Neuropharmacological Profile of Hydroethanolic Extracts of Leaves of *Morus alba* Linn in Animal Model

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ABSTRACT

Neurons are the design squares of the tangible sensory network which fuses the cerebrum and the spinal cord. Depression is the second most common psychiatric ailment, affecting 21 percent of the worldwide people. The age range is rapidly shrinking, from 40-50 years old to 25-35 years old, as seen around the world. *Morus alba*, also called as white mulberry, is a quickly developing, little to average-sized mulberry tree. Moranoline, albafuran, albanol, morusin, kuwanol, calystegin and hydroxymoricin are confined to various biochemical mixtures from mulberry plants that assume a significant role in the drug industry. The purpose of the current review was to evaluate the neuropharmacological effect of hydroethanolic lef extract of *Morus alba* by using various behavioral models of rodent animals. The observation suggests that the phenolic intensification present in the concentrate could be responsible for the rummaging effect of nitric oxide. The high antioxidant activity of the HEMA may because of their phenolic constituents. Anxiolytic capability of HEMA at 200 and 400 mg/kg was practically significant to that of diazepam as indicated by elevated plus maze model. Tail suspension models were utilized for the assessment of anxiolytic action of HEMA. Results reported that 200 and 400 mg/kg dosage of HEMA indicated potent antidepressant effect that was like that of imipramine. Furthermore, the outcomes of photoactometer test affirm considerable antidepressant capability of HEMA. The sedative properties of *Morus alba* Linn leaves might be relevant to its future therapeutic application.

Keywords: Morus alba, antioxidants, toxicity, elevated plus maze, photoactometer.

1. INTRODUCTION

Neurons are the design squares of the tangible sensory network which fuses the cerebrum and the spinal cord. Neurons consistently won't reproduce or override themselves, so when they become hurt or fail horrendously, they can't be replaced by the body.[1] Depression is the second most common psychiatric ailment, affecting 21 percent of the worldwide people. The age range is rapidly shrinking, from 40-50 years old to 25-35 years old, as seen around the world. Affective disorder is defined by a change in mood that is accompanied by behavioral changes, motivation, hunger, sleeping, and obesity.[2,3]

Dopamine appears to play a role in the pathogenesis and therapy of depression, according to clinical evidence.[4] Several medications to treat depression have been found in recent decades, but all of them have major side effects, such as sleeplessness, stress, and weight gain (Figure 1). Nature is well-known as the best and safest source of all medicines. As a result, it's worthwhile to look for a new antidepressant prescription that comes from a natural source and has fewer side effects and difficulties.[5,6]

