ANTIDOPAMINERGIC EFFECT OF METHANOLIC EXTRACT OF *MORUS ALBA* LINN LEAVES

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Summary

The effect of methanolic extract of *Morus alba* L. leaves was investigated on dopaminergic function. In this study, we evaluated effect of methanolic extract of *Morus alba* L. leaves on haloperidol induced catalepsy, metoclopramide induced catalepsy, foot shock induced aggression, amphetamine induced stereotyped behavior and phenobarbitone induced sleeping in mice. In each of these tests extract was administered in doses of 50, 100 and 200 mg/kg, i.p. 30 min before performing the test in mice. The extract produced significant dose dependant potentiation of haloperidol (1mg/kg, i.p.) and metoclopramide (20 mg/kg, i.p.) induced catalepsy in mice. The extract significantly reduced number of fights and increased latency to fights in foot shock induced aggression, also decreased amphetamine (1mg/kg, i.p.) induced stereotyped behavior in dose dependant manner. The sleeping time induced by phenobarbitone (50 mg/kg, i.p.) was also prolonged. Further the inhibitory effect of extract on dopamine was studied using isolated rat vas deferens. The extract inhibited contractions produced by dopamine (100 µg/ml) on isolated rat vas deferens. The results suggest that the methanolic extract of *Morus alba* L. possess antidopaminergic activity. Further neurochemical investigation can explore the mechanism of action of the plant drug with respect to antidopaminergic functions and help to establish plant as antipsychotic agent.

Key words: Catalepsy, Dopamine, Haloperidol, *Morus alba*, Stereotyped, Vas deferens

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Introduction

Morus alba L. (Moraceae) is a moderately sized tree 3 to 6 m. high, native of India, China and Japan, and is occasionally cultivated elsewhere in Europe, North America, and Africa. *Morus alba* (MA) is a medicinal herb reputed to be beneficial in the traditional system of medicine, which is commonly known as a white mulberry. White mulberry is cultivated throughout the world wherever silkworms are raised, which utilizes the leaves as their main food source.[1] The white mulberry has a long history of medicinal use in Chinese medicine; almost all parts of the plant are used in one way or another.[2] Traditionally the Mulberry fruit has been used as a medicinal agent to nourish the blood, benefit the kidneys and treat weakness, fatigue, anemia and premature graying of hair. It is also utilized to treat urinary incontinence, tinnitus, dizziness and constipation in the elderly and anemic.[3] The medicinal uses of the plant reported so far include analgesic, antiasthmatic, antirheumatic, antitussive, astringent, diaphoretic, diuretic, emolient, expectorant, hypotensive, hepatoprotective, hypoglycemic and brain tonic.[4] The plant extract has been demonstrated to posses free radical scavenging activity.[5] The antioxidant potency of some phenolic compounds (Flavonoids, stilbenes and 2-arylbenzofurans) from MA has been reported.[6] A decoction of leaves is being used as a gargle for inflammation of throat. Besides it shows antiviral and antimicrobial effect.[7] The plant is reported to contain the phytoconstituent tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, benzofuran derivatives, morusimic acid, anthocyanins, anthoquinones, iridoid glycosides, and oleanolic acid as main active principles. [8-11]

Although several medicinal uses have been reported for MA; no investigative reports exists pertaining to its central nervous system activity. Hence here an attempt has been made to evaluate the antidopaminergic activity of the plant.

Materials and Methods

Extract preparation

Fresh leaves of the plant (1kg.) were collected from local area in Nashik, and authenticated at Botanical survey of India; Pune. A voucher specimen of the plant has been deposited (voucher No.NVMA2). The leaves were washed and cut into pieces and air dried. The powered plant material was defatted using petroleum ether (60-80ºC) by Soxhlet extractor. The marc was further extracted by methanol for 72 h. to obtain the extract. Extract was filtered and

evaporated to dryness on rotary evaporator under reduced pressure. The yield of methanolic extract of *Morus alba* L. (MAE) leaves was found to be 2.2% w/w. Before use, the extract was dissolved in distilled water for administration intraperitoneally (i.p.).

Phytochemical screening

Phytochemical investigations of the extract for the presence of phenolic compounds, flavonoids, tannins, anthocyanins, anthroquinones and sterols were carried out using methods previously described by Kokate, Trease and Evans.[12,13] The presence of alkoloids and saponins was also ascertained.

