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Inhibitory Effect of *Bacopa monniera* on morphine Induced Pharmacological Effects in Mice

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Abstract – The effects of the alcoholic extract of *Bacopa monniera* (BMA) on morphine-induced pharmacological activities were studied in mice. Oral administration of the extract (40 mg/kg) significantly inhibited morphine-induced analgesic tolerance, withdrawal symptoms, hyperactivity, reverse tolerance, Dopamine receptor supersensitivity and apo-morphine-induced climbing behaviour in mice. The results of this study showed that, alcoholic extract of *Bacopa monniera* (BMA) exerted inhibitory effect against morphine-induced pharmacological effects, suggesting that the extract could be useful in the treatment of morphine toxicity.
Keywords – *Bacopa monniera*, morphine toxicity, hyperactivity, analgesic tolerance, supersensitivity, climbing behaviour

Introduction

Bacopa monniera (Linn) wettst. (Syn. *Herpestis monniera* (Linn) H.B & K is a small creeping herb known as brahmi in the Ayurvedic System of Medicine. It is a potent nervine tonic used in the treatment of epilepsy, insanity, hysteria, and other mental disorders. It is claimed to improve memory and mental functions (The wealth of India Raw Materials, 1988). The saponin fraction exhibited barbiturate hypnosis potentiation in rats and clinical trial showed it to be an anti anxiety agent with adaptogenic effect (Singh and Singh, 1980). The drug was shown to have tranquilizing effect with improvement in mental function (Singh *et al.*, 1979). The saponins designated as bacosides A and B improved the performance of rats in several learning tests as manifested by better acquisition, consolidation, and retention of newly acquired behavioural responses (Singh and Dhawan, 1982). Bacosine, a triterpenoid isolated from the plant showed potent analgesic activity (Vohora *et al.*, 1997). The plant extracts also exhibited antiepileptic (Martis *et al.*, 1992), antioxidant (Tripathi *et al.*, 1996), adrenergic (Khanna and Ahmed, 1992), and anti-cancer (Elangovan *et al.*, 1995) activities. The facilitatory effect of bacosides on hippocampus, hypothalamus, and cerebral cortex have been demonstrated together with their safety in clinical trials (Singh and Dhawan, 1997). Hepatoprotective activity (Sumathi *et al.*, 2001), protective effect on morphine-induced brain mitochondrial enzyme activity (Sumathi *et al.*, 2002), and inhibitory effect on *in-vitro* effects of morphine withdrawal in guinea pig ileum (Sumathi *et al.*, 2002) have also been reported for this plant extract. *Bacopa monniea* has been reported for its use in improving mental ability and used as a nervine tonic, an attempt has been made to evaluate its efficacy against morphine toxicity, since morphine has been reported to cause a variety of neurological disorders. *Bacopa monniera* belongs to a group of Ayurvedic drugs classified as "Medhya Rasayana Drugs" which improve memory and mental function. Already the effect of *Withania somnifera* which is a Medhya Rasayana Drug has been studied on morphine-induced toxicity symptoms (Kulkarni and Ninan, 1997). Here, the effect of another Medhya Rasayana Drug, *Bacopa monniera* against morphine toxicity has been studied.

Experimental

Plant material and extraction procedure – The plant material was collected at Chennai, Tamil Nadu and was authenticated by Dr.P.Brindha, Botanist, Captain Srinivasa Murti Drug Research Institute for Ayurveda, Arumbakkam,

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Chennai. The shade-dried and coarsely powdered whole plant material (1 kg) was extracted with 90% ethanol in the cold (48 hrs). The extract was filtered and distilled on a water bath to get a dark green syrupy mass. It was finally dried, in vacuo, (yield 52 gm). It was dissolved in water and given orally as an aqueous suspension.

Drugs – Morphine hydrochloride used in the present investigations was obtained from Moti and Company Ltd., Chennai. Naloxone hydrochloride and Apomorphine hydrochloride were obtained from Sigma Chemical Co., St. Louis, U.S.A. All drugs were dissolved in physiological saline.

