One-way ANOVA followed by Student–Newman–Keuls test).

Table 1
Effect of MAR on L-DOPA-induced hyperactivity and aggressive behavior (Data are expressed as mean ± S.E.M., $N=8$ in each group)

Groups	Behavioral score
Control	2.0 ± 0.19
MAR (100 mg/kg)	2.5 ± 0.27

3.3. 5-HTP-induced head twitches

Fig. 3 illustrates the effect of MAR and IMP on 5-HTP-induced head twitches in mice. One-way ANOVA showed a significant difference in drug treatment between the groups [$F(3,28)=15.502$, $p<0.01$]. Post-hoc analysis revealed that both the doses of MAR (100 mg/kg and 200 mg/kg, $p<0.01$) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 200 mg/kg was more effective than 100 mg/kg ($p<0.05$). Similarly, IMP treated group showed significant increase ($p<0.05$) in the 5-HTP-induced head twitches compared to control. However, the effect of 200 mg/kg of MAR was significantly higher than IMP ($p<0.05$). Therefore, MAR enhances the serotonergic mediated behavior indicating the involvement of serotonergic pathway in the antidepressant activity.

3.4. L-DOPA-induced hyperactivity and aggressive behavior (LHA)

The effect of MAR on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 1. The Unpaired Student t test reveals that the MAR did not have any significant effect on L-DOPA-induced hyperactivity and aggressive behavior in mice ($t=1.528$, df −14; $p>0.05$). Hence, the effect of MAR may not involve the dopaminergic pathway.

3.5. Clonidine-induced aggression

Fig. 4 indicates the effect of MAR (100 and 200 mg/kg) on the latency to first attack and the number of bouts in the clonidine-induced aggressive behavior in mice. One-way ANOVA reveals that there were significant differences in the latency to first attack among the experimental groups [$F(3,28)=64.083$, $p<0.05$]. Post-hoc analysis

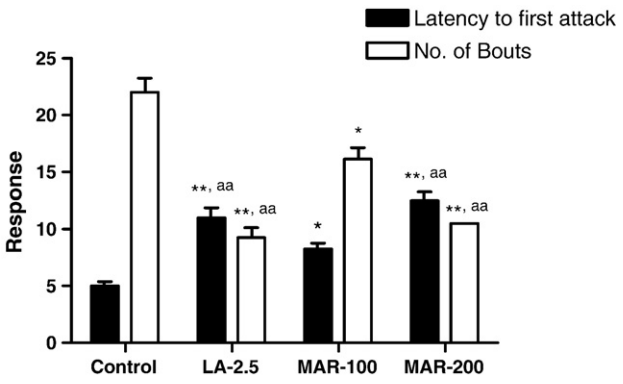

Fig. 4. Effect of MAR (100 and 200 mg/kg) and lorazepam (LA; 2.5 mg/kg) on clonidine-induced aggression in mice. MAR pretreatment for 7 days significantly increased the latency to first attack and decreased the number of bouts. The dose of 200 mg/kg was more effective than the dose of 100 mg/kg. Each column represents mean ± S.E.M. of response of 8 mice in each group. *, [a]$p<0.05$, **, [aa]$p<0.01$; *compared to control and [a]compared to MAR 100 mg/kg (One-way ANOVA followed by Student–Newman–Keuls test).

showed that MAR (100 mg/kg, $p<0.05$ and 200 mg/kg, $p<0.01$) significantly increased the latency to first attack compared to control. In comparison, the 200 mg/kg of MAR was more effective than 100 mg/kg and the effect was comparable to that of lorazepam ($p<0.01$). Statistical analysis by One-way ANOVA shows that there were significant differences in the number of bouts between the experimental groups [$F(3,28)=54.881$, $p<0.05$]. Post-hoc analysis showed that MAR (100 mg/kg, $p<0.05$ and 200 mg/kg, $p<0.01$) significantly decreased the number of bouts compared to control. Furthermore, the effect of 200 mg/kg of MAR was comparable to that of lorazepam ($p<0.01$) and was more effective than the dose of 100 mg/kg of MAR. Thus, the antidepressant activity of MAR may also involve the adrenergic system.

3.6. Spontaneous motor activity (SMA)

The effect of the MAR given in the dose of 100 mg/kg on spontaneous locomotor activity is shown in Fig. 5. Unpaired Student *t* test reveals that the MAR significantly decreased spontaneous locomotor activity ($t=7.526$, $df-8$; $p<0.001$) compared to that of vehicle treated group.

