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Protective effects of Majun Brahmi on aluminium-induced cognitive impairment in rats: Biochemical and behavioral changes

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ABSTRACT

Background: The Unani formulation Majun Brahmi (MB), a combination of herbs, is used in India as a brain tonic and memory enhancer. Aluminium deposition in the brain is associated with the development of neurodegenerative diseases. **Aims and Objectives:** The present study was designed to observe that the effects of MB have been evaluated on aluminium trichloride or aluminium chloride ($AlCl_3$)-induced cognitive impairment in an experimental rat model. **Materials and Methods:** Twenty male Wistar albino rats were divided into four groups of five rats each. $AlCl_3$ was administered orally for 30 days to induce cognitive impairment. Group I received saline, Group II-$AlCl_3$ (100 mg/kg b.wt), Group III-MB (1027.77 mg/kg b.wt), and Group IV-$AlCl_3$ + MB (100 mg/kg b.wt + 1027.77 mg/kg b.wt). At the end of the experiment, rats were subjected to behavioral and biochemical assessments. **Results:** Animals treated with $AlCl_3$ showed a significant increase in time to reach the platform in the Morris water maze test (MWM), prolonged transfer latency (TL) in the elevated plus maze, and decreased step-down latency in the passive avoidance test, as compared to controls ($P < 0.01$). Cotreatment with MB resulted in a reduced time to reach the platform in MWM, increased step-down latencies, and decreased TL. $AlCl_3$ induction significantly increased malondialdehyde and decreased superoxide dismutase, glutathione reductase, glutathione, total antioxidant capacity, and catalase levels. Concomitant administration of MB significantly attenuated the effects $AlCl_3$ on lipid peroxidation and restored the reduced antioxidant parameters. **Conclusion:** The study provides strong evidence for the potential use of MB in the treatment of neurodegenerative disorders like Alzheimer's disease.

Keywords: Cognitive impairment; Morris water maze; Passive avoidance test; Elevated plus maze; Majun Brahmi; Oxidative stress

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INTRODUCTION

Aluminum (Al) is present abundantly in the biosphere and can enter the systemic circulation through several routes, such as dermal absorption, ingestion, and intramuscular injection.[1] Al has been reported to be involved in neurotoxicity and as a major risk factor for development of Alzheimer's disease.[2] Chronic exposure to Al has been implicated in the emergence of cognitive impairment in welders accidentally exposed at their workplace and may interfere with many biochemical functions in brain including acetylcholine synthesis.[3] Hence, Al-induced animals are being used as models for Alzheimer's disease. Previous animal studies have proved that exposure to Al is responsible for central nervous system damage including neurochemical and neurobehavioral changes. Most notable changes are poor learning and behavioral functions, which involve change in acetylcholinesterase activity that deteriorates the learning ability of experimental rats.[3] Interest in the use of herbal products is reported to

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have increased dramatically in developed countries as well as western world.[4] In the mythology of Indian medicine, several herbs have been used traditionally as a brain and nerve tonic.

The Unani system of medicine evolved in Greece and was first introduced to India by the Arabs. Majun Brahmi (MB) is a commercially available and highly popular polyherbal Unani formulation prescribed as a brain tonic and is claimed to enhance memory. All of its ingredients (Table 1) are common constituents of the human diet and are used as spices or condiments in India. The naturally occurring drugs used in Unani system are usually free from any side effects. Hence, MB is considered to be safe for human consumption. The major constituent of MB is Brahmi (*Bacopa monnieri*) from which it derives its name. *B. monnieri* has been reported to enhance memory.[5] Similarly, a number of other ingredients of MB such as *Prunus amygdalus*,[6] *Pistacia*,[7] *Cinnamomum zeylanicum*,[8] and *Foeniculum vulgare*[9] have been reported to have neuroprotective and cognition enhancing effects. The imbalance between reactive oxygen species (ROS) and reactive nitrogen species generation results in oxidative stress. Endogenous antioxidant enzymes systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione-related enzymes take part in removal of oxidative stress.[10] It has been reported that MB extract modulates the expression of enzymes involved in generation and scavenging of ROS in rat brain.[11] The other ingredients of MB that has been reported to have antioxidant properties are *C. Zeylanicm*[12] and *Coriandrum sativum*.[13]