Animals and treatment schedule – Male albino mice weighing 20 - 25 g were used. The apomorphine was dissolved in saline containing 0.1% ascorbic acid, just before the experiment. They were administered to mice, subcutaneously (s.c). BMA extract was dissolved in water and given orally as an aqueous suspension, 2 h before the injection of morphine. The mice were divided into 4 groups.

Group I Mice received saline and served as control.

Group II Mice received morphine hydrochloride, (10 mg/kg, subcutaneously) for 10 days (Kim *et al.*, 1999).

Group III Mice received BMA extract (40 mg/kg)orally 2 h before the administration of morphine (Singh and Dhawan, 1997).

Group IV Mice received BMA extract alone for 10 days.

Treatment was carried out for 10 days, on day 11, analgesic tolerance, withdrawal symptoms, reverse tolerance and DA receptor supersensitivity were measured whereas, hyperactivity and apomorphine induced climbing behaviour were recorded on day 1, since these pharmacological effects resulted from acute administration of morphine (single day treatment) while other effects resulted from chronic administration of morphine (10 days treatment).

Experimental design

Measurement of the development of analgesic tolerance induced by morphine – To test the development of analgesic tolerance, morphine was administered (10 mg/kg) to mice once a day for 10 days and BMA (40 mg/kg) was given orally 2 h before the daily injection of morphine. The inhibitory degree of development of morphine-induced analgesic tolerance of BMA was evidenced by an increase in analgesic response to morphine (5 mg/kg s.c) and estimated by tail-flick method described by D'Armour and smith (1942) 24 h after the final injection of morphine on day 11. The tail-flick latencies were expressed in seconds.

Measurement of the development of morphine-induced physical dependence – To assess the morphine withdrawal mice were injected with naloxone (1 mg/kg, i.p.) (Kim *et al.*, 1999) immediately after the tail-flick test on day 11. The withdrawal syndrome was assessed by placing each mouse in a 30 cm high plexi glass box and recording the incidence of escape jumps. Additional signs include paw shakes, burrows, and writhings were also measured. The withdrawal sign was measured for 15 min after the injection of naloxone on day 11, 24 hrs after the final injection of morphine (10 mg/kg). BMA (40 mg/kg) extract has given orally for 10 days 2 hrs before administration of morphine.

Measurement of morphine-induced hyperactivity – The hyperactivity of the mice was measured by a photoactometer (Inco, Ambala, India). Each mouse was placed in an activity cage, and after an adaptation period of 10 min, morphine (10 mg/kg s.c) was administered to mice. The mice were pretreated with BMA extract orally (40 mg/kg) 2 h before the injection of morphine. The ambulatory activity was measured every 10 min for 1 h after the acute administration of morphine.

Measurement of the development of morphine-induced reverse tolerance – Reverse tolerance to the ambulatory activity of morphine (10 mg/kg) was developed significantly within a period of 10 days. Thus, to induce reverse tolerance, 10 mg/kg of morphine (s.c) was administered once a day for 10 days. BMA extract was given orally once a day 2 h before the injection of morphine for 10 days. To test the degree of the development of reverse tolerance to morphine, mice were injected only with 10 mg/kg of morphine on day 11, 24 h after the final injection of morphine, to avoid any residual effects of the test drugs themselves. The morphine-induced ambulatory activity was measured for 1 h by using a photoactometer. The mice were first allowed to perambulate for 10 min in the activity cages followed by a 1 h test period immediately after the injection of morphine. The development of reverse tolerance was evidenced by measuring the enhanced response to morphine and the inhibition of the reverse tolerance was evidenced by a lesser ambulatory activity produced by morphine.

Measurement of the development of dopamine receptor supersensitivity in morphine-induced reverse tolerant mice – To determine the development of DA (Dopamine) receptor supersensitivity in the reverse tolerant mice, morphine (10 mg/kg, s.c) was administered once a

day for 10 days. The degree of the development of morphine-induced DA receptor supersensitivity was evidenced by measuring the enhanced ambulatory activity induced by apomorphine on day 11, 24 hr after the final injection of morphine. Mice were first allowed to preambulate for 10 min and were given apomorphine (2 mg/kg, s.c) which produced significant increase in ambulatory activity (Protais *et al.*, 1976). The ambulatory activity following apomorphine treatment was measured for 20 min.