3.7. Antioxidant activity

The effect of MAR on lipid peroxidation (LPO) and superoxide dismutase (SOD) in hippocampus and striatum is depicted in Fig. 6A and B respectively. One-way ANOVA showed that the level of LPO in stress with or without drug and vehicle treatment is significantly different in hippocampus [$F(3,20)=66.853$, $p<0.001$] and striatum [$F(3,20)=48.608$, $p<0.001$]. Post-hoc analysis revealed that there was a significant increase in LPO levels ($p<0.001$) in the FST group compared to control rats. This effect was significantly mitigated ($p<0.001$) by pretreatment with MAR (100 mg/kg, oral) for 7 days. There was a significant decrease in LPO levels ($p<0.001$) both in the hippocampus and striatum compared to the stress group. Similarly, IMP significantly decreased ($p<0.001$) LPO levels compared to stress group both in the hippocampus and striatum. Statistical analysis by One-way ANOVA to study the effect of MAR and IMP on the levels of SOD showed a significant effect of treatment both in the hippocampus [$F(3,20)=21.5$, $p<0.01$] and striatum [$F(3,20)=35.861$, $p<0.01$]. Post-hoc analysis revealed that the antioxidant enzyme SOD levels were significantly reduced ($p<0.01$) with stress compared to control and this change was significantly augmented with pretreatment with MAR both in the hippocampus and striatum. A significant increase in SOD levels ($p<0.01$) was observed in MAR treated group compared to stress group. Similarly, IMP significantly elevated SOD levels both in the hippocampus and striatum ($p<0.05$) compared to stress group. The effect of MAR on the antioxidant enzyme catalase (CAT) level in hippocampus and striatum are depicted in Fig. 7A and B respectively. One-way ANOVA revealed that there was significant difference in hippocampus [$F(3,20)=34.83$, $p<0.001$] and striatum [$F(3,20)=37.558$, $p<0.001$] CAT levels between the experimental groups. Post-hoc analysis showed that the level of CAT was significantly reduced ($p<0.001$) with stress and this change was significantly improved ($p<0.001$) both in the hippocampus and striatum with the pretreatment with MAR and IMP. Thus, MAR decreases elevated LPO level, ameliorates decreased SOD and CAT levels in stress, indicating antioxidant action in the hippocampus and striatum.

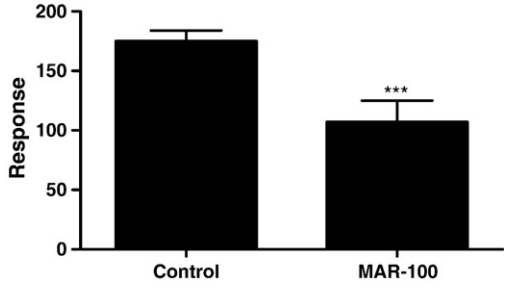

Fig. 5. Effect of MAR on spontaneous motor activity in mice. MAR pretreatment for 7 days with 100 mg/kg of MAR significantly decreased spontaneous motor activity. Each column represents mean±S.E.M. of 6 mice per group respectively. ***$p<0.001$ compared to control (Unpaired Student's-*t* test).

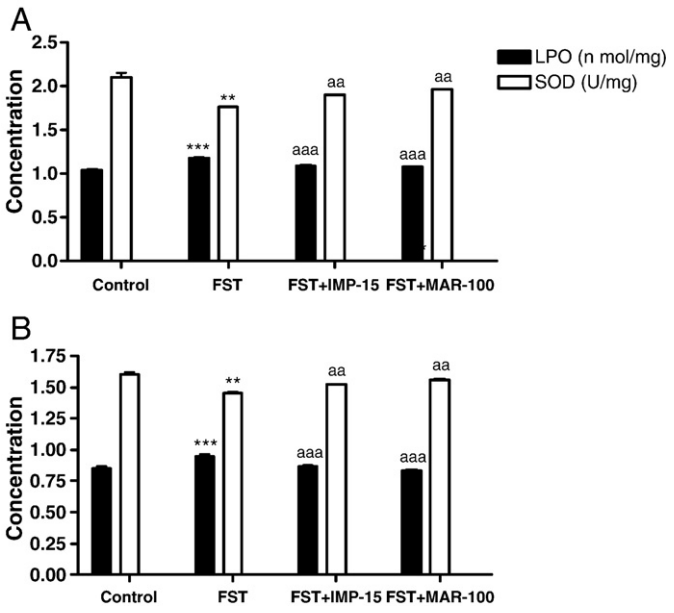

Fig. 6. Effect of MAR and IMP on hippocampal (A) and striatal (B) LPO and SOD levels in FST exposed rats. Each column represents mean±S.E.M. of the concentration of LPO and SOD of 6 animals per group. **, $^{aa}p<0.01$ and ***, $^{aaa}p<0.001$; * compared to control and ^{a}compared to FST (One-way ANOVA followed by Student–Newman–Keuls test).