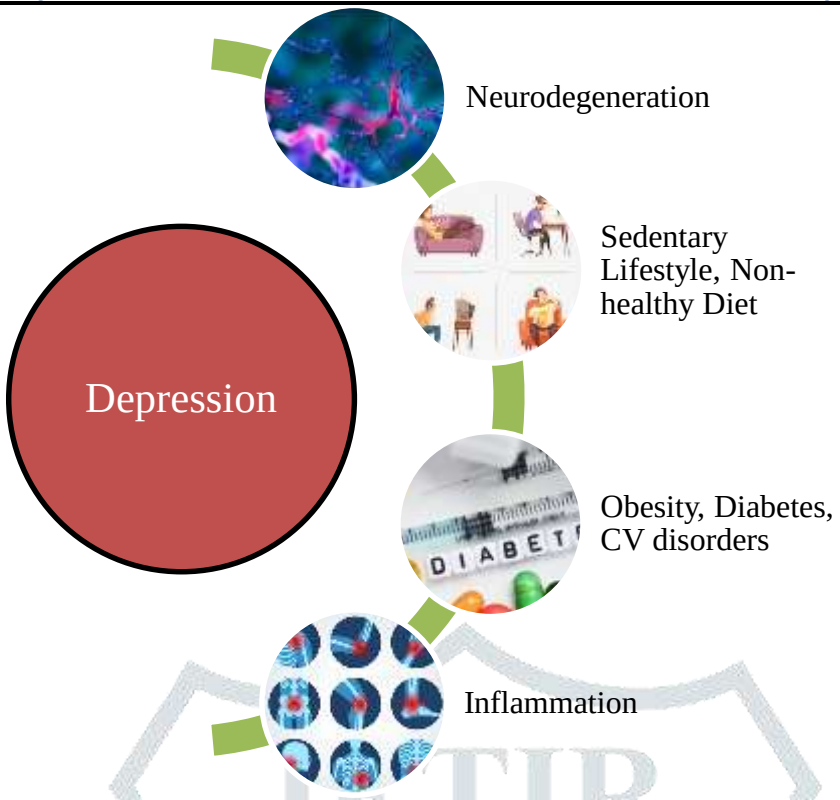

Figure 1: Causes of Depression

Morus alba, also called as white mulberry, and silkworm mulberry, is a quickly developing, little to average-sized mulberry tree. The white mulberry is broadly developed to take care of the silkworms utilized in the business creation of silk. Its berries are consumable when ready. The organic commodity is often consumed, dried daily, or made into wine.[7] Rutin, quercetin and apigenin are found in *Morus alba* leaves as bioactive constituents. 1-deoxynojirimycin is one of the significant components of *Morus alba*. In macrophages, nitric corrosive, prostaglandin E2 and cytokines were found to develop *Morus alba* leaf separately.[8]

Moranoline, albafuran, albanol, morusin, kuwanol, calystegin and hydroxymoricin are confined to various biochemical mixtures from mulberry plants that assume a significant role in the drug industry. Audit indicates the existence of starches, protein, thiamine. Tannins, phytosterols, sitosterols, saponins, triterpenes, flavonoids, benzofuran subordinates, morusimic corrosives, anthocyanins, anthroquinones, glycosides and oleanolic corrosives are found in the plant as the main dynamic quality corrosive.[6] The natural substance is used in traditional Chinese medicine to cure rash silver hair, "tonify" the blood, and treat blockage and diabetes.[9,10] The bark is used to cure coughing, wheezing, oedema, and to encourage peeing. It is also used for the prevention of flu, migraines, and dry and irritated red eyes.[11]

The purpose of the current review was to evaluate the neuropharmacological effect of hydroethanolic lef extract of *Morus alba* L by using various behavioral models of rodent animals.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

5,5'-Dithio-bis-2-nitrobenzoic acid (DTNB) sample was acquired from Loba Chemicals Ltd. and hydrogen peroxide from Qualigens Fine Chemicals Ltd., Mumbai, India. All other solvents of analytical grade were procured from S. D. Fine Chem. Ltd., Mumbai, India. Diazepam (Ranbaxy, India), Sodium Nitrite (SD-fine-chemicals, Mumbai, India) and phenytoin (Sigma, USA) were included purchased and used in the study.

2.2 Collection and Authentication

Leaves of *M.* alba L were collected in the month of January, 2016 from village area of Andhra Pradesh, India. The plant was authenticated by the Joint Director, Botanical Survey of India (BSI), Howrah, India. A voucher specimen CNH/1(64)/2014-Tech.II/1680 was deposited at our laboratory PPRL/DP /DPT/04/ 2014 for future reference.

2.3 Extraction

500 gm of powdered leaves (40 #sizes) of each plant were first extracted with 3 liters of petroleum ether (60-80ºC) in a soxhlet extraction apparatus. The extraction was carried out until the process was completed and then the solvent was recovered by distillation under reduced pressure using rotary vacuum evaporator (Buchi type, Mumbai, India) and finally lyophilized to ensure complete removal of solvent. Resultant brownish mass of hydroethanolic extract of *Morus alba* (HEMA) was preserved in a well closed container for various pharmacological studies.[12]

2.4 Determination of Total Phenolic Content

The quantitative estimations of total pheloic, flavonoid and alkaloid content was done as per the standard methods and procedures.[13-15]

2.5 Toxicity Testing

2.5.1 Acute Oral Toxicity Study (OECD 423)

OECD guideline 423 was adopted in order to conduct the oral toxicity analysis. For each progression, it's a stepwise technique for three animals with a solitary sex. Depending on the mortality or likely (50, 300, 2000 mg/kg body weight) and the findings cause a drug to be placed as dreariness of the animals whether it is necessary to pass judgement on the harmfulness of the test substance in a few stages. Due to negligible use of aniamls when considering worthy results, this approach has advantage over distinct strategies.[16]

2.5.2 Sub Acute Toxicity Study (OECD407)

OECD guideline 407 was followed for the sub-acute toxicity analysis. The research period was 28 days. With a portion level of 200 mg/kg, the rodents were handled. Ten animals are included in each category (5 animals/sex/group). The drug was prescribed orally for 28 days, once a day. The animals were anaesthetized on the 29th day and blood was extracted by retro orbital wounds. Hematological limitations have been determined. The serum was isolated and tested for biochemical parameters. Animals were forfeited, organs harvested and weighed. The organs were preserved in 10 percent formalin and used for histo-obsessive analysis.[17]