Measurement of apomorphine-induced climbing behaviour – Climbing behaviour has been used as a simple method to assess striatal DA activity and to screen DA agonists (or) antagonists, since apomorphine-induced climbing is reduced after destruction of the striatum and is enhanced by 6-OHDA induced lesions of DA input into the striatum which presumably induce receptor supersensitivity. In the previous experiment, chronic experiment with BMA inhibited development of the DA receptor supersensitivity induced by morphine. Therefore the apomorphine-induced climbing behaviour in mice treated with a single dose of BMA acutely, was determined to investigate the acute behavioural effects of BMA on the postsynaptic dopaminergic receptor. Climbing behaviour in mice was measured by modifying the method of Protais *et al.* Immediately after the injection of apomorphine (2 mg/kg, s.c), the mice were put into individual cylindrical cages (12 mm in diameter and 14 cm in height), with walls of vertical metal bars (2 mm in diameter; 1 cm apart). After a 5-min period of exploratory activity, climbing behaviour was measured at 10, 20 and 30 min after the injection of apomorphine and 3 scores were averaged. The scores of this behaviour were evaluated as follows: four paws on the floor = O points; fore-feet holding the wall = 1 point; and four paws holding the wall = 2 points. BMA (40 mg/kg) was administered orally to mice 2 h before the injection of apomorphine.

Statistical analysis – Values are expressed as mean ± S.D. (n = 6). The statistical significance of difference between the mean values were analysed by Student *t*-test. ***P < 0.001; **P < 0.01; *P < 0.05, P value of *** < 0.001 was considered to be significant.

Results

Inhibitory effect of BMA on morphine - induced analgesic tolerance – The morphine-induced analgesic effect was significantly decreased ($p < 0.01$) by chronic administration of morphine (10 mg/kg/day, s.c) for 10 days, thus suggesting the development of tolerance to morphine. The group pretreated with BMA showed significant inhibition ($p < 0.001$) of the development of morphine-induced analgesic tolerance when compared with the morphine group. The inhibitory effect of BMA on the development of morphine-induced analgesic tolerance was evidenced by the increase in analgesic response to morphine. Mice given BMA alone did not show any changes in their tail-flick latencies (Fig. 1).

Inhibitory effect of BMA on morphine-induced withdrawal symptoms – Animals that had received

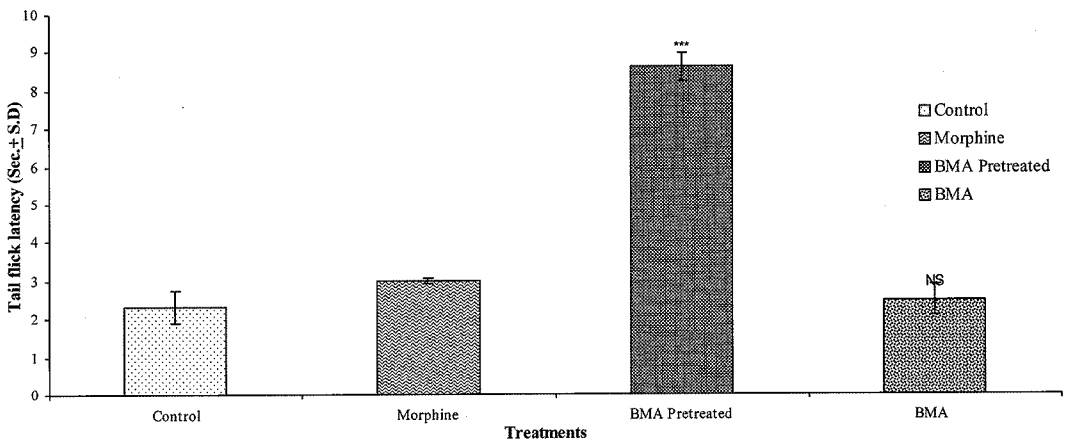

Fig. 1. Inhibitory effect of BMA on the development of morphine induced analgesic tolerance in mice.
Analgesic tolerance was tested on day 11, after the final injection of morphine.
*** $p < 0.001$; NS-Not significant values are mean ± S.D (n = 6).
Morphine group was compared with control group.
BMA pretreated group was compared with morphine induced group.
BMA group was compared with control group.

