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ANTI-PSYCHOTIC EFFECT OF AQUEOUS LEAVES EXTRACT OF *MORUS ALBA* IN ANIMAL MODELS

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ABSTRACT

There are number of patients suffering from of psychiatric condition such as depression, anxiety, schizophrenia, migraine, anti-impulsivity, cognition. Serotonin is the most important neurotransmitters that have been implicated in etiology of number of these psychiatric conditions. Ethyl acetate soluble fraction and insoluble of leaves extracts of morus Alba was carried out on different in-vitro methods. Laboratory animals were given lithium sulphate to causes serotonin blockage from serotonergic neurons. They were treated with different higher concentration clozapine (active comparator) and EASF and EAISF then the psychotic symptoms were monitored to see which group exhibited the best response. The highest dose extract (100mg/kg) was comparable to clozapine in lowering psychotic behavior characterized by head twitches, stereotypy and 5-hydroxytryptophan potention hence both morus Alba and clozapine can be used to treat psychosis induced by amphetamine. The anti-psychotic effect of morus alba leaves may be in dose graded fashion.

Keywords: Morus Alba, anti-psychotic effect, lithium sulphate, serotonin.

INTRODUCTION

Psychosis is the term used to describe a mental state in which the individual experience a distortion or loss of contact with reality and clouding of consciousness. It is characterized by the presence of depression, delusion, hallucination, anxiety, sleep disturbance, thought disorder, social withdrawal and impaired role functioning. Psychosis can be caused by drug intoxication metabolic and schizoaffective disorder. Psychosis is characterized by stereotyped (sniffing, licking, gnawing and repetitive behavior) and rotational behaviors.

Psychosis in both human and rats are characterized by anorexia and agitation among others. Serotonin is important neurotransmitters that have been implicated in etiology of number of these psychiatric conditions. Many people taking powerful psychiatric medications that increase their risk of weight gain and diabetes are prescribed those drugs when there's little evidence that they will get any benefit from them. Even when these atypical antipsychotics are prescribed as recommended, they may not be safer or more effective than the less expensive, older medications that they've apparently replaced. Allopathic medicine causes inhibitory effects of polyphenol compounds on lipid peroxidation caused by antipsychotics (haloperidol and amisulpride) in human plasma in vitro. It is extensively cultivated throughout the plains of India and hilly areas of Himalayas.

The tree is moderate in size, upto 3m high and 1.8m in girth. Leaves are ovate and serrate, flowers greenish, fruits white to pinkish white, purple or dark purple in color. The leaves are diaphoretic, emollient and antibacterial. *Morus Alba* is posses neuroprotective, antioxidant, Hypolipidemic, antimicrobial and antidiabetic activity. Because of the side effects of modern antipsychotic drugs and renewed interest in the use of traditional medicine there is need to investigate our indigenous plants that have long standing claims by our indigenous traditional medical practitioners of antipsychotic properties. Therefore, this study was designed to

investigate the antipsychotic effect of ethyl acetate soluble fraction of leaves of morus Alba extract in different laboratory animals. Preliminary studies were carried out which reveled methanolic extract of *morus Alba* exhibited inhibition of serotonin induced contraction on isolated rat fundus. The methanolic extract was further fractioned into Ethyl acetate soluble fraction and insoluble fraction and further tested for in-vitro study. In-vitro studies for ethyl acetate soluble fraction were shown to have inhibitory effect on serotonin activity and it may have an effect on CNS disorders.

MATERIAL AND METHODS

Plant material: The leaves of morus Alba were collected from local area of Junagadh district, Gujarat and shed dried. A herbarium of morus alba plant was authenticated at the department of Pharmacognosy, N. R. Vekaria institute of pharmacy, Junagadh, were a voucher specimen was submitted (voucher no.-nrvip-15). Dried powder leaves of plant extracted with hot Soxhlet extraction with following solvents, petroleum ether, methanol, and ethyl acetate.

Experimental Animals: Male Swiss albino mice (22-25g) and Male Wistar rats (160-180g) were used for the study. Animals were housed in a temperature (21± 2^0C) and light controlled room under a 12:12 h light dark cycle. Food and water were provided ad libitum. The experiment was carried out according to guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and the Institutional Animal Ethical Committee (IAEC) approved protocol of this study.