Animals

Albino Male Swiss mice (18-22g) and male Wistar rats (180-220g) were used for the study. Animals were housed in colony cages and maintained under the standard environmental conditions; temperature 25 ± 2ºC, 12 h light: 12 h dark cycle and 45-55% relative humidity with free access to food and water ad libitum. Food but not water was deprivated overnight and during the experiment. All experiments were carried out during the light period (08.00-16.00h.). The Institutional Animal Ethical Committee of N.D.M.V.P.S. College of pharmacy, Nashik, India approved the protocol of the study.

Drugs

All drug solutions viz. Haloperidol (RPG Life sciences, India), metoclopramide (Ipca Lab, India), d-amphetamine (Sigma, USA), phenobarbitone (Samarth Life Sciences, India) and Dopamine HCl (VHP Life Sciences, India) were prepared in distilled water. Distilled water was used as a vehicle.

Acute toxicity test

The extract was administered orally and i.p. in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice (n=6). Mortality rate was observed and recorded for 24h. period .

Haloperidol induced catalepsy

Haloperidol (1mg/kg) was injected intraperitoneally (i.p.) to mice (n=6) pretreated with vehicle or MAE (50, 100 and 200 mg/kg, i.p.). The vehicle or MAE was administered 30 min prior to administration of haloperidol. The duration of catalepsy was measured at 0, 30, 60, 90, 120, 150 and 180 min. using the Bar test.[14] Both the forepaws of mouse were placed on a horizontal bar raised 3 cm from the table, and the time required to remove the forepaws

from the bar was recorded as the duration of catalepsy. In all the experiments the scorer was blind to the treatment given to the mice. Between experiments, the animals were returned to their home cages.

Metoclopramide induced catalepsy

Metoclopramide is a potent dopaminergic blocking agent.[15,16] Metoclopramide induces catalepsy in mice by blocking D_2 receptors.[17] Metoclopramide (20 mg/kg) was injected intraperitoneally (i.p.) to mice (n=6) pretreated with vehicle or MAE (50, 100 and 200 mg/kg, i.p.) and duration of catalepsy was measured as described in haloperidol induced catalepsy.

Foot shock induced aggression (FSIA)

FSIA behavior is induced in pair of mice by administering a train of impulses through an electronic stimulator to a grid floor for 3 min. The animals were divided into 5 groups of 12 mice (6 pairs of male mice) per group. The vehicle, haloperidol as a standard and MAE (50, 100 and 200 mg/kg) were administered i.p. 30 min before start of experiment. Aggressive behavior was noted in pair of mice by using two parameters viz. number of fights and latency to fight.[18]

Amphetamine induced stereotyped behavior in mice

Mice were allowed a maximum of 30 min to acclimatize to the observation cage prior to the experiment. The amphetamine induced stereotype was scored blind by an independent observer every 5 min for 30 min. The stereotyped activity was scored by means of the method described by Abiodun O. Ayoka.[19]

Stereotypy scoring 0, absence of stereotyped behavior; 1, intermittent sniffing; 2, constant sniffing; 3, constant sniffing with intermittent liking and/ or false biting; 4, constant licking or false licking; 5, constant licking; 6, constant biting and moving around; 7, constant biting and resisted to a small area in the cage; 8, rearing was used.

Animals were divided into 5 groups each containing 6 animals. They were treated with vehicle or extract 50, 100 and 200 mg/kg and placed individually into the cage. Amphetamine (1 mg/kg i.p.) was given 30 min after extracts were administered. The stereotyped behavior was recorded.

Phenobarbitone induced sleeping in mice

Phenobarbitone (50 mg/kg) was injected i.p. to mice (n=6) pretreated with vehicle or MAE 50, 100 and 200 mg/kg. Haloperidol (1 mg/kg, i.p.) was used

as positive control (standard Neuroleptic drug). The vehicle, MAE and haloperidol were administered 30 min prior to administration of phenobarbitone. Immediately after phenobarbitone administration, each animal was placed in an individual cage and observed. The latency to the loss of righting reflex (induction time in min) and the time required to recover righting reflex or awakening (sleeping time in min) were noted for each animal.[20, 21]

Effect of MAE on dopamine–induced contraction of isolated rat vas deferens

Adult male Wistar rats were sacrificed by cervical dislocation and the vas deferens was removed and kept in Krebs-Henseleit solution. The dose-responses to dopamine (10, 20, 40, 80 and 160 µg/ml) was recorded on the vas deferens. Dose-response to dopamine was latter repeated in presence of MAE (0.5 ml of 25 mg/ml). The contact time between the dopamine and the tissue was maintained 60 sec.[22]

Statistics

Results are expressed as mean ± S.E.M., and the statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability level less of 0.05 was considered statistically significant.