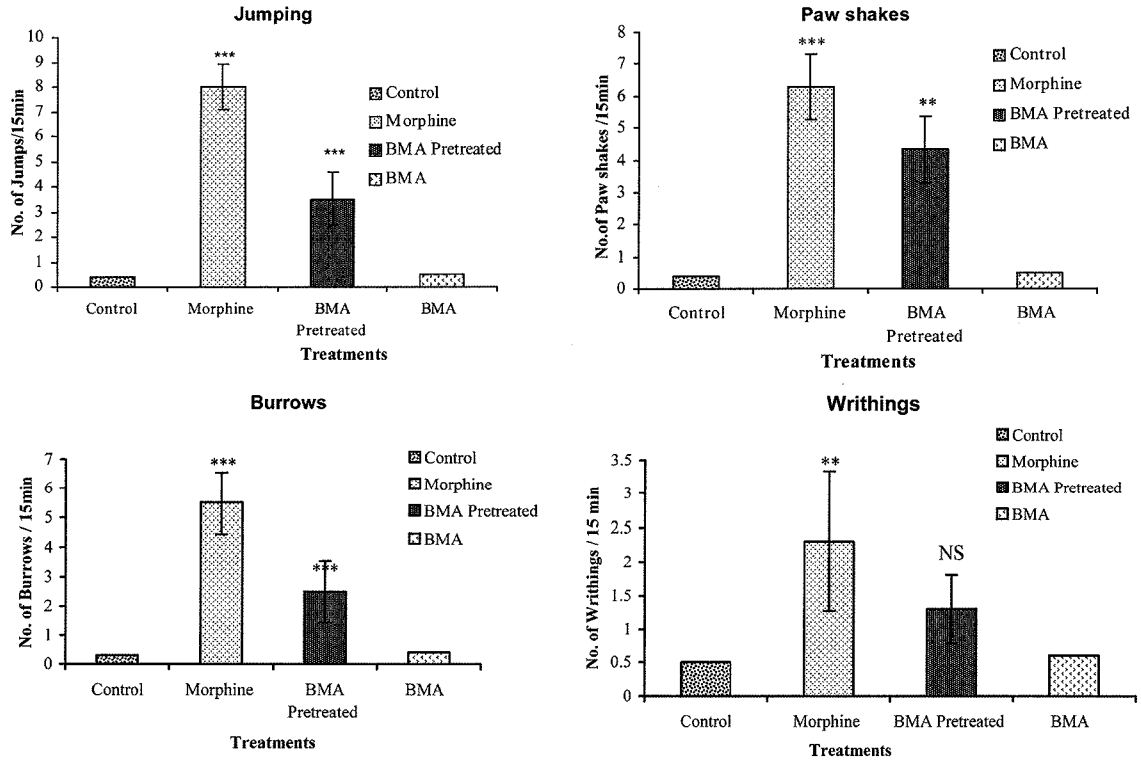

Fig. 2. Inhibitory effect of BMA on naloxone-precipitated withdrawal signs in morphine dependent mice.
The withdrawal syndrome was measured for 15 min after an injection of naloxone on day 11, 24 hrs after the final injection of morphine.
*** $p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS-Not significant-Each bar represents a mean ± S.D ($n = 6$).
Morphine group was compared with control group.
BMA pretreated group was compared with morphine induced group.
BMA group was compared with control group.

repeated administration of morphine (10 mg/kg) displayed numerous withdrawal symptoms viz., jumping, paw shakes, burrows, ($P < 0.001$) and writhings ($P < 0.01$) is response to an injection of naloxone (1 mg/kg, i.p). The groups pretreated with BMA showed a significant inhibition of naloxone induced withdrawal signs when compared with the morphine group, (Fig. 2). In BMA pretreated group, the naloxone induced withdrawal signs viz., jumping ($P < 0.001$), burrows ($P < 0.01$) and paw shakes ($P < 0.05$) were significantly reduced when compared with morphine -induced group. There was no significant reduction in writhing sign (NS) of BMA pretreated group observed, whereas mice given BMA alone resembled the control.