Antipsychotic Activity:

$LiSO_4$ induced head twitches:

Animal: male Wistar rats weighing 120-150g.
The animals were divided into 6 groups (n=5).
Group 1: Control (without any treatment)
Group 2: Vehicle $LiSO_4$ (200mg/kg, i.p.)
Group 3: Standard Clozapine(2mg/kg, i.p.)+ $LiSO_4$(200mg/kg, i.p.)
Group 4: EASF of Morus alba extract (25mg/kg, p.o.) + $LiSO_4$(200mg/kg, i.p.)
Group 5: EASF of Morus alba extract (50mg/kg, p.o.) + $LiSO_4$(200mg/kg, i.p.)
Group 6: EASF of Morus alba extract (100mg/kg, p.o.) + $LiSO_4$(200mg/kg, i.p.)

The animals were treated with test compound (25, 50,100 mg/kg, i.p.) 45min and standard (0.5mg/kg, i.p.) 30 min prior to $LiSO_4$ dosage. $LiSO_4$ was injected intraperitoneally with dose 200mg/kg. The animals were placed in separate plastic cage and number of head twitches was counted for 1 hr immediately after administration of $LiSO_4$ [2].

$LiSO_4$ induced stereotypy:

Animal: Male Wistar rats weighing 125-130g.
The animals were divided into 6 groups (n=5).
Group 1: Control (without any treatment)
Group 2: Vehicle $LiSO_4$ (200mg/kg, i.p.)
Group 3: Standard Clozapine(2mg/kg, i.p.)+ $LiSO_4$(200mg/kg, i.p.)
Group 4: EASF of Morus alba extract (25mg/kg, p.o.) + $LiSO_4$(200mg/kg, i.p.)
Group 5: EASF of Morus alba extract (50mg/kg, p.o.) + $LiSO_4$(200mg/kg, i.p.)
Group 6 : EASF of Morus alba extract (100mg/kg, p.o.) + $LiSO_4$(200mg/kg, i.p.)

The animals were treated with test compound (25, 50,100 mg/kg,i.p.) 45min and standard (0.5mg/kg,i.p.) 30 min prior to $LiSO_4$ dosage. $LiSO_4$ was injected intraperitoneally with dose 200mg/kg. The animals were placed in separate plastic cage. A half hr observation period was used to measure the presence of stereotypic activity such as sniffing, licking, biting, rearing, etc; immediately after $LiSO_4$administration [3-4].

5-Hydroxytryptophan potentiating in mice:

Animal: male Wistar rats weighing 125-130g.
The animals were divided into 6 groups (n=5).
Group 1: Control (without any treatment)
Group 2: Vehicle 5-HTP (10mg/kg, i.v.)
Group 3: Standard Clozapine(2mg/kg, i.p.)+ 5-HTP (200mg/kg, i.v.)
Group 4: EASF of Morus Alba extract (25mg/kg, p.o.) +5-HTP (10mg/kg, i.v.)
Group 5: EASF of Morus Alba extract (50mg/kg, p.o.) +5-HTP (10mg/kg, i.v.)
Group 6: EASF of Morus Alba extract (100mg/kg, p.o.) +5-HTP (10mg/kg, i.v.)
The animals were treated with test compound (25,50,100mg/kg,p.o) 45 min and standard (0.5mg/kg i.p) 30 min prior to 5-HTP dosage. 5-HTP was injected intravenously with dose 10mg/kg. The animals were placed in separate plastic cage and number of head twitches was counted for 1 hr immediately after administration of 5-HTP [5].
Toxicity study:

Acute toxicity study: Oral administration of methanolic, ethyl acetate soluble fraction (EASF) and acetate insoluble fraction (EAISF) of Morus Alba leaves up to 2000 mg/kg did not produce any toxic effect and no mortality was observed in mice. This

suggests that LD_{50} of extracts of Morus Alba leaves was found to be above 2000 mg/kg.

Sub acute toxicity study: During this study, no deaths were observed; no significant clinically relevant changes were observed in general behavior and other physiological activities in the present study.

Body weight, food, water intake, and blood pressure: No significant differences were observed in control or treated group in various parameters like Body weight, food, water intake, and blood pressure.