2.6 Evaluation of Neuropharmacological Profile

Tests were performed on each rodent to establish the effect of HEMA on brain. Given the animals were administered hydroethanolic leaves concentrate of *Morus alba* Linn (HEMA), 100 and 200 mg/kg (p.o) suspended in 1% carboxy methylcellulose. Assessments such as spontaneous activity, sound, touch, pain responses, latency to groom, locomotion, postural, righting, pinna reflexes and grip strength were done between 8 am 11pm.[18]

2.7 Evaluation of Antidepressant and Anxiolytic Activities

Swiss albino mice of either sex weighing 20-25 g raised in institutional animal house were used for the study. They were kept up under standard research facility condition on 12 h day/night cycle and with free admittance to food and water. The animals were adjusted to the lab conditions preceding experimentation. All the analyses were completed between 10h to 16h at encompassing temperature. The animals were drawn aimlessly for test and control groups (Table 1).

Groups	Treatment	Dose (mg/kg)
I	Control	Distilled water (10 ml/kg) p.o.
II	Negative (DZ)	Diazepam (2 mg/kg) p.o.
III	Standard (DZ+IMI)	Diazepam (2 mg/kg) p.o. + Imipramine (20 mg/kg)
IV	HEMA I	200 mg/kg, p.o + Diazepam (2 mg/kg) p.o.
V	HEMA II	400 mg/kg, p.o + Diazepam (2 mg/kg) p.o.

Table 1: Grouping of Animals

2.7.1 Elevated Plus Maze Test (EPM)

The elevated plus maze test was performed according to the method of Komada et al., 2008. The test apparatus consisted of two enclosed arms (length 30 cm × width 5 cm × height 15 cm), two open arms (length 30 cm × width 5 cm), and a central platform (5 cm × 5 cm). The maze was elevated 45 cm above the floor level. Mice were placed individually into the center of the maze, facing one of the open arms. The number of open arms entries and the time spent in the enclosed and open arms were recorded for 5 min with a video camera. Increased activity in the open arms was indicative of less anxiety. Entry into an arm was defined when mice placed all four feet into the arm. The maze was cleaned with 10% ethanol solution after each test.[19,20]

2.7.2 Tail Suspension Test (TST)

The tail suspension test was conducted as previously described by Can et al., 2012. Briefly, mice were suspended 50 cm above the floor on the edge of the table with the aid of sticky tape positioned approximately 1 cm from the tip of the tail.The all out length of fixed status prompted by tail suspension was recorded during a 6 min period. The animals were viewed as fixed when it didn't show any development of the body aside from those needed for breath and hanged latently.[21,22]

2.7.3 Digital Photoactometer

Mice were set in the advanced photoactometer (MAC, New Delhi, India) 1 h after medication organization. A persistent light emission from six lights was made to fall on comparing photoelectric cells, the photoelectric cell got initiated when ananimal crossed the light emission and subsequently removes the beams of light falling on it. These shorts were checked naturally for a time of 10 min and the figure was taken as a proportion of the locomotor action of the animals.[23,24]

2.8 Statistical Analysis

The data was expressed as mean ± SEM (n=6) and one direction ANOVA followed by Dunnet's t test was conducted by statistical analysis. *P* values < 0.05 were deemed statistically relevant (95% confidence limit).

3. RESULTS

3.1 Quantitative Estimation

It was found that the overall phenolic content in HEMA was 155.46 ± 0.45 mg/g respectively. The cumulative quantity of HEMA flavonoids was found to be 28.48 ± 0.52 mg quercetin equivalent/g plant extract. The overall content of alkaloid found for HEMA was 0.26 ± 0.03 mg of ascorbic acid/g of plant extract.

3.2 Toxicity Studies

The acute oral toxicity test based on OECD recommendations 423 showed the non-toxicity of *Morus alba* Linn hydroethanolic leaf extract (HEMA). In rats, the findings made during the research are shown in Table 2.