Although MB is claimed to enhance memory to a large extent, the authors have not come across any scientific studies to authenticate this assertion. Hence, the present study was designed to evaluate the neuroprotective effect of MB in aluminium trichloride or aluminium chloride ($AlCl_3$)-induced cognitive impairment and oxidative stress in experimental animals.

Table 1: Ingredients of Majun Brahmi*

Each dose of 10 g contains	(mg)/10g of MB
Agar (*Aquilaria agallocha* Roxb.)	84
Badiyan (*Foeniculum vulgare* Mill.)	168
BrahmiBooti (*Bacopa monnieri* Linn.)	837
Banslochan (*Bambusa arundinacea* Willd.)	168
IlaichiKhurd (*Elettaria cardamomum* Maton.)	84
Darchini (*Cinnamomum zeylanicum* Nees.)	42
KishnizKhushk (*Coriandrum sativum* Linn.)	168
MastagiRoomi (*Pistacia lentiscus* Linn.)	84
MaghzBadamShirin (*Prunus amygdalus* Batsch.)	419
MaghzPista (*Pistacia vera* Linn.)	419
Qiwam shaker (Sugar)	7530

*National formulary of Unani medicine, Department of AYUSH, ministry of health and family welfare, Government of India

Aims and objectives

The aim of the study was to evaluate the neuroprotective effect of MB in AlCl3- induced cognitive impairment and oxidative stress in experimental rat models with the help of following objectives-a. Induction of cognitive impairment in Wistar albino male rats with AlCl3; b. Assessment and comparison of cognitive function of rats in MB treated and control groups with the help of Morris Water Maze, Passive Avoidance and Elevated Plus Maze test; c. Assessment and compare of oxidative stress parameters (SOD,MDA,TAC and GR activity) in brain tissue homogenate of rats in MB treated and control groups.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry and Department of Pharmacology, University College of Medical Sciences and GTB Hospital, Delhi.

Chemicals and reagents

The Unani formulation, MB, was prepared and supplied by the Central Research Institute of Unani Medicine, Ministry of AYUSH, Government of India. The constituents of MB are listed in Table 1.[14] $AlCl_3$ anhydrous powder sublimed (M.wt. 133.34 g/moL.) was procured from Merck Life Sciences Private limited, Mumbai, India. EDTA, DTNB, CDNB, Metaphosphoric acid, and NaCl were obtained from Sigma-Aldrich Company (USA). All other chemicals used were of analytical grade and obtained either from Sisco chemicals or Qualigens fine chemicals (Mumbai, India).

Animals and treatments

Male Wistar strain albino rats (pathogen-free) weighing 100–150 g were selected for the study randomly from the central animal house facility of University College of Medical Sciences, Delhi, India. Rats were housed under standard laboratory conditions in clean well-ventilated polypropylene cages. All animals received humane care in compliance with the committee for the purpose of control and supervision of experiments on animals, in India. Standard food pellets obtained from M/S Hindustan Lever Ltd. Mumbai, India and drinking water were provided ad libitum throughout the period of study. Animals were maintained as per the conditions: Light: Dark cycle, 14–10 h, temperature was maintained at 22±2°C, and humidity was 40–45%. Rats were acclimatized to the experimental environment at least 1 week before the initiation of the experiment. Necessary approval for the study was obtained from the Institutional Animal Ethics Committee (IAEC) (Approval No.: IAEC/2016-02 dated April 23, 2016).