Inhibitory effect of BMA on morphine-induced hyperactivity – Hyperactivity was induced by acute administration of morphine and evaluated by measuring the ambulatory activity after every 10 min. for 1 h. Morphine-induced mice showed significant increase ($P < 0.001$) in ambulatory activity, showing 1233 counts, 1008 counts more than the 225 counts of the control group. There was no significant difference in ambulatory activity observed in BMA alone treated mice when compared with control mice. Meanwhile, the group pretreated with BMA showed a significant inhibition ($P < 0.001$) in ambulatory activity yielding 591 counts, 642 counts less than that of the morphine group (Fig. 3).

Inhibitory effect of BMA on morphine-induced reverse tolerance – Chronic administration of morphine for 10 days produced enhanced ($p < 0.001$) ambulatory activity, showing 2218 counts, 1091 counts more than the 1127 counts of the control group, thus suggesting the development of reverse tolerance to morphine. The degree of development of morphine-induced reverse tolerance was evidenced by enhanced ambulatory activity caused by morphine. Ambulatory activity caused by morphine was measured on day 11, for 1 h. The groups pretreated with BMA extract for 10 days showed significant inhibition ($P < 0.001$) of morphine-induced ambulatory activity when compared with the morphine-induced group, whereas BMA treated animals had no significant ambulatory activity induced by morphine when compared with the control group (Fig. 4).

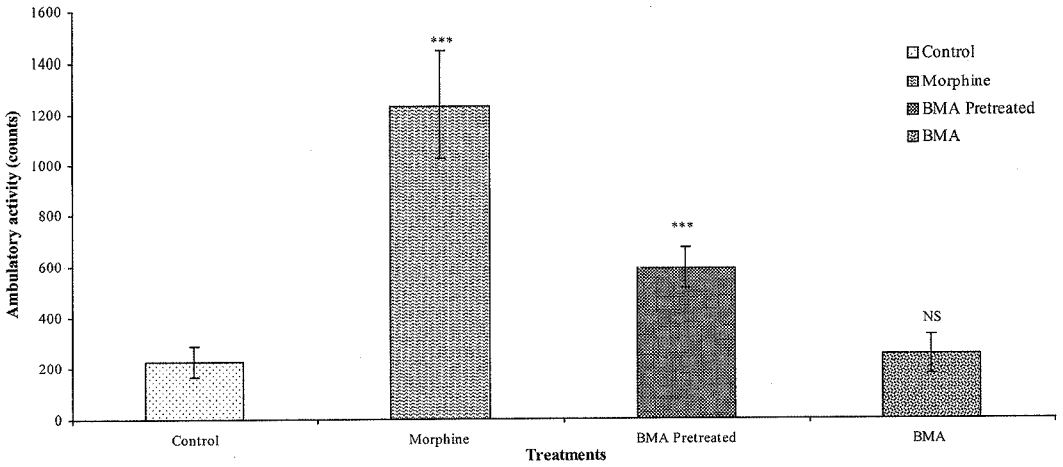

Fig. 3. Inhibitory effect of BMA on morphine induced hyperactivity in mice.
The ambulatory activity was measured every 10 min for 1 hr after the actue injection of morphine.
*** $p < 0.001$; NS- Not significant. Each bar represent mean ± S.D. ($n = 6$).
Morphine group was compared with control group.
BMA pretreated group was compared with morphine induced group.
BMA group was compared with control group.