Parameters observed	Ist hr	IInd hr	IIIrd hr	IVth hr
Piloerection	-	-	-	-
Edema	-	-	-	-
Urine stains	-	-	-	-
Alopecia	-	-	-	-
Loss of writing reflex	-	-	-	-
Circling	-	-	-	-
Nasal sniffing	+	+	+	+
Lacrimation	-	-	-	-
Seizures	-	-	-	-
Righting reflex	+	+	+	+
Grip strength	+	+	+	+
Eye closure at touch	+	+	+	+

Rearing	+	+	+	+
Straub tail	-	-	+	-

Table 2: Acute Toxicity Study at Dose 2000 mg/kg

3.3 Screening of Neuropharmacological Activity

General behavioural profile surveys of rodents handled with HEMA are listed in Table 3, indicating little change in their overall behaviour. It was common for unregulated movement, mindfulness and sharpness, sound reactions, contact reactions, pain reaction, grooming delay, sensory tests, traditional grooming, motor action and intensity of grip. Over the cognition time frame, the animals showed no signs of despondency. General behaviour research suggests that there is no neurotoxicity of both hydroethanolic extricates.

Observation	**HEMA (200 mg/kg)**	**HEMA (400 mg/kg)**	**Observation**	**HEMA (200 mg/kg)**	**HEMA (400 mg/kg)**
Spontaneous Activity	Normal	Normal	**Locomotion**	Normal	Normal
Sound Response	Normal	Normal	**Postural Measure**	Normal	Normal
Touch Response	Normal	Normal	**Righiting Reflex**	Normal	Normal
Pain Response	Normal	Normal	**Pinna Reflex**	Normal	Normal
Latency to Groom	Normal	Normal	**Grip Strength**	Normal	Normal

Table 3: Neuropharmacological Profile of HEMA

3.4 Evaluation of Neuropharmacological Activity

3.4.1 Elevated Plus Maze Test

Table 4 shows that diazepam decreased time spent on the open arms when compared with the control group. The results revealed that HEMA pretreatment in 200 and 400 mg/kg enhanced significantly ($P<0.05$) the time spent on the open arms when compared with diazepam alone treated groups. Results suggest that HEMA in a dose-dependent manner significantly diminished depression-like behaviour.

Groups	**Treatment**	**Dose (mg/kg)**	**No. of SAP (Time spent)**		**No. of HD (Time spent)**		**No. of entries (Time spent)**	
			Open Arms	**Closed Arms**	**Open Arms**	**Closed Arms**	**Open Arms**	**Closed Arms**
I	**GA**	**10 ml/kg**	23.12 ± 6.55	228.80± 6.51	0.28±0.02	4.85±1.65	15.62±5.72	9.93±6.48
II	**DZ**	**2**	75.85±4.3	110.30± 4.74	4.50±1.62	10.50±6.1	8.32±2.62	3.85±1.66
III	**DZ+IMI**	**20**	31.75±2.32*	220.65±2.42	1.26±1.32	3.75±5.22	12.98±2.53	8.28±3.52**
IV	**DZ+HEMA**	**200**	32.73±5.37	183.88± 7.38	1.02±2.14*	6.26±7.25	11.82±1.65	7.68±4.39
V	**DZ+HEMA**	**400**	45.62±4.82*	168.00± 8.65	1.56±2.26*	8.93±2.24	10.46±3.42**	7.12±1.85*

Table 4: Effects of HEMA on the Elevated Plus Maze Test

GA: Gum acacia; DZ: Diazepam; IMI: Imipramine; SAP: Stretch attend postures, HD: Head dips

Data are expressed as mean ± SEM (n=6) and treated with one way ANOVA followed by Dunnet's t test. **P*<0.05, ***P*<0.01 represents stastically significant when compared to the diazepam trated group.

3.4.2 Tail Suspension Test

The level of decrease in immobility with diazepam (2 mg/kg) was significant (*P*<0.01) declined in immobility when contrast with control. On the other hand, extracts of HEMA (200 and 400 mg/kg) indicated portion subordinate abatement in immobility individually. The qualities were exceptionally significant (*P*<0.01) (Table 5).

3.4.3 Digital Photoactometer Test

The level of decrease in locomotor movement with diazepam (2 mg/kg) wassignificant (*P*<0.01) declined in locomotor movement contrast with control, where as portion of HEMA (200 and 400 mg/kg) indicated portion subordinate increase in locomotor action individually. The qualities were exceptionally significant (*P*<0.01) (Table 5).

Groups	Treatment	Dose (mg/kg)	Duration of Immobility (s)	Locomotor Activity in 10 Mins	
				Before	After
I	**GA**	10 ml/kg	182.67±1.26	250.18±5.64	-
II	**DZ**	2	90.84±1.24$^{\beta}$	280.32±6.32$^{\beta}$	92.44±2.65$^{\beta}$
III	**DZ+IMI**	20	75.21±1.24**	270.54±5.98**	240.24±3.32**
IV	**DZ+HEMA**	200	131.24±2.57*	375.31±6.32*	214.163.54±*
V	**DZ+HEMA**	400	153.67±2.15**	384.21±4.35**	225.75±5.64**

Table 5: Effects of HEMA on the Immobility and Locomotor Activity

Data are expressed as mean ± SEM (n=6) and treated with one way ANOVA followed by Dunnet's t test. $^{\beta}$*P*<0.01 represent stastically significant when compared to the control group. **P*<0.05, ***P*<0.01 represents stastically significant when compared to the diazepam treated group.