Results

Phytochemical screening

The Phytochemical screening of MAE revealed the presence of phenolic compounds, flavonoids, tannins, anthocyanins, anthroquinones, sterols, alkoloids and saponins.

Acute toxicity test

Oral and i.p. administration of MAE up to 2 gm/kg did not produce any toxic effects in mice. No mortality was observed and MAE was found to be safe at given doses.

Haloperidol induced catalepsy

In vehicle treated animals, haloperidol (1 mg/kg. i.p.) produced maximum catalepsy after 90 min (236.2 ± 5.275 s).The MAE (50, 100, 200 mg/kg, i.p.) significantly potentiated haloperidol induced catalepsy at each time interval in

dose dependent manner. MAE at dose 50, 100 and 200 mg/kg showed maximum cataleptic score 275.8 ± 9.998, 290.3 ± 5.852 and 291.2 ± 5.288 s. respectively at 120 min (*P<0.01*) in haloperidol treated animals. The MAE (200 mg/kg, i.p.) treated mice did not exhibit any catalepsy and appeared same as the normal animals. (Fig.1).

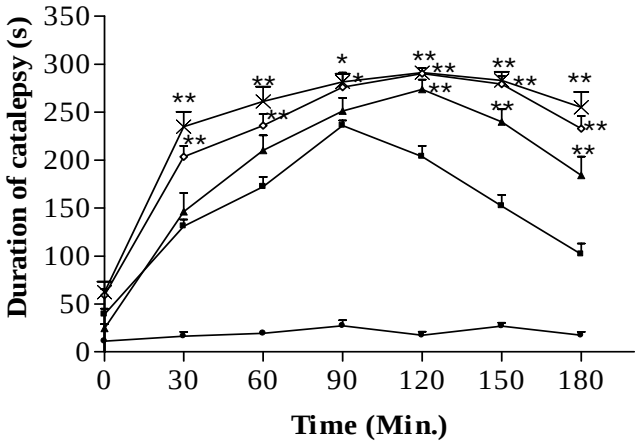

FIG.1. Effect of methanolic extract of Morus alba Linn.(MAE) on haloperidol induced catalepsy in mice.Each point indicates the mean ± S.E.M. of six mice.

—▪— Haloperidol (1mg/kg i.p.)
—▲— MAE (50mg/kg,i.p) + Haloperidol (1mg/kg,i.p)
—◇— MAE (100mg/kg,i.p) + Haloperidol (1mg/kg,i.p)
—×— MAE (200mg/kg,i.p) + Haloperidol (1mg/kg,i.p)
—●— MAE (200mg/kg,i.p)

* $P < 0.05$,** $P < 0.01$ vs vehicle
(one-way ANOVA followed by Dunnett's test)

Metoclopramide induced catalepsy

MAE 200 mg/kg i.p. treated animals did not exhibit any obvious behavioral syndrome and appeared same as the normal animals and did not induce catalepsy. Pretreatment with MAE (100 and 200 mg/kg, i.p.) was found to be significantly potentiated the cataleptic effect of metoclopramide (20 mg/kg, i.p.) at each time interval, however MAE (50 mg/kg, i.p) potentiated metoclopramide induced catalepsy up to 90 min which is not statistically significant. (Fig.2).