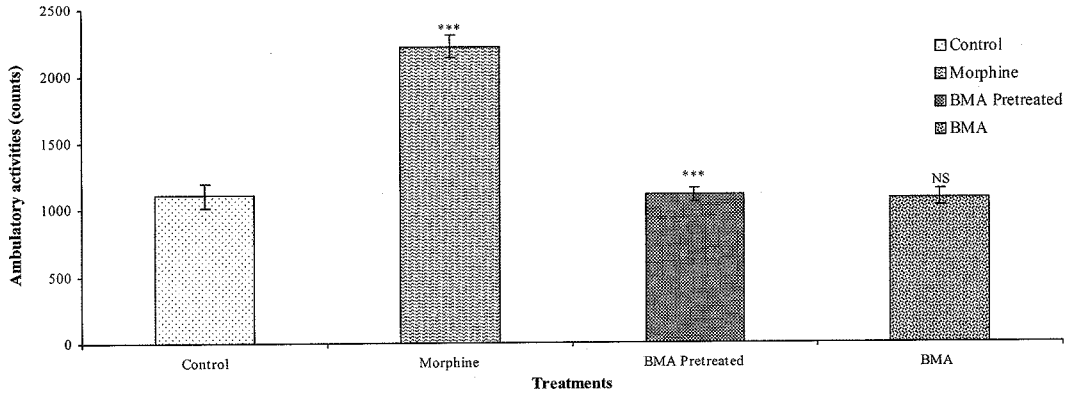

Fig. 4. Inhibitory effect of BMA on the development of morphine induced reverse tolerance in mice.
The degree of development of morphine induced reverse tolerance was evidenced by enhanced ambulatory activity caused by morphine. Ambulatory activity caused by morphine was measured for 1 h, on day 11, 24 h after the final injection of morphine.
*** $p < 0.001$; NS- Not significant. Each bar represent mean ± S.D. ($n = 6$).
Morphine group was compared with control group.
BMA pretreated group was compared with morphine induced group.
BMA group was compared with control group.

Inhibitory effect of BMA on morphine-induced DA receptor supersensitivity – The mice induced with morphine for 10 days showed a significant ($P < 0.001$) increase in ambulatory activity in response to 2 mg/kg of apomorphine given on day 11, yielding 342 counts, 152 counts more than the 190 counts of the control group. Meanwhile, the group pretreated with BMA showed a significant inhibition ($P < 0.001$) of the enhanced ambulatory activity caused by apomorphine when compared with the apomorphine-induced group. BMA treated mice, resembled the control mice (Fig. 5).

Inhibitory effect of BMA on apomorphine-induced climbing behaviour – The climbing behaviour was induced by acute administration of apomorphine and measured at 10, 20 and 30 min after apomorphine injection (Fig. 6). The groups pretreated with BMA produced a significant ($P < 0.01$) inhibition of apomorphine-induced climbing behaviour, resulting in 1.30 score against 1.93 score of apomorphine induced group ($p < 0.001$), while BMA treated animals did not show any significant changes in their climbing behaviour induced by apomorphine when compared with control. In this study, pretreatment with