4. Discussion

MAR pretreatment for 7 days given in the doses of 100, 200 and 400 mg/kg showed antidepressant activity in the forced swim and learned helplessness tests. MAR significantly reduced immobility

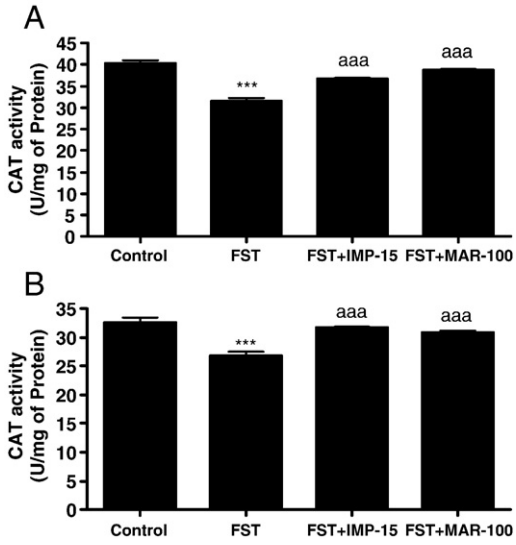

Fig. 7. Effect of MAR and IMP on hippocampal (A) and striatal (B) CAT levels in FST exposed rats. Each column represents mean±S.E.M. of the concentration of CAT of 6 animals per group. **, $^{aa}p<0.01$ and ***, $^{aaa}p<0.001$; *compared to control and ^{a}compared to FST (One-way ANOVA followed by Student–Newman–Keuls test).

period in the forced swimming test indicating antidepressant activity. Immobility is thought to reflect either a failure to persist in escape directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli (Kirby and Lucki, 1997). Several antidepressants reduce the immobility after forced swimming (Porsolt et al., 1978; Borsini and Meli, 1988). To further validate the antidepressant activity of MAR, the learned helplessness (LH) model of depression was used. LH is a validated animal model of stress-induced behavioral depression in which prior exposure to inescapable stress produces deficits in escape testing (Willner, 1991). Antidepressants reverse behavioral deficits induced by LH (Petty et al., 1979; Sherman et al., 1982). Although, this model mimics certain aspects of human depression such as changes in behavioral, biochemical, physiological and hormonal parameters, it satisfies criteria for face and predictive validity (Maier 1984; Willner 1986). In the LH test, rats exposed to inescapable and unpredictable electric shocks, at particular time frame losses the motivation to escape in spite of the possibility to do so; this is termed as escape failures. This coping deficit is decreased by potential antidepressant drugs. The animals also are not able to avoid the impending danger by escaping to the safe chamber, which is termed as avoidance responses, which are concomitantly increased. MAR and IMP significantly decreased escape failures and increased avoidance responses indicating antidepressant activity. Thus, the combined observations from both FST and LH indicate the antidepressant activity of MAR.

Depression has been linked to perturbations in the neurotransmission involving brain 5-HT, norepinephrine (NE) and dopamine activity (Maes and Meltzer, 1995; Posener et al., 1994). Studies implicating serotonin in the pathogenesis of depression have been widely studied both in preclinical and clinical investigations (Blier, 2003; Anguelova et al., 2003a,b; Drevets, 2001). Serotonin mechanisms are known to be involved in the development, maintenance, and reversal of LH (Petty et al., 1994). In the present study, MAR significantly increased the frequency of 5-HTP-induced head twitches indicating increase in serotonergic activity in rodent brain *in vivo*. Increase in stereotypical behavior by serotonin precursor 5-HTP is reported to involve increase in extracellular serotonin and subsequent activation of 5-HT_{2A} receptors (Schreiber et al., 1995). Although, it can be assumed that there is a favorable disposition of 5-HT in MAR treated animals, the exact mechanism of MAR-induced changes in serotonin levels can only be confirmed after *ex vivo* or *in vivo* experiments involving 5-HT depletion studies and/or direct 5-HT measurements.

It has been reported that psychostimulants which are clinically ineffective as antidepressants, however show antidepressant like effects in the FST (Sherman et al., 1982). To discount the possibility of false positives, MAR was evaluated for its effects on spontaneous motor activity. MAR did not increase spontaneous motor activity in rats but in fact had anti-immobility effect. It is of interest to note that several established antidepressants decrease locomotor activity (Hemby et al., 1997). In the LH model, the general activity is independent of antidepressant activity as amphetamine increased general activity without reversing escape failures (Sherman et al., 1982). MAR significantly reduced escape failures and augmented avoidance responses. Thus, it is unlikely that the antidepressant activity of MAR is due to the effects on motor activity.