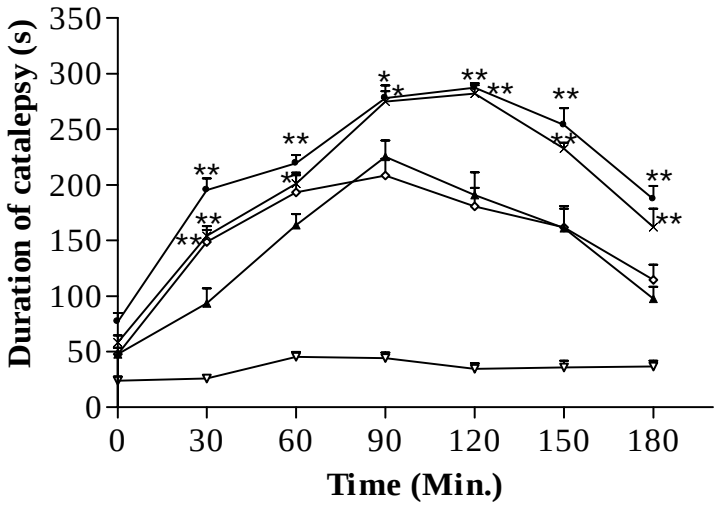

Fig. 2: Effect of methanolic extract of Morus alba Linn on Metoclopramide induced catalepsy in mice.Each point indicates the mean ± S.E.M.of six mice.

—◇— MAE (50 mg/kg, i.p) +Metoclopramide (20 mg/kg, i.p)

—▲— Metoclopramide (20 mg/kg, i.p)

—×— MAE (100 mg/kg, i.p) +Metoclopramide (20 mg/kg, i.p)

—•— MAE (200 mg/kg, i.p) +Metoclopramide (20 mg/kg, i.p)

—▽— MAE (200 mg/kg, i.p)

**P*<0.05, ***P*<0.01 vs.vehicle.
(one-way ANOVA followed by Dunnett's test).

Foot shock induced aggression (FSIA)

The intraperitoneal (i.p.) administration of MAE (50, 100 and 200 mg/kg, i.p.) showed significantly (*P*<0.01) dose dependent decrease in number of fights in foot shock induced aggression compared with vehicle. Further MAE (100 and 200 mg/kg, i.p.) significantly (*P*<0.01) increased latency to fight, but 50 mg/kg did not produce significant increase, however haloperidol (standard, 1 mg/kg, i.p.) treated group showed statistically significant (*P*<0.01) decrease in number of fights and increase latency to fight. (Table 1).

Table 1. Effect of *Morus alba* L. on foot shock induced aggression

Treatment	Latency to fight (sec.)	Number fights
Vehicle	12.33 ± 2.765	53.50 ± 2.872
Haloperidol (1 mg/kg, i.p.)	249.3 ± 17.47**	6.00± 2.769**
MAE (50 mg/kg, i.p.)	29.67± 4.302	33.33 ± 2.807**
MAE (100 mg/kg, i.p.)	82.67 ± 9.091**	22.67 ± 2.974**
MAE (200 mg/kg, i.p.) +	110.8± 9.105**	13.0 ± 3.215**

n=6. ***P*<0.01vs.Vehicle (one-way ANOVA followed by Dunnett's test)

Amphetamine induced stereotyped behavior in mice

Amphetamine (1 mg/kg) induced a stereotyped behavior characterized by intermittent sniffing or constant sniffing, intermittent or constant licking, intermittent or constant biting, moving around, resisted to a small area in the cage and rearing. Administration of MAE (50, 100 and 200 mg/kg, i.p.) significantly (*P*<0.01) decreased amphetamine induced stereotyped behavior. (Fig.3).

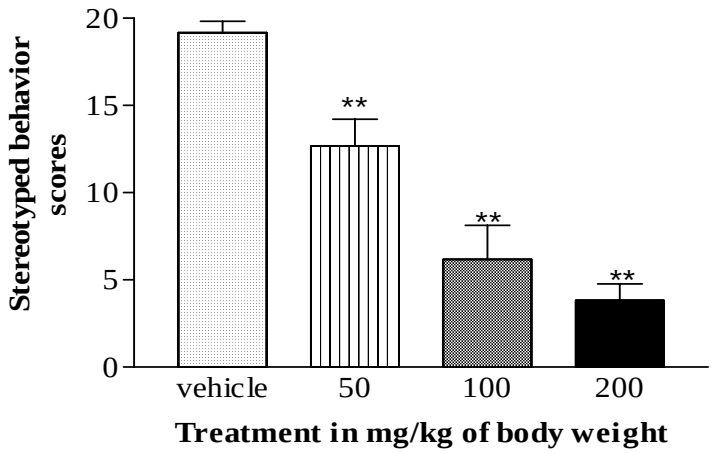

Fig. 3: Effect of MAE on amphetamine (1mg/kg) induced stereotyped behaviour in mice. Each Column represents the mean ± S.E.M. (n=6).
Vehicle + amphetamine (1 mg/kg, i.p.)
MAE (50 mg/kg, i.p.) + amphetamine (1 mg/kg,i.p.)
MAE (100 mg/kg,i.p.) + amphetamine (1 mg/kg, i.p.)
MAE (200 mg/kg,i.p.) + amphetamine (1 mg/kg,i.p.)
**P<0.01,with respect to vehicle (one-way ANOVA followed by Dunnett's test)