Hematological and plasma biochemical data: No significant changes were observed in blood analtsis of hemoglobin, red blood cell, white blood cells in extract treated groups compared to the control group (Table). These were no significant differences observed in any of the biochemical parameters examined in either the control or treated group rats in biochemical analysis (Table).

RESULTS

Antipsychotic Activity:
L iSO_4 Induced Head Twitches: Effect EASF of Morus Alba leaves on lithium sulphate induced head twitches in experimental animals. The number of head twitches was significantly increased ($p<0.001$) in Group II as compared to Group I. The number of head twitches was significantly decreased in ($p<0.001$) in Group III, IV, V and VI as compared to Group II.

L iSO_4 Induced Stereotyped Behavior: Effect EASF of Morus Alba leaves on lithium sulphate induced stereotyped behavior in mice. Stereotyped behavior scores were significantly increased in Group II ($p<0.001$) as compared to Group I. Stereotyped behavior scores were significantly decreased in Group III ($p<0.001$) as compared to Group II L iSO_4 in stereotyped behavior in mice. Stereotyped behavior scores were significantly decreased in Group IV ($p<0.001$) as compared to Group II L iSO_4 in stereotyped behavior in mice. Stereotyped behavior scores were significantly ($p<0.001$) decreased in GroupV and VI as compared to Group II L iSO_4 in stereotyped behavior in mice.

5-Hydroxytryptophan (5HTP) Induced Head Twitches: The number of head twitches was significantly increased in ($p<0.001$) in Group II as compared to Group II in 5-HTP induced head twitches in experimental animals. The number of head twitches was significantly decreased in ($p<0.001$) in Group II as compared to Group II in 5-HTP induced head twitches in experimental animals. The number of head twitches was significantly decreased in Group IV ($p>0.05$) as compared to Group II in 5-HTP induced head twitches in experimental animals. The number of head twitches was significantly decreased in Group V ($p<0.05$) as compared to Group II in 5-HTP induced head twitches in experimental animals. The number of head twitches was significantly decreased in Group IV ($p<0.001$) as compared to Group II in 5-HTP induced head twitches in experimental animals.

Tissue analysis: There was no significant difference between control and treated groups in the organ weights of rat.

DISCUSSION

Previous studies done on Morus Alba (moraceae) have strongly proved that it has wide ranging effects on CNS, like antioxidant [6], anxiolytic [7], antidopaminergic [8]. Mulberry leaves can be used as protein source in food formulation and its neuroprotective functions can be used against neurodegenerative disorders such as Alzheimer's disease and Parkinsonism [9]. The fruit of Morus Alba also shows promoting effect and recovery activity from physical strees [10]. Alone with these neuronal activities, Morus Alba herb is also useful in improving skin tone, as a Hypolipidemic, and an anti-microbial [11]. So it is likely that these neuronal activities may be attributed to change in concentration of monoamines, both centrally as well peripherally.

The present investigation was carried out to evaluate the involment serotonin in various neuropharmacological activities of Morus Alba. Acute toxicity study by Up and Down procedure (UDP) is the principle tests to estimate its median lethal dose. The extract of Morus Alba did not show any toxic symptoms, changes in behavior or mortality in mice even at the maximum dose 2 g/kg p.o. This suggests that the plant extract are safe even at the highest tolerable doses suggesting a LD_{50} above 2.0 g/kg by i.p. and p.o. route. The substances that present LD_{50} higher than 2.0 g/kg by oral route can be considered as practically non toxic.

The present study demonstrated the ability of Ondansertron, a prototype 5-HT_3 receptor antagonist to ameliorate the performance (i.e. acquisition and memory) in young mice impaired with Scopolamine water maze spatial navigation task. The result are in accordance with the published reports of the cognition enhancing activity of Ondansertron in

Morris water maze spatial model used to examine learning and memory [12]. Stereotypy is frequent mechanical repetition of a movement. It is a isolated motor acts or partial sequence of more complex behavioral patterns from the repertoire of the species, occurring out of the context and with an abnormally high frequency [13]. Stereotypy behavior is well established model foe Schizophrenia. In present study Lithium Sulphate salt of lithium was used as serotonin enhancing agent. Lithium Sulphate significantly induced stereotyped behavior in animals. EASF of methanolic extract of Morus Alba (25, 50,100 mg/kg, p.o.) significantly blocked lithium induced stereotyped behavior in mice which was compared with standard Clozapine. It significantly blocked lithium sulphate induced stereotyped behavior in mice.