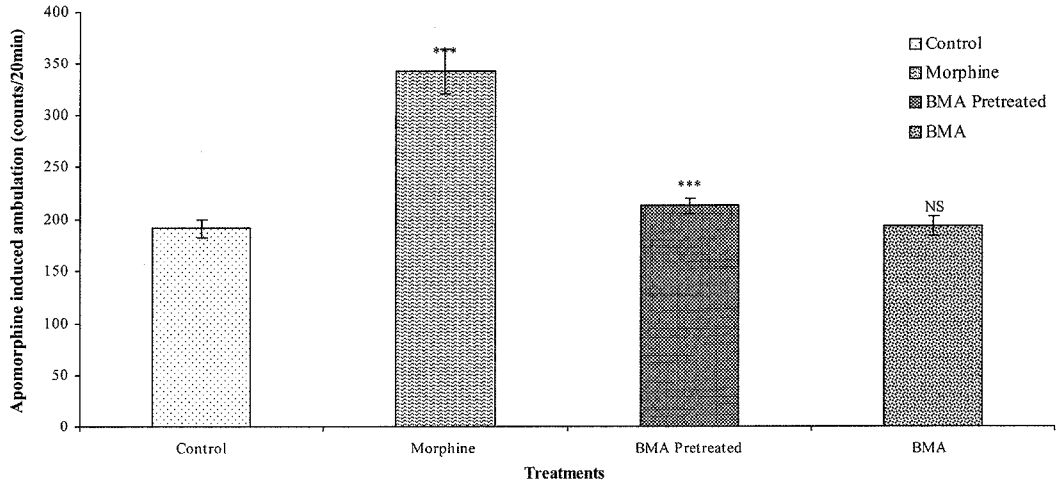

Fig. 5. Inhibitory effect of BMA on the development of DA receptor supersensitivity in morphine induced reverse tolerant mice.
Apomorphine induced ambulations were measured for 20 min.
*** $p < 0.001$; NS- Not significant. Each bar represent mean ± S.D. (n = 6).
Morphine group was compared with control group.
BMA pretreated group was compared with morphine induced group.
BMA group was compared with control group.

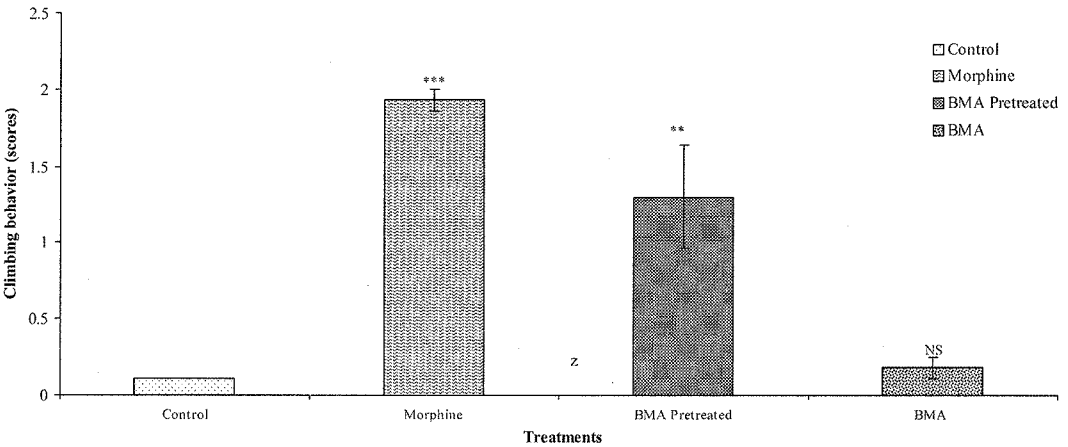

Fig. 6. Inhibitory effect of BMA on apomorphine-induced climbing behavior in mice.
The climbing behavior was measured at 10, 20 and 30 min after acute apomorphine administration.
*** $p < 0.001$; NS- Not significant. Each bar represent mean ± S.D. (n = 6).
Morphine group was compared with control group.
BMA pretreated group was compared with morphine induced group.
BMA group was compared with control group.

BMA extract 2 h before the injection of morphine inhibited the chronic morphine effects namely, development of analgesic tolerance, development of dependence, reverse tolerance and postsynaptic DA receptor supersensitivity. BMA also inhibited the acute effect of morphine namely hyperactivity, and apomorphine-induced climbing behaviour. Thus the results indicate that BMA inhibited both acute and chronic pharmacological adverse effects of morphine.

Discussion

Morphine, by continuous administration produced development of tolerance and dependence. Interactions between the glutamatergic and dopaminergic transmissions were also observed in relation to the development of morphine tolerance and dependence (Huang *et al.*, 1997). It was also reported that the GABA-ergic system plays an important role in the morphine physical

tependence (MPD) and/or morphine tolerance (Bartlett and Smith, 1996). In our study, pretreatment with BMA inhibited the development of tolerance and dependence induced by morphine. *Bacopa monniera* has been reported to be an anti-epileptic drug (Martis *et al.,* 1992). Being an anti-epileptic drug, *Bacopa monniera* might have prevented morphine-induced dependence, through stimulating the GABA-ergic transmission, as it has been reported that GABA-ergic system plays an important role in MPD.