Phenobarbitone induced sleeping in mice

Pretreatment with haloperidol (1 mg/kg, i.p.) and MAE (200 mg/kg, i.p.) produced significant prolongation of phenobarbitone (50 mg/kg, i.p.) induced sleeping time. MAE (100 mg/kg, i.p.) also prolonged phenobarbitone induced sleeping time, but not statistically significant. Further haloperidol and MAE (100 and 200 mg/kg) also significantly reduced induction time of sleep. MAE in dose 50 mg/kg did not alter induction and sleeping time as compared to vehicle group. (Table 2).

Table 2 . Effect of *Morus alba* L. on phenobarbitone induced sleeping time

Treatment	Induction time in minute	Sleeping time in minute
Vehicle + Phenobarbitone (50 mg/kg, i.p.)	26.45 ± 2.027	84.50 ± 4.667
Haloperidol (1 mg/kg, i.p.) + phenobarbitone (50 mg/kg, i.p.)	5.007 ± 0.5456**	207.2 ± 6.539**
MAE (50 mg/kg, i.p.) + phenobarbitone (50 mg/kg, i.p.)	25.99 ± 1.786	87 ± 11.15
MAE (100 mg/kg, i.p.) + phenobarbitone (50 mg/kg, i.p.)	13.06 ± 1.637**	113.8 ± 10.14
MAE (200 mg/kg, i.p.) + phenobarbitone (50 mg/kg, i.p.)	13.10 ± 1.129**	132.2 ± 12.82**

n=6. ***P*<0.01vs.Vehicle (one-way ANOVA followed by Dunnett's test).

Effect of MAE on dopamine–induced contraction of isolated rat vas deferens

Dopamine produced dose dependant contractions of rat vas deferens. MAE reduced amplitude of contraction produced by dopamine on rat vas deferens. (Fig 4).

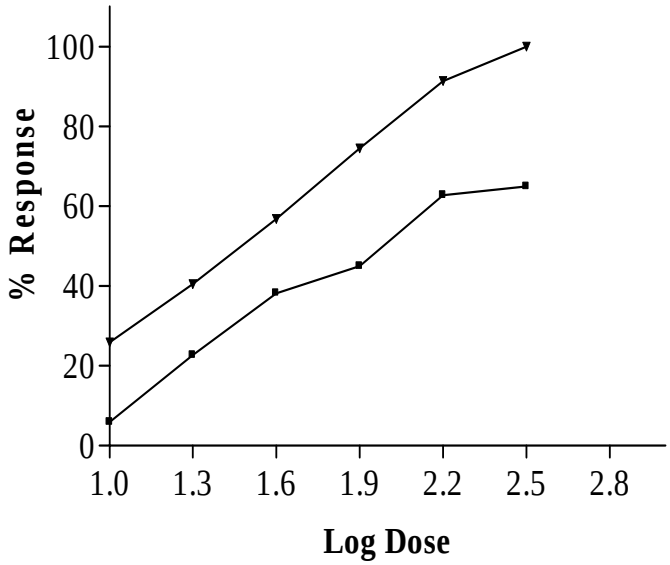

Fig.4: Effect of MAE on dopamine -induced contraction of isolated rat vas deferens. each point indicate the mean ± S.E.M.(n=6).

—▪— MAE (25 mg/ml, 0.5 ml) +Dopamine

—▾— Dopamine (100 μg/ml)

Discussion

The methanol extract of *Morus alba* leaves produces a dose-dependant potentiation of haloperidol and metoclopramide induced catalepsy, decreased number of fights and increased latency to fight in foot shock induced aggression, antagonized amphetamine induced stereotyped behavior and potentiated pentobarbitone induced sleep time.

Furthermore, the extract inhibited contractions induced by dopamine on rat vas deferens. The outcome of the present study demonstrated that MAE possessed anti-dopaminergic activity.