CONCLUSION

The present investigation indicates that EASF of Morus Alba L. has not shown any toxic effect up to 2000 mg/kg p.o. in both acute and sub-acute toxicity studies. The present investigation indicates that EAISF of Morus Alba have significant serotonin potentiating activity, therefore it can be used as anti-psychotic agent. EASF of Morus Alba inhibit serotonin activity, therefore it can be used as remedy in disorders caused by excess of serotonin e.g. Psychosis, memory. It was concluded that EASF and EAISF of methanolic extract of Morus Alba have anti-psychotic pharmacological activities by modulating serotonin mediated behaviors. Thus EASF was found to be safe.

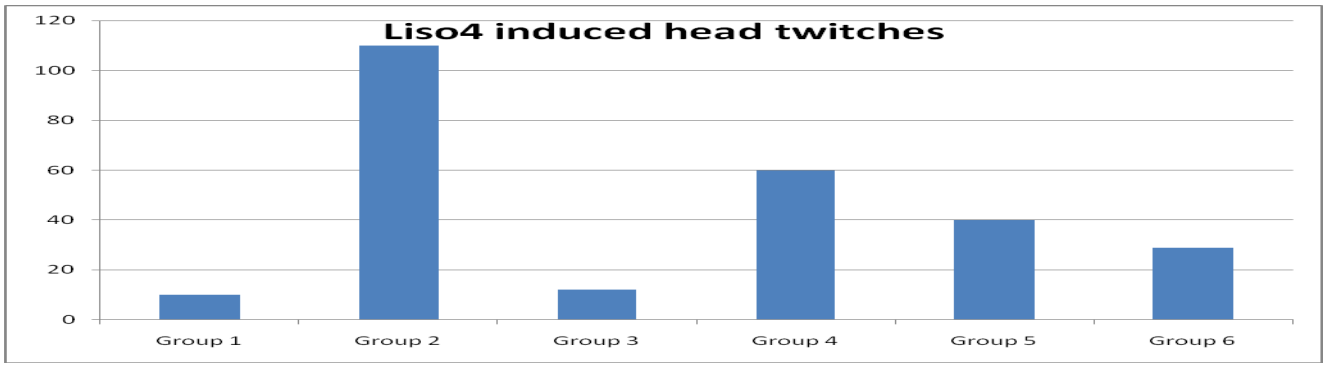

Figure 1

Group I: Control, Group II: $LiSO_4$ (200 mg/kg, i.p.), Group III: $LiSO_4$ + Clozapine (2 mg/kg, i.p.), Group IV: $LiSO_4$ + EAISF (25mg/kg, p.o.) , Group V: $LiSO_4$ + EAISF (50mg/kg, p.o.) , Group VI: $LiSO_4$ + EAISF (100mg/kg, p.o.) Values are expressed as mean±SEM (n=5) Group II was compared to Group I Groups II, III, IV and V compared to Group II. Non significant, *p<0.05 , **p<0.01, ***p<0.001. One-way ANOVA followed by Dunnett's test