4. DISCUSSION

Since days of yore, plant products have been vital for phytomedicines. These can be collected from any part of the plant, such as bark, leaves, blooms, seeds, and so on, i.e. dynamic sections can be found in any part of the plant.[25] Free radicals are extraordinarily sensitive compound organisms systematically generated by ordinary natural responses. Oxidative stress can be exacerbated and cells are destroyed by decreased antioxidant enzymes. Diabetes mellitus, stroke, myocardial infraction, atherosclerosis, neurodegenerative disorders and ageing result in elevated oxidative stress.[26]

Morus alba Linn contain considerable amounts of biologically active ingredients that might be associated with some potential pharmacological activities that are beneficial for health. Therefore, they have been traditionally used in traditional medicine. Studies have reported that the presence of bioactive components in mulberry fruits, including alkaloids and flavonoid, are associated with bioactivities such as antioxidant. Therefore, the hydroethanolic leaf extract of *Morus alba* Linn was used in the present studu to assess its neuropharmacological profile.

DPPH is a generally stable free radical which is extinguished as proton suppliers, such as cell reinforcements, undergo it and the absorption decreases.[27] Nitric oxide is a diffusible free radical that in various organic structures like neuronal messenger, vasodilation, antimicrobial and antitumor exercises as a fundamental work as an effector particles. Nitric oxide foragers compete with oxygen, causing reduced nitric oxide production. Nitric oxide overabundance is associated with a few disorders, such as adjuvant joint pain, neurodegenerative problems and malignancy.[28,29]

Hydroxyl radical can produce hydroxyl radical reaction by fenton and reach the cell layer, causing harmful influence of tissue. Accordingly, H_2O_2 expulsion is essential for the safety of cancer preventive agents in cells or food frameworks. H_2O_2 may cross films and different mixes can be oxidised.[30,31] In the present study, *Morus alba* Linn hydroethanolic (HEMA) concentrates have shown tremendous *in-vitro* scavenging activity. HEMA were fit for hydroxyl radical rummaging in a dose portion dependent manner and conditionally scan for the NO free radical section. The observation suggests that the phenolic intensification present in the concentrate could be responsible for the rummaging effect of nitric oxide. The high antioxidant activity of the HEMA may

because of their phenolic constituents.

In the current investigation, no mortality was seen during sub-acute toxicities assessment in the different portions controlled. No signs or side effects of poisonousness were noticed. The aftereffects of the examination uncover that HEMA ought to be viewed for all intents and purposes as nonpoisonous. The concentrates have not developed any adverse side effects or mortality in rodents up to the portion stage of 2000 mg/kg, and the drugs have then been deemed healthy for further pharmacological screening.

The neuroprotective effects of HEMA in animals with neurodegenerative disease model was evaluated in the present study. Anxiolytic capability of HEMA at 200 and 400 mg/kg was practically significant to that of diazepam as indicated by elevated plus maze model. Oral administration of HEMA at both 200 and 400 mg/kg significantly increased the total time spent in the open arms compared with that of diazepam treated group which indicated the anxideprassant like effect of drug and further, the results are quite comparable to imipramine which is the standard antideprassant drug.

Tail suspension models were utilized for the assessment of anxiolytic action of HEMA. Results reported that 200 and 400 mg/kg dosage of HEMA indicated potent antidepressant effect that was like that of imipramine. Furthermore, the outcomes of photoactometer test affirm considerable antidepressant capability of HEMA. Promising mechanisms for antidepressant effect of crude extracts of the plant accounted may be due to inhibitory action on monoamine oxidase. Imipramine showed marked antidepressant potential due to selective inhibition of serotonin reuptake.

5. CONCLUSION

Since the above findings of the present investigation, it can be concluded that hydroethanolic extract of *Morus alba* L leaves has a CNS-depressant action, mostly similar to that of imipramine and other psychopharmacological agents. This study supports the central sedative and relaxant properties of *Morus alba* Linn leaves and its possible application in anxiety conditions. The sedative properties of *Morus alba* Linn leaves might be relevant to its future therapeutic application.

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