Although, SSRIs have been the mainstay in the treatment of depression, it is interesting to note that only 30% of patients are responsive to SSRIs (Nemeroff, 1998). Further, in experimental models such as the LH paradigm, selective serotonergic lesions did not reverse escape failures by antidepressants and noradrenergic lesions of the hippocampus delayed the reversal of escape deficits by the imipramine (Soubrie et al., 1986, 1987). This suggests that the antidepressant activity can be sustained by extracellular norepinephrine levels. It is also reported that although post-synaptic 5-HT agonist reversed learned helpless behavior, the action of SSRIs was independent either 5-HT_{1A} receptor function or extracellular 5-HT (Zazpe et al., 2007). These observations suggest that serotonergic system may not be the sole transmitter mediating antidepressant activity and that NE may have an important role to play. Clinical studies show that combined 5-HT and NE reuptake inhibitor is more effective than used alone (Anderson, 1998; Nelson et al., 2004). Based on these observations, we evaluated the role of NE in the antidepressant effect of MAR. MAR significantly increased the clonidine-induced aggressive behavior indicating increased activity of noradrenergic system (Ozawa et al., 1975; Maj et al., 1981). Hence, the antidepressant activity of MAR may involve both serotonergic and noradrenergic systems. Several studies have shown that the antidepressant effect involves augmentation of dopaminergic neurotransmission (D'Aquila et al., 2000, 2001, 2003; Serra et al., 1990). However, MAR did not alter L-DOPA-induced aggressive behavior indicating absence of effect on the dopaminergic system. Even though there are no earlier reports indicating sedative effects of MAR, in the present study MAR significantly reduced spontaneous motor activity. Hence, it is possible that this may be the reason for absence of any significant effect of MAR on L-DOPA-induced aggression.

The above results indicate that MAR has antidepressant activity by virtue of its action on serotonergic and noradrenergic systems based on behavioral experimental evidence. Generally, repeated treatment with antidepressants has been reported to facilitate both serotonergic and/or noradrenergic transmission (Blier and De Montigny, 1994; Hajos et al., 2000). The dual action of MAR may have several advantages over SSRIs. Although, SSRIs are widely used, side effects such as nausea, sexual dysfunction and sleep disorders (Goodnick and Goldstein, 1998) may limit their potential use. Moreover, clinical studies indicate that they may not offer too many advantages over classical antidepressants (Goodnick and Goldstein, 1998). Further, clinical studies have shown that mixed 5-HT and NE reuptake inhibitors (SNRIs) are effective and well-tolerated antidepressants (Tran et al., 2003; Zajecka and Albano, 2004). Although, the specific mechanism of action of MAR needs to be explored before coming to any conclusions on its mechanism of action, preliminary investigations indicate that MAR may potentially have the more desirable dual action on 5-HT and NE.

It is pertinent to note that although serotonergic and adrenergic drugs have been successfully used in the treatment of depression, never the less there exist no casual relationship between reduced serotonin and depression. Clinically, a decrease in monoamine levels including serotonin does not necessarily lead to depression in undepressed individuals nor does further depletion of monoamines worsen behavioral symptoms in depressed patients (Delgado, 2000a). However, depletion of 5-HT or NE leads to rapid relapse in SSRI and NRI treated patients respectively implying that monoamines may not have a critical role in development of depressive symptoms, but however are important for response of antidepressant drugs (Delgado, 2000a,b, 2006). It is therefore important to evaluate other factors involved in development of depression. Oxidative free radicals increase during chronic depression and are reported to play an important role in the pathogenesis of depression (Bilici et al., 2001). Subchronic administration of SSRIs reversed oxidative damage and this may also be responsible for its antidepressant activity (Bilici et al., 2001). Several natural antidepressant drugs such as *B. monneira* and *W. sominfera* (Sairam et al., 2002; Bhattacharya et al., 2000) have been reported to have antioxidant activity including MAR (Sairam et al., 2002). In the present study, MAR significantly reduced free radical production in rats subjected to forced swim in terms of decrease in LPO activity both in the hippocampus and striatum. It is well established that the limbic system including the hippocampus is the active site for antidepressive effect of antidepressants (Duncan et al., 1986; Dremencov et al., 2003). The hippocampus mediates adaptation to stress (Deakin et al., 1992) and receives dense serotonergic (Vertes et al., 1999) and noradrenergic projections (Loy et al., 1980; Samson et al., 1990), which are modulated by

antidepressants. The antioxidant effect of MAR may be due to augmentation of antioxidant enzyme protective system as MAR increased SOD and CAT levels both in hippocampus and striatum which were compromised due to FST.

The present study establishes the antidepressant activity of MAR in rodent models of depression. Further, results from behavioral experiments indicate that this activity may be due to the facilitatory effect on both serotonergic and noradrenergic system apart from the antioxidant activity.

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