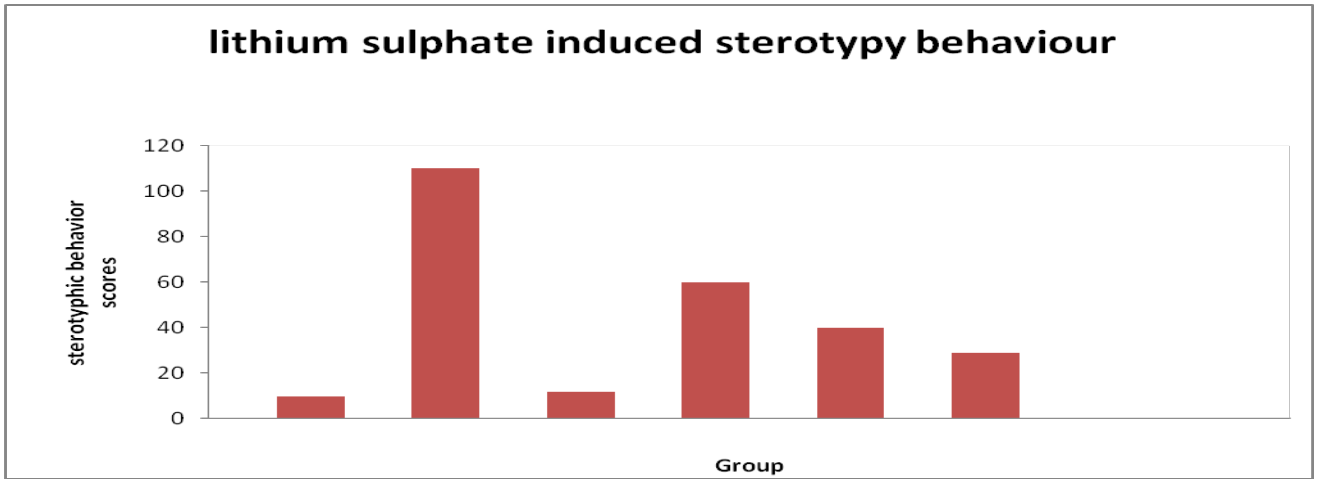

Figure 2

Group I : Control, Group II : $LiSO_4$ (200 mg/kg, i.p.), Group III: $LiSO_4$ + Clozapine (2 mg/kg, i.p.), Group IV: $LiSO_4$ + EAISF (25mg/kg, p.o.), Group V : $LiSO_4$ + EAISF (50mg/kg, p.o.) , Group VI: $LiSO_4$ + EAISF (100mg/kg, p.o.)
Values are expressed as mean±SEM (n=5) Group II was compared to Group I Groups II,III,IV and V compared to Group II. ns-non significant, *p<0.05 , **p<0.01, ***p<0.001.
One-way ANOVA followed by Dunnett's test

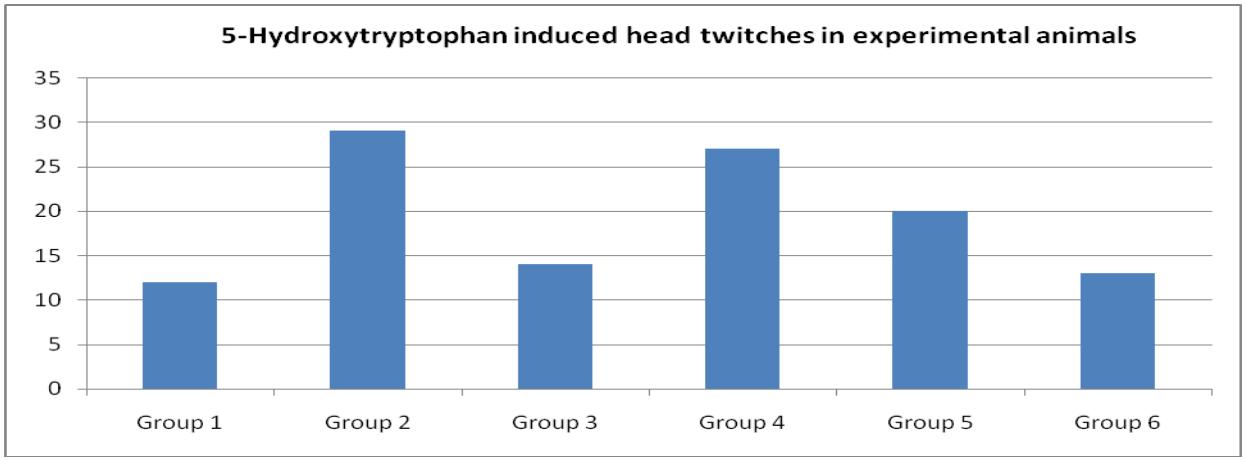

Figure 3

Group I : Control, Group II : 5-HTP (10 mg/kg, i.p.), Group III: 5-HTP + Clozapine (2 mg/kg, i.p.),Group IV: 5-HTP + EAISF (25mg/kg, p.o.) , Group V : 5-HTP + EAISF (50mg/kg, p.o.) ,Group VI: 5-HTP + EAISF (100mg/kg, p.o.)
Values are expressed as mean±SEM (n=5) Group II was compared to Group I Groups II,III,IV and V compared to Group II. Ns-non significant, *p<0.05, **p<0.01, ***p<0.001.
One-way ANOVA followed by Dunnett's test

