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Effect of *Moringa oleifera* on stress induced brain lipid peroxidation in rats

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ABSTRACT

Moringa oleifera Lam. (Moringaceae) is a medicinal plant used in the treatment of many clinical conditions in India. In the present study effect of ethanolic and methanolic extracts of *Moringa oleifera* on chronic stress induced lipid peroxidation was evaluated by measuring the levels of malondialdehyde (MDA) in brain. Restraint stress was accomplished by immobilizing rats three hours in restrainers daily for twenty one days. The stress procedure induced an increase in MDA level. The extracts were able to reduce the thiobarbituric acid reactive substances (MDA level) significantly, when compared with a standard drug. The protective effect of *Moringa oleifera* against chronic stress induced brain lipid peroxidation might be due to the presence of glycosides, phytosterols and polyphenols in it.

Key words: *Moringa oleifera*; chronic restraint stress; rat brain; lipid peroxidation.

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INTRODUCTION

Stressful stimuli can disrupt the physiological homeostasis and inabilitiy to cope with such aversive inputs have widespread deleterious effects on the biological system [1]. Stress increases generation of free radicals and lipid peroxidation [2- 4]. Lipid peroxidation is an auto catalytic free radical mediated chain reaction, involves the introduction of a functional group containing two catenated oxygen atoms O-O into unsaturated fatty acids [5]. Lipid peroxidation of biological membrane polyunsaturated fatty acids may adversely affect many functionally important parameters such as membrane fluidity, permeability, electrical potential and controlled transport of metabolites across the membrane [6]. The end products of lipid peroxidation malondialdehyde and 4-hydroxynonenal have the additional ability to inactivate phospholipids, proteins and DNA by bringing about cross-linking between these molecules [7]. These damaging events are of particular significance in the brain which is rich in oxidizable polyunsaturated fatty acid [8]. The tissue damages could be determined using the measurement of these end products [9]. Thiobarbituric acid reactive substances (TBARS) is well established assay for screening and monitoring lipid peroxidation.

Moringa oleifera Lam belongs to the family Moringaceae, which accounts for 14 species. A native of north India and Arabia, it has been used in nutritional, industrial and medical fields since a long time ago in Indian and African societies. Several studies reported that *Moringa oleifera* contains amino acids, fatty acids, vitamins, nutrient elements, isothiocyanates, carbamates, thiocarbamates and polyphenols [10-13]. It has anti-oxidant [14, 15], anticancer [16], antimicrobial [11, 12, 17, 18], antihypertensive [13], antiulcer [19], anti-inflammatory [20], hepatoprotective [20] and thyroid status regulator properties [21]. However no studies have been carried out to investigate *Moringa oleifera* protective effect on restraint stress induced brain lipid peroxidation. Hence, in the present study it was planned to investigate it.

MATERIALS AND METHODS

Plant material

The leaves of *Moringa oleifera* were collected from the fields around the campus in the month of July. The collected material was authenticated by botanist. The voucher specimen was deposited in the laboratory for future reference.

Extraction

The fresh leaves were shade dried, pulverized and extracted with 95% ethanol and 80% methanol in a Soxhlet apparatus. The extracts obtained were concentrated under vacuum at 40^0C and dried over anhydrous sodium sulphate. The resultant extract yield was 22.5% and 7% for methanolic and ethanolic extracts respectively. A suspension of extracts in 1% w/v gum acacia was prepared before oral administration to the animal for pharmacological studies.

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Experimental animal

Adult Swiss albino mice (20-25 gm) and adult wistar rats (180-200 gm) of either sex procured from Venkateshwara Enterprises, Bangalore were acclimatized for 10 days before being used for experiments. Standard pelleted diet and water were given ad libitum. Animals were maintained under a constant 12 hours light and dark cycle and an environmental temperature of 24-26 ^{0}C. Experiment was carried out according to the guidelines and approval of the animal ethics committee (Registration number 131/ 99/ CPCSEA).

Chemicals

Geriforte (Himalaya drugs) was used as a standard drug which is a multiconstituent ayurvedic drug with 35 herbal and natural constituents like *Withania somnifera, Chichorium intybus, Asparagus racemosus, Myristica fragrans, Caesalpinia digyna, Glycyrrhiza glabra*, Shilajeet etc. Thiobarbituric acid was procured from Sigma–Aldrich Company, USA. All other chemicals used in the study were of analytical grade.

EXPERIMENTAL