Induction of cognitive impairment

Cognitive impairment in rats was induced by $AlCl_3$. $AlCl_3$ (10 mg/mL) was dissolved in normal saline and each rat was given an oral dosing (gavage) of 100 mg/kg body wt.[15] The human (therapeutic) effective dose (HED) of MB is 10g/day. For rats, the therapeutic ED (TED) was calculated using the following formula:[16]

HED (mg/kg body weight) = Animal dose (mg/kg) × Animal k_m/Human k_m

Where k_m is the basal surface area. For rats, k_m is six and for humans, it is 37. For rats, the calculated TED of MB is 1027.77 mg/kg b.wt.

Grouping and treatment

Twenty Wistar albino male rats of weight 100–150 g were randomly selected and divided into four groups of five animals each and treated as follows:

- Group 1-Control group (Saline)
- Group 2-$AlCl_3$ (100 mg/kg)
- Group 3-MB (1027.77 mg/kg)
- Group 4-$AlCl_3$ +MB (100 mg/kg+1027.77 mg/kg).

Animals were treated once daily for a period of 30 days. Food consumption and general condition and any other symptoms were observed daily and body weight was recorded once a week.

Assessment of cognitive function

Spatial memory on Morris water maze (MWM)

The acquisition and retention of memory were evaluated using the MWM. The MWM consisted of a large circular tank (150 cm in diameter, 60 cm in height, filled to a depth of 45 cm with water at 28±1°C) and divided into four equal quadrants with the help of two threads fixed at right angles to each other. The tank was placed in an illuminated room. A circular platform (4.5 cm diameter) was placed in one quadrant of the pool, 1 cm above the water level during the acquisition phase. The same platform was placed 1 cm below the water level for the retention phase. Each animal was subjected to four consecutive trials with a gap of 5 min. The animal was gently placed in the water between quadrants facing the wall of the pool, with the drop location changed for each trial. The animal was then allowed 120 s to locate the platform. Next, the animal was allowed to stay on the platform for 20 s. If the animal failed to reach the platform within 120 s, it was guided to the platform and allowed to remain there for 20 s.[17]

During the assessment of retention, the time taken by each rat to locate the target quadrant (quadrant in which the platform was placed during training) was noted and measured as retention latency (RL). The time spent in the target quadrant where the platform was placed was recorded over a period of 120 s.

The training session was continued for 4 days before the start of dose regimen. The probe test was conducted on day 1 before administration of $AlCl_3$. Similarly, the training session post-treatment was provided from day 27 onward until day 30 followed by the probe test on day 31.

Step-down latency (SDL) in passive avoidance apparatus

SDL was assessed in a passive avoidance apparatus which consists of an insulated wooden platform (6 cm×9.6 cm×3 cm) placed in the center of a metallic grid floor. The platform serves as a shock-free zone. In training sessions, the animals received a 0.3-mA, 2-s foot shock immediately on stepping down. A 180-s ceiling was imposed on test session latency measurements and the time taken for the rat to step down was measured as SDL. This training period represented the acquisition phase and the SDL measured at this point was considered as Initial SDL (ISDL).[18]

After 24 h, the same procedure was repeated without electric shock. The time taken by the rat to step down on the metallic grid floor was measured as retention SDL (RSDL). A cutoff time of 180 s was selected, that is, for the animal which did not step down in this period, SDL assigned was 180 s.

The ISDL was measured at 0 and 30th day of treatment whereas the RSDL was measured at 1st and 31st day of treatment.

Transfer latency (TL) on elevated plus maze

The elevated plus maze consisted of two open arms (50 cm×10 cm) and two closed arms (50 cm×10 cm×40 cm) with an open roof. The apparatus was placed at a height of 50 cm above the floor. The test was performed according to the method described by Itoh et al.[19] On the first trial training on day 0, the rat was placed at the end of one of the open arms facing away from the central platform, and the time the rat took to move from the open arm to enter either of the closed arms was recorded as acquisition TL (ATL). If the rat did not enter the closed arm within 90 s, it was pushed gently to guide its entrance in the closed arm, and in such case, the TL was assigned as 90 s. Then, the rat was gently taken out of the plus-maze after 10 s as it entered the closed arm and was returned to its home cage. Twenty-4 h later (on day 1), the second trial retention TL (RTL) was performed. To accomplish this, the rat was again put into the plus-maze, and TL was recorded up to a maximum of 90 s.

ATL was measured at 0 day, that is, 24 h before start of treatment and 30th day, that is, 24 h before termination of treatment. Similarly, RTL was measured on 1st day and 31st day of treatment.

Tissue sample preparation

After assessment of the behavioral parameters, at the end of the treatment period, all the animals were sacrificed using ether anesthesia. The brains were rapidly excised, washed with ice-cold saline, weighed and stored on ice, and were processed further within an hour of dissection. The brains were homogenized with phosphate buffer saline in a volume of 10 times the weight of the tissue to prepare a 10% brain homogenate. The homogenate was centrifuged at 800 rpm for 30 min at 4°C and supernatant was collected and used for the estimation of oxidative stress parameters on the same day.

Assessment of oxidative stress parameters

The SOD activity was assessed by the xanthine oxidase method. Catalase activity was conducted according to the hydrogen peroxide method. The malondialdehyde (MDA) levels were estimated by the thiobarbituric acid colorimetric method. The total antioxidant capacity (TAC) was determined colorimetrically, using standard Trolox. All these assays were performed according to instructions included in the commercial assay kits (Bioassay systems, USA). The glutathione reductase (GR) activity was determined by the method of Goldberg and Spooner.[20] The total glutathione (GSH) content was measured by the method of Tietze.[21]

Statistical analysis

The obtained raw data were subjected to statistical analysis using GraphPad Prism software version 6.0. The data on biochemical parameters and neurobehavioral test estimations are expressed as mean ± standard error of mean (SEM). Group differences in the escape latency in the MWM test were analyzed using two-way analysis of variance (ANOVA) and the remaining data were analyzed with one-way ANOVA followed by Tukey's *post hoc* test.

RESULTS

Effect of oral administration of MB on behavioral parameters in $AlCl_3$-induced cognitive impairment

Spatial memory

Animals orally intoxicated with $AlCl_3$ exhibited a significant increase ($P<0.01$), while MB administration, along with $AlCl_3$ exposure, showed a significant decrease ($P<0.01$) in the time to reach the platform (RL) in MWM when compared to the control as well $AlCl_3$-treated group. Treatment with MB alone resulted in a highly significant decrease in the RL as compared to all other groups (Table 2).

Table 2: Retention latency during probe test in Morris water maze task

Groups	Retention latency (s)	
	Day 1	Day 31
Control	98.40±22.19	91.60±23.56
$AlCl_3$	93.40±13.26	112.2±7.36[a,c]
MB	99.60±18.12	56.80±31.87[a,b,c]
$AlCl_3$+MB	91.40±8.79	86.60±5.59[a,b]

[a]$P<0.01$ as compared to normal control, [b]$P<0.01$ as compared to $AlCl_3$ control, [c]$P<0.05$ as compared to the corresponding group of day 1. Day 1 and day 31 represent the retention phase, values are expressed as mean±SEM, $n=5$. The intergroup variation between various groups was conducted by Prism 6.0 software using one-way ANOVA followed by Tukey's *post hoc* test. $AlCl_3$: Aluminium trichloride, MB: Majun Brahmi, SEM: Standard error of the mean

SDL

In the passive avoidance task (Table 3), day 0 and day 30 represent the acquisition phase (ISDL), whereas day 1 and day 31 represent the retention phase (RSDL). On day 0, there was no significant difference in the ISDL of control and treated groups. $AlCl_3$ treatment resulted in a significant decrease in the ISDL on day 30, which was ameliorated by treatment with MB. MB treatment alone resulted in a significant increase in the ISDL.

No significant differences were observed in the RSDL of control and treated rats (Day 1). On day 31, there was a significant decrease in the RSDL of rats treated with $AlCl_3$, as compared to the control. These observations show that rats treated with $AlCl_3$ stayed for a lesser time on the wooden platform and stepped down earlier as compared to the control. Cotreatment with MB resulted in a significant increase in RSDL as compared to the $AlCl_3$-treated group. Treatment with MB alone resulted in a significant increase in the RSDL, as compared to all other groups indicating an enhancement in retention memory.

TL

At day 0, no significant differences were found among the TL values of all the studied groups. A significant increase in both acquisition as well as retention in TL paradigm was found in the $AlCl_3$-treated group at day 30th and 31st as compared to TL values of both controls of day 0th and 1st ($P<0.01$). Administration of MB in combination with $AlCl_3$ resulted in significant reduction in TL values on day 30th and 31st as compared to the $AlCl_3$-treated group ($P<0.05$). Treatment with MB alone resulted, in a significant decrease in the acquisition as well as retention TLs (Table 4).

Effect of oral administration of MB on oxidative stress parameters in $AlCl_3$-induced cognitive impairment

The results of the oxidative stress parameters are depicted in Figure 1. The activities of the antioxidant enzymes SOD, GSH reductase, and CAT as well as TAC and GSH levels reduced while MDA levels were significantly increased in $AlCl_3$-treated rats as compared to normal control group. MB administration, along with $AlCl_3$, resulted in

Table 3: Step-down latency during probe test in passive avoidance test

Groups	Step-down latency (s)			
	Day 0	Day 30	Day 1	Day 31
Control	107±20.02	102.6±19.39	101.2±20.58	100.8±19.52
$AlCl_3$	106±18.62	60.80±15.42[a,b]	104.4±21.89	60.20±15.37[a,c]
MB	108±18.43	144±14.37[a,b]	105.8±18.63	139.8±20.99[a,b]
$AlCl_3$+MB	105.6±17.95	70.80±15.56[a,c]	104±17.73	68.40±14.15[a,c]

[a]P<0.05 as compared to the control group of day 0 and day 1, [b]P<0.01 and [c]P<0.05 as compared to day 0 of the corresponding group, [b]P<0.01 and [c]P<0.05 as compared to day 1 of the corresponding group. Day 0 and 30 represent the acquisition phase and day 1 and day 31 represent the retention phase, values are expressed as mean±SEM, *n*=5. The intergroup variation between various groups was conducted by Prism 6.0 software using one-way ANOVA followed by Tukey's *post hoc* test. $AlCl_3$: Aluminium trichloride, MB: Majun Brahmi, SEM: Standard error mean

Table 4: Transfer latency during probe test in elevated plus Maze task

Groups	Transfer latency (sec)			
	Day 0	Day 30	Day 1	Day 31
Control	20.60±10.24	19.20±8.70	21±8.88	20±7
$AlCl_3$	19.40±11.41	48.20±13.03[a,b]	21.60±3.84	50.20±13.14[a,c]
MB	19.80±8.01	13.60±5.98[a,b]	20.60±8.84	12.60±4.72[a,b]
$AlCl_3$+MB	20.20±9.25	44.80±15.83[a,b]	20.60±7.57	43.40±17.66[a,b]

[a]P<0.05 as compared to the control group of day 0 and day 1. [b]P<0.05 as compared to day 0 of the corresponding group, [b]P<0.05 and [c]P<0.01 as compared to day 1 of the corresponding group. Day 0 and 30 represent the acquisition phase and day 1 and day 31 represent the retention phase, values are expressed as mean±SEM, *n*=5. The intergroup variation between various groups was conducted by Prism 6.0 software using one-way ANOVA followed by Tukey's *post hoc* test. $AlCl_3$: Aluminium trichloride, MB: Majun Brahmi, SEM: Standard error mean

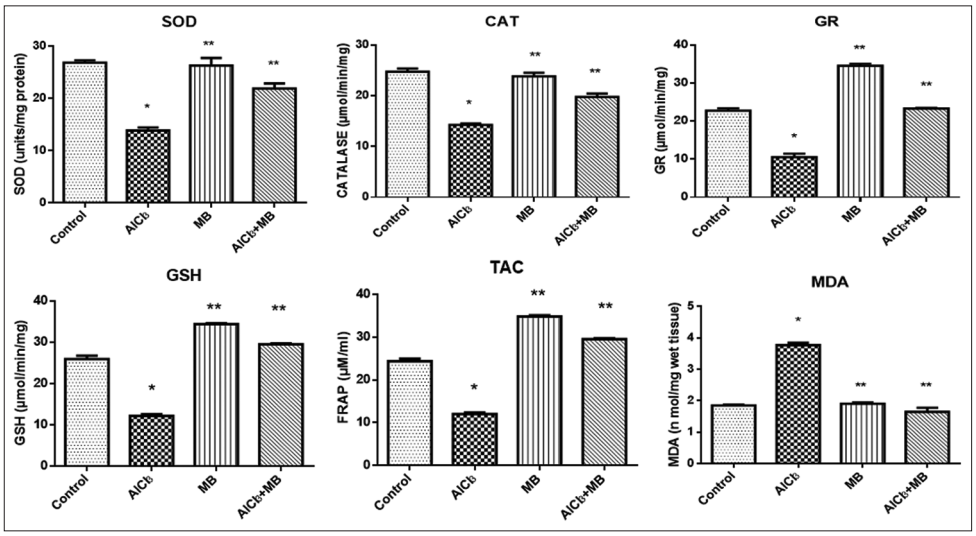

Figure 1: Effect of Majun Brahmi on oxidative stress parameter in aluminium chloride-treated animals. Values are expressed as mean±SEM, n=5. The intergroup variation between various groups was conducted by 6.0 software using one-way ANOVA followed by Tukey's *post hoc* test. *P<0.05 as compared control; **P<0.05 as compared to $AlCl_3$ and control. SOD: Superoxide dismutase, GR: Glutathione reductase, GSH: Glutthione content, TAC: Total antioxidant capacity, MDA: Malondialdehyde

a significant increase in SOD, GR, GSH, TAC, catalase, and a significant decrease in MDA levels as compared to the $AlCl_3$-treated group. Treatment with MB alone caused a significant decrease in MDA levels and enhanced of all other antioxidant parameters as compared to controls.

DISCUSSION

Al has been implicated in the etiology of a number of neurodegenerative disorders and cognitive impairment, by increasing oxidative damage in the brain.[22] $AlCl_3$ partially mimics the pathophysiological changes of AD, and it is reported to be a major risk factor for the cause and development of AD.[23] The present study was designed to study the effect of the Unani formulation MB on $AlCl_3$-induced cognitive dysfunction and oxidative stress in experimental animals. Our observations indicate that cotreatment with MB attenuated learning and cognitive impairments as well as oxidative stress caused by $AlCl_3$ treatment in the brain of rats.

In animal models, several learning and memory assessment tests have been used to study the pathogenesis of AD. MWM test is used to test spatial memory. In our study, we observed that chronic $AlCl_3$ administration resulted in progressive deterioration of spatial memory in the MWM task. It has been reported earlier that $AlCl_3$ administration resulted in memory impairment in the MWM task in rabbits,[24] which is in agreement with our findings. Al is known to interfere with downstream effector molecules such as cyclic GMP which is involved in long-term potentiation[25] and this disruption can explain the observed memory impairment and behavioral changes in the $AlCl_3$-treated rats. Treatment with MB was found to attenuate $AlCl_3$-induced cognitive impairment as evidenced by a reduction in the latency time to reach the hidden platform (Table 2) *Bacopa monniera*, the major constituent of MB has been reported to reverse cognitive deficits in animal models of Alzheimer's disease.[26]

The passive avoidance test and the elevated plus maze test are used for the evaluation of learning and memory retention in rodents. The present results indicated that $AlCl_3$-treated rats showed impaired performance in the passive avoidance task as evidenced by decreased SDL (Table 3) and enhanced retention TL in the elevated plus maze test (4). Al causes disturbances in cholinergic neurotransmission which may be associated with altered memory and learning processes.[27] Coadministration of MB was found to reverse memory loss due to Al intoxication, which may be attributed to a decrease in oxidative stress. Extract of *B. monnieri*, the major constituent of MB, has been reported to have neuroprotective effect against Al-induced oxidative stress in the hippocampus of rat brain.[28] Another major constituent of MB *P. amygdalus*, has been shown to enhance memory function in control rats and attenuate memory impairment in animal model of amnesia by increasing the brain levels of the neurotransmitter acetylcholine.[6] One other constituent of MB – *Pistacia vera*, has been reported to enhance memory functions which can be attributed to its cholinesterase and anti-inflammatory activity.[7] The aqueous extract of another constituent of MB – *C. zeylanicum* is reported to have cognition-enhancing effects in an animal model of AD.[8]

The brain is more susceptible to free radical damage due to its low glutathione content and high PUFA in the membranes. In our study, treatment with $AlCl_3$ increased lipid peroxidation (increase in MDA levels) (Figure 1) which possibly may affect peroxidative damage. Al has been reported to increase generation of ROS.[29] Decrease in MDA levels after treatment with MB indicates the antioxidant effects of this Unani formulation. The antioxidant enzymes SOD, CAT, GR, and GSH are important antioxidants that protect the brain from H_2O_2-induced neuronal damage. SOD converts superoxide anions to the less toxic H_2O_2, which is then detoxified to H_2O by CAT, GPx, and GR using GSH. In the present study, the antioxidant enzymes SOD, CAT, GR, and GSH were significantly decreased in $AlCl_3$-treated rats. Hence, increased MDA levels may be due to the inhibition of these antioxidant enzyme activities as well as GSH levels. Cotreatment of MB to $AlCl_3$-treated rats decreased the MDA levels and increased the activities of antioxidant enzymes as well as GSH. This is in agreement with earlier studies, where flavonoids with antioxidant properties were used in the treatment of various neurodegenerative diseases such as AD.[30] The TAC, which is an overall measure of the total antioxidant capability of the tissue, was also found to be increased by treatment with MB (Figure 1).

In the present study, Al was found to affect memory parameters, cognitive function, as well as oxidative stress adversely. This clearly indicates a corelation between oxidative stress and memory impairment. Treatment with MB improves behavioral and memory function in the Al-treated brain which could be correlated at least partially to its antioxidant properties. Moreover, treatment with MB alone resulted in considerable enhancement of cognitive function in normal healthy rats, as assessed by all the neurobehavioral paradigms under study. This study for the 1st time reports the memory enhancing effects of this Unani formulation and, thereby, justifies its use as a brain tonic for improvement of memory. Although, we have come across several studies reporting the neuroprotective, cognition enhancing, and antioxidant properties of quite a few of the individual constituents of MB, this study for the 1st time reports the neuroprotective functions of this formulation in an animal model of AD and advocates its potential use in the treatment of memory loss and cognitive impairment.

Further studies on the effect of this formulation on anticholinesterase activity, BDNF, and inflammatory cytokines need to be carried out and are the subject of current studies in our laboratory.

Limitations of the study

This study does not provide details on the effect of MB formulation on anticholinesterase activity, BDNF and inflammatory cytokines involved.

CONCLUSION

Results of the present study indicate that chronic exposure of experimental animals to Al resulted in cognitive impairment and increased oxidative stress in the brain. Cotreatment with MB, a polyherbal Unani formulation,

resulted in amelioration of cognitive dysfunction and reduced oxidative stress which might be responsible for the observed neuroprotection. Treatment with MB alone resulted in considerable enhancement of cognitive functions in normal healthy rats, indicating formulation's neurobehavioral role. This study, for the 1st time, provides scientific evidence for the traditional use of this drug as a brain tonic, and its potential use in the prevention/ treatment of Alzheimer's diseases.

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