EFFECT OF MORINGA OLEIFERA IN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE: ROLE OF ANTIOXIDANTS

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Abstract

Effect of chronic treatment of standardized aqueous extract of Moringa oleifera (MO) leaves (250mg/Kg; PO) on colchicine infused Alzheimer model in rats, behavioral testing (Radial Y arm maze task) and brain antioxidants (superoxide dismutase, SOD, Catalase and Lipid peroxidase, LPO) level was studied in Holtzman strain adult albino rats. The result revealed that pretreatment with MO markedly increased the number of correct choices in a radial Y arm maze task. Chronic treatment with MO significantly increased the SOD and Catalase activity and decreased LPO activity in the cerebral cortex (CC). MO leaves may help to scavenge free radicals either by non enzymatic defenses like vitamins or by bioactive compounds like flavanoids or both. Thus it can be concluded that MO leaves may give protection against this devastating disease like Alzheimer's.

Key Words: Colchicine, Radial Y-arm maze, Moringa Oleifera,

Introduction

The nervous system, including the brain, spinal cord and peripheral nerves, is rich in both unsaturated fats and iron (1). The high lipid content of nervous tissue coupled with its high metabolic activity makes it particularly susceptible to oxidative damage.

There is substantial evidence that oxidative stress is a causative factor in the pathogenesis of major neurodegenerative diseases. Evidence of oxidative stress is in the form of increased lipid peroxidation and oxidation of DNA, RNA and proteins as seen in Alzhemier's disease (2). A number of in vitro studies have shown that antioxidants, both endogenous and dietary, can protect nervous tissue from damage by oxidative stress (3).

Most in vitro and clinical studies on the effects of lipid soluble antioxidants supplementation on neurological diseases have been focused on vitamin E, B carotene, vitamin A, niacin, riboflavin etc. which was found to prevent cell death in rat neurons which leads to Alzheimer's disease including hypoxia (4). It has been suggested that Alzheimer's disease may be linked to diet, which can be reduced with diet high in antioxidants (3).

Moringa oleifera (MO) leaves are rich source of vitamins and antioxidants. They contain good amount of proteins, minerals, vitamin A, vitamin B complex, essential amino acids and a high content of vitamin E (5). Studies reveal that these compounds not only have antioxidant property but also have memory facilitating effect (6). So, the present study has been undertaken to determine the memory facilitating effect of MO leaves on an experimentally validated model of Alzheimer's disease in rats.

Materials and Methods

Animal use and maintenance: Forty-five male Holtzman strain rats weighing between 200-250g were used throughout the experiment. The rats were housed individually in a photoperiod cycle of 12h: 12h (Light and dark), at room temperature (around 28° C) and constant humidity (60%) with standard laboratory diet, which supplemented the necessary proteins, carbohydrates and minerals. Drinking water was supplied ad libitum. Body weight of the rats were recorded every day and maintained in the laboratory throughout the experimental period.

Collection and Preparation of ethanolic extract from the leaves of Moringa oleifera (MO): Fresh, young, healthy leaves of MO after identification were shade dried and ground with the help of an electrical grinder to get a free flowing powder. This powder was subjected to extraction with dehydrated alcohol at room temperature for 24 hours. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40-50° C to get a free flowing powder. This powder was subjected to extraction with dehydrated alcohol at room temperature for 24 hours. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40-50° C to get a dry powder, which was dissolved in double distilled water for final use (7).

Treatment: The extract of leaves of MO was given orally at the standard dose of 250mg per kg body weight for 14 consecutive days (between 10:00 and 11: 00 am). The dose was standardized in the laboratory.

After 14 days, the animals were sacrificed by cervical dislocation and the cerebral cortex was isolated for antioxidants estimation.

Grouping of animals : The animals were divided into 3 groups:

1. Control
2. Experimental Alzheimer's model's group
3. MO treated experimental Alzheimer group

Preparation of experimental Alzheimer's model by colchicine

Prior to surgery, all the animals were subjected to overnight fasting though drinking water was not withdrawn. The rats were anaesthetized with anaesthetic ether (Kabra drugs Ltd., India). The anesthetized animals were mounted on stereotaxic instrument (INCO, India Ltd.) equipped with a custom-made ear bar that prevents the damage of the tympanic membrane. Head was fixed in such a position that lambda and bregma sutures were in the same horizontal plane by introducing the incisor bar properly attached to the mouth. All surgery was performed under strict aseptic conditions. The scalp was incisioned in the midline and the pericranial muscles and fascia were retracted laterally. After retracting the nuchal musculature, the overlying bone was drilled

at the specific loci in the lateral ventricle following the co-ordinates of the stereotaxic atlas (8) [According to the coordinates: 0.6 mm posterior to bregma, 1.8 mm lateral to the midline and 2.6 mm below the cortical surface] Colchicine (15 ugm of Colchicine/ 5ul of artificial CSF or ACSF) was then slowly infused (0.125 ul/ min) into the lateral ventricle.