Haloperidol, a typical Neuroleptic produces catalepsy in rodents and extrapyramidal side effects in human.[23] Haloperidol induced catalepsy is one of the animal models to test the extrapyramidal side effects of antipsychotic drugs.[24] The haloperidol, (a non-selective D_2 dopamine antagonist) induced catalepsy is primarily due to blockade of dopamine receptors in the striatum. The agent, increasing dopamine transmission inhibits neuroleptic induced catalepsy. [25] The striatum and nucleus accumbens have been implicated as the major brain structures involved in antipsychotic induced catalepsy, which appears due to the blockade of dopamine neurotransmission.[26] In the present study MAE (50, 100 and 200 mg/kg, i.p.) significantly ($P<0.05$) potentiated dose dependently haloperidol induced catalepsy. The potentiation of catalepsy is indicative of the ability of the drug to decrease dopamine level in striatum.

Metoclopramide is a potent dopaminergic blocking agent [25], which has been shown to induce catalepsy in mice. Metoclopramide induced catalepsy was found to be mediated through selective blocking of D_2 dopamine receptor.[17] In this study, MAE (50, l00 and 200 mg/kg) significantly potentiated metoclopramide induced catalepsy. Thus, we confirm that MAE shows antidopaminergic activity.

Central monoaminergic neurons appear to play an essential role in modulation of aggressive behavior. Central D_2 dopamine receptors are involved in the modulation of foot shock aggression in mice. Brain dopamine level is increased on foot shock induced aggression. [18] The MAE (50, 100 and 200 mg/kg, i.p) significantly ($P<0.01$) reduced number of fighting attacks and increased latency to fight, indicating antidopaminergic activity in FSIA.

Amphetamine induced stereotyped behavior is a well established model for schizophrenia. [27] Amphetamine is an indirectly acting sympathomimetic agent, which releases dopamine, induces characteristic stereotypy behavior.[18] Amphetamine induced stereotyped behavior is a measure of dopamine D_2 receptor reactivity. It is known that amphetamine induced stereotyped behavior is mediated by hyperactivity of dopaminergic mechanism in the nigrostriatal and mesolimbic pathway. [28] The MAE (50, 100 and 200 mg/kg, i.p.) significantly ($P<0.01$) blocked amphetamine induced stereotyped behavior in mice. This suggests that the extract contain antidopaminergic compound(s). Majority of antipsychotic drugs (Phenothiazines, butyrophenones) used in the management of psychosis are known to have preference for D_2 receptor,[25] which reduces or abolish amphetamine induced stereotyped behavior.

Since the MAE has antidopaminergic potential which need further investigations in the treatment of psychosis.

Earlier reports of chemical constituents and their pharmacology suggest that the plants containing flavonoids, saponins, tannins, possess activity against many CNS disorders.[29] Phytochemical tests of MAE revealed the presence of flavonoids, tannins, saponins which may be responsible for the antidopaminergic potential of the extract.

Drug decreasing dopaminergic transmission prolongs barbiturate induced sleep. Haloperidol, a typical antipsychotic is reported to potentiate barbiturate induced sleep, by decreasing the activity of the nigrostriatal and mesolimbic dopaminergic system involved in cortical activation and behavioral arousal and sensitizes the CNS to the depressant action of barbiturate.[14] In this study, pretreatment with MAE was found to prolong phenobarbitone induced sleeping time in mice; suggest that MAE by decreasing dopaminergic transmission, increases the sensitivity of the CNS to the depressant action of phenobarbitone, which prolongs sleeping time.

Dopamine D_2 receptors are predominantly present in vas deferens.[22] Dopamine produces dose dependant contractions of vas deferens. The result of the in-vitro test indicates that MAE inhibits dopamine induced contractions on rat vas deferens. Thus, it is concluded that the MAE possess antidopaminergic activity through dopamine D_2 receptor blocking.

In acute toxicity test MAE did not produce any detectable toxicity on oral and i.p. administration. No mortality was found, which is reflected by high LD_{50} of MAE.

Conclusion

The present investigation concludes that the methanolic extract of *Morus alba* L. leaves contains constituents that inhibit dopaminergic neurotransmission and block dopamine D_2 receptor. Thus MAE possesses antidopaminergic activity. The results suggest that leaves of *Morus alba* L. have potential clinical application in the management of psychiatric disorders.

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