Ca^{2+} channel antagonists inhibit the development of dependence on several addictive substances. Kuzmin *et al.* (1992) reported the inhibitory effect of Ca^{2+} channel antagonists on the development of morphine dependence. Inhibitory effects of Ca^{2+} channel antagonists on development of dependence were described in *in vivo* and *in vitro* models (Littleton and Brennan, 1993). It has been reported that *Bacopa monniera* acts as a Ca^{2+} channel antagonist on vascular and intestinal smooth muscles of rabbit and guinea pig (Dar and Channa, 1999), it could act through interruption of calcium influx via both voltage and receptor operated calcium channels, and a critical role of the voltage-dependent Ca^{2+} channels are involved both in analgesia and in the acute abstinence syndrome, while the development of tolerance and dependence seems to involve Ca^{2+} channels (Dar and Channa, 1999). As evident from the report of Littleton and Brennan (1993) that, the calcium antagonists are involved on the development of morphine dependence both in *in vivo* and *in vitro* models, it can be suggested from the results that, the inhibitory effect of morphine-induced dependence by BMA extract could also be due to the presence of its calcium antagonistic activity (Dar and Channa, 1999).

A single administration with morphine in mice produces hyperactivity (Babbini and Davis, 1992). Morphine produce hyperactivity by releasing dopamine from presynaptic neurons in the striatum, thereby increasing dopaminergic activity (Kuschinski and Hornykiewiez, 1974). It has been postulated that the drugs which reduce the availability of catecholamines in the presynaptic neuron or which block the action of catecholamines on postsynaptic receptors, attennate the behavioural effects, such as the hyperactivity and reinforcing effects of psychomotor stimulants in the monkey and rat (Wilson and Schuster, 1972).

Pretreatment with BMA inhibited the hyperactivity induced by morphine in mice. The inhibitory effect of BMA extract on morphine induced hyperactivity could have been possible through its receptor mediated action on reducing the availability of catecholamines in the presynaptic neurons (or) blocking the action of catecholamines on postsynaptic receptors. Several reports have been postulated that BMA is able to modulate the dopaminergic system. The action of BMA could have been possible at postsynaptic DA receptor by inhibiting the apomorphine-induced postsynaptic dompaminergic behaviour and cage climbing. In addition to this BMA has also been reported to have a number of neuropharmacological activities (Singh and Dhawan, 1997). BMA inhibition on morphine-induced hyperactivity could be closely related with the inhibition of morphine-induced dopaminergic activation at the Postsynaptic DA receptors as well as at the presynaptic DA receptors via direct or indirect effects.

Morphine increases hyperactivity in mice and this effect was progressively enhanced by the repeated administration of morphine indicating the development of reverse tolerance. The repeated administration of morphine leads to increased ambulatory activity, that is called the development of reverse tolerance (Kuribara and Tadokoro, 1989). The phenomenon of reverse tolerance is a model for studying the psychotoxicity of dependence liable drugs (Robinson and Becker, 1986). It has been reported that morphine-induced reverse tolerance appears to involve the activation of the dopaminergic system in brain (Wood and Altar, 1988). In accordance with this report the postsynaptic DA receptor supersensitivity to apomorphine, a DA receptor agonist was developed after repeated administration of morphine (Bhargava, 1980).

The inhibitory effect of BMA on morphine-induced reverse tolerance and postsynaptic DA receptor supersensitivity could be closely related to the recovery of dysfunction in the dopaminergic system produced by morphine in the central nervous system.

To conclude the results of the present study show that the BMA administered orally exhibited significant inhibitory effects on both acute and chronic pharmacological investigations and could have a promising role to play in the treatment of morphine toxicity, hence it could be safely used as anti-dote for morphine addiction. Further, morphine and its brain receptor level investigations are required to understand the precise mechanism of inhibitory action exhibited by BMA.

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