Table I: Effect of ethyl acetate soluble fraction of methanolic extract of Morus Alba on Body weight, food, water intake parameters in sub acute toxicity studies in rat

Parameters	Day		
Body weight(g)	0 Day	189.15 ± 2.01	187.39 ± 2.94^{NS}
	7th Day	198.54 ±1.95	195.6 ± 2.64^{NS}
	14th Day	205.29 ± 1.77	203.76 ±1.84^{NS}
Food consumption (g/day)	All 15 Day	31.16 ± 0.33	30.69 ± 0.46^{NS}
Fluid intake (ml/day)	All 15 Day	79.05 ± 0.32	79.85± 0.40^{NS}

Table II: Effect of ethyl acetate soluble fraction of methanolic extract of Morus Alba on blood pressures in sub acute toxicity studies in rat

Day		
0	105.98 ± 1.66	106.45 ± 1.5 ^{NS}
14	106.37 ± 0.74	104.42 ± 1.97^{NS}

Data are expressed as mean ± S.E.M., (n=6). No statistical difference between control and Morus Alba.

Table III: Effect of ethyl acetate soluble fraction of methanolic extract of Morus Alba on Hematological parameters in sub acute toxicity studies in rat

Hematological parameters	Control (DW 0.5 ml/100g p.o)	EASF of Morus alba (2g/kg)
RBC ($\times 10^6$ $mm^{-3)}$.	7.95 ± 0.285	7.88 ± 0.27^{NS}
WBC ($\times 10^6$ $mm^{-3)}$	4.1 ± 0.42	4.09 ± 0.36^{NS}
Hemoglobin (gm/dl)	14.96 ± 0.39	15.13 ± 0.34^{NS}

Table IV: Effect of ethyl acetate soluble fraction of methanolic extract of Morus Alba on Biochemical parameters in sub acute toxicity studies in rat

Biochemical parameters	Control (DW 0.5 ml/100g p.o)	EASF of Morus alba (2g/kg)
Glucose (mg/dl)	85.66 ± 3.75	82.52± 3.66^{NS}
Cholesterol (mg/dl)	78.12 ± 3.56	77.27 ± 4.13 ^{NS}
Triglyceride (mg/dl)	75.35 ± 5.69	75.38 ±9.80 ^{NS}
HDL (mg/dl)	31.62 ± 2.95	30.41 ±2.79 ^{NS}
VLDL (mg/dl)	15.07 ±1.13	15.07 ±1.96 ^{NS}
LDL (mg/dl)	61.56 ± 4.62	61.94 ±6.03 ^{NS}
SGPT (IU)	36.83 ± 2.06	35.38 ±3.39 ^{NS}
SGOT (IU)	58.55 ±0.94	58.78 ± 0.79 ^{NS}

Data are expressed as mean ± S.E.M., (n=6). No statistical difference between control and Morus Alba.

Table V: Effect of ethyl acetate soluble fraction of methanolic extract of Morus Alba on organ weights (g) in sub acute toxicity studies in rat

Biochemical parameters	Control (DW 0.5 ml/100g p.o)	EASF of Morus alba (2g/kg)
Heart	0.83 ± 0.02	0.84 ± 0.02^{NS}
Brain	1.75 ± 0.02	1.76 ± 0.03 ^{NS}

Liver	6.54 ± 0.28	6.58 ±0.28^{NS}
Kidneys	1.79± 0.03	1.77±0.03^{NS}
Pancreas	1.21±0.2	1.19 ±0.01^{NS}
Stomach	1.60± 0.03	1.61 ±0.03^{NS}
Spleen	0.52± 0.2	0.53 ±0.02^{NS}
Adrenal	0.17±0.009	0.18 ± 0.008 ^{NS}
Lung	1.47±0.04	1.48 ± 0.04 ^{NS}

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