Post operative care: After surgery, all aseptic measures were taken for different periods and particular care was taken for feeding until they recovered from surgical stress. Antibiotic was given post operatively to all animals for 3 consecutive days by intramuscular route. After 3 days of surgery, experimental sessions started and continued routinely until sacrificed. The same procedure was repeated thrice each at an interval of two days.

Behavioral testing by radial y-arm maze training: Animals were preoperatively trained to perform a standard radial-arm maze (RAM) task. Rats were given 5 days of habituation trials in which food pellets (chocolate chips) were scattered throughout the maze and the rat was allowed to freely explore for 5mins. Following habituation session, the animals were trained for 10 daily trials on RAM task (10 trials per day). In this task, an animal was placed in the centre of the maze and allowed to visit each of the 4 arms, which were baited with single food pellet. Entry into an arm previously visited within any daily trial was scored as an error. Animals not reaching this criterion were discarded from the study (9).

Biochemical estimation: Rats were sacrificed by cervical dislocation on day 14, immediately after behavioral testing. The cerebral cortex was dissected out. The tissue was weighed and homogenized in ice-cold phosphate buffer and prepared for biochemical estimation.

1. Catalase activity was estimated by spectrophotometric method of Cohen et al (1970) (10).
2. Superoxide dismutase (SOD) was estimated by spectrophotometric method of Mishra et al (1972) (11).
4. Lipid Peroxidase (LPO) was estimated Spectrophotometrically by the method of Bhattacharaya et al. (2001) (12).

Statistical analysis: Students t-test was done for statistical evaluation of the data. A probability level of 0.05 was accepted as being statistically significant.

Results

Behavioral analysis by RAM training

Prior to surgery, all rats acquired the RAM task and were making 8-9 correct choices (>90% accuracy) in their first 4 arms selections (acquisition). Intracerebroventricular infusion of colchicine (15 ugm/5ul of ACSF) produced significant impairments in the RAM performance (reacquisition) after 3 days of surgery compared to sham control group. The correct choices out of 10 daily trials, was decreased to 3. (67% decrease) and the latency time was increased to about 200 seconds (~50% increase). There was a significant effect after ICV infusion of colchicine ($P<0.001$ in case of making correct choices as well as latency period) Moreover treatment with colchicine exhibited less accurate performance than the sham control group. Pretreatment with MO leaves extract for 14 days improved RAM performance significantly 3days after surgery by increasing the correct choices (by 57%) and decreasing the latency time by around 30%. ($P<0.01$ in case of making correct choices in daily trials in radial arm maze and $P<0.05$ in case of latency time). The result is shown in Table 1.

Table1: Effect of Moringa oleifera in behavioral parameters (RAM test) induced by infusion of colchicine (15 ugm of colchicine /5ul of ACSF).

	Acquisition		Reacquisition	
	No. of trials (out of 10)	**Latency (in secs)**	**No. of trials (out of 10)**	**Latency (in secs)**
Sham control	9.0 ± 0.2	100.0 ± 1.2	9.0 ± 0.4	110.0 ± 0.6
Colchicine	9.0 ± 0.4	110.0 ± 1.4	3.0 ± 0.6*	200.0 ± 1.2
MO+ Colchicine	8.0 ± 0.3	105.2 ± 1.2	7.0 ± 0.6 ##	148.6 ± 0.5#

*$P<0.001$; Compared to Sham control; ## $P<0.01$, # $P<0.001$; Compared to colchicine infused rats

Biochemical estimations:

Estimation of SOD, Catalase and LPO activity:

In experimental Alzheimer's rat model, SOD and Catalase level was decreased significantly (31% in case of SOD and 30% in case of Catalase) whereas LPO level was significantly increased (58%) in comparison to sham control. Students t-test revealed significant effects after ICV infusion of colchicine ($P<0.01$ in case of both SOD and Catalase and $P<0.05$ in case of LPO) Pretreatment with MO leaves for 14 days significantly increased SOD and Catalase levels (24.3% and 20% increase respectively) and decreased LPO level (48% decrease) as compared to Colchicine group ($P<0.01$ in case of SOD, $P<0.05$ in case of Catalase and LPO). The results are shown in details in Table2.

Table 2: Changes in SOD, Catalase and LPO in cerebral cortex of Moringa oleifera treated colchicine (15 ugm /5ul of ACSF) infused Alzheimer rats).

	SOD (% inhibition unit)	Catalase (% inhibition unit)	LPO (nMol of TBARS gm mol of tissue)
Sham control	13.8 ± 1.2	14.2 ± 1.0	3.4 ± 1.2
Colchicine	20.1 ± 1.5*	20.2 ± 1.7*	8.0 ± 1.4**
MO+ Colchicine	15.2 ± 0.7##	16.2 ± 0.8#	4.2 ± 1.2#

Values are expressed in Mean *P<0.01, **P<0.05; Compared to sham control, # P<0.05, ## P<0.01; Compared to colchicine group

Discussion

Antioxidant enzymes help to improve alertness, memory and overall brain function. It prevents brain cell damage by inhibiting the formation of free radicals and lipid peroxidation. Vitamin E, Vitamin C, B carotene are such examples, which helps to retard aging and improves brain function (13). In the present study, preparation of rat model of Alzheimer's disease by intracerebroventricular infusion of colchicine produced a decrease in the learning behavior by decreasing the correct choices in daily trials, and increasing the latency period together with a decrease in SOD and Catalase and an increase in the LPO activity. It has been reported that central administration of colchicine causes impairment in learning and memory by altering the antioxidant enzymes like SOD, Catalase and LPO (14). Parle et al (15) also reported that Vitamin C has a powerful memory improving effect, which may be attributed to its antioxidant property. In our study, ICV infusion of colchicine produced impairments in the performance of RAM task. Out of 10 daily trials, the correct choices decreased and the latency period increased significantly. The inter correlation between behavioral and antioxidant activity revealed significant correlation. Colchicine impaired memory as is evidenced by learning and memory behavior in RAM task, possibly by depleting the cellular defending enzymes like SOD and Catalase and increasing the LPO activity. Treatment with MO leaves for 14 days (250mg/kg b.w) helped to improve memory (which is evident from the RAM performance) by increasing the correct choices in daily trials and decreasing the latency time. Antioxidant property of MO leaves may be a contributing factor for this improved memory which is evident from increased SOD and Catalase activity and decreased LPO activity.

Neurodegenerative diseases are a group of illnesses with diverse clinical importance and etiologies. Alzheimer's disease (AD) is one of those diseases. Numerous epidemiological and experimental studies provide many risk factors of this disease, of which oxidative stress is the key one (16).

Induction of Alzheimer's disease can occur when the generation of free radicals is augmented, scavenging of free radicals or repair of oxidative modified macromolecules decreases, or both (17).

There are different endogenous defending systems, which maintain a balance between reactive oxygen species (ROS) production and detoxification. Antioxidants like SOD and Catalase are such enzymes. Exogenous sources including metals and neurotoxins trigger excess ROS production thereby disturbs this homeostasis which leads to ROS accumulation, predominantly (O2-) and OH-) species. Apart from the hydroxyl radical, H2O2 which is generated by the action of SOD, is highly toxic by itself and can generate hydroxyl radicals by Fenton reaction, by reacting with ferrous ions. Hydroxyl radicals are highly toxic and induce lipid peroxidation of cell membranes. H2O2 is neutralized by the enzyme Catalase (18). In the present study in Colchicine induced experimental Alzheimer model in rats, the generation of such free radicals may have occurred thereby decreased the SOD and Catalase activity significantly and increased the lipid peroxidase (LPO) activity. Decreased SOD and Catalase activity led to the generation of more and more ROS, which caused the peroxidation of membrane lipids, which is evident from increased activity of LPO.

Treatment with MO leaves for 14 days increased SOD and Catalase activity. Since it is a rich source of antioxidants, specially B carotene, vitamin B, C and E it may be capable of scavenging peroxyl and superoxyl radicals (4) (non enzymatic defences). Besides this, the major bioactive compound of phenolics found in MO leaves is of groups such as quercetin and kaempferol. MO leaves may help to scavenge free radicals either by non-enzymatic defenses like vitamins or by bioactive compounds like flavonoids or both. Further study is required to clarify this mystery.

MO leaves increases RAM performance in rats and this increase may be related to suppression of lipid peroxidation and activation of SOD and Catalase. Thus MO leaves helps to improve memory by enchancing the activity of SOD and Catalase and depressing the LPO activity, which is evident from the behavioral test like RAM performance.

So, from the present study it can be concluded that MO leaves may give protection against this devastating disease like Alzheimer's by its free radical scavenging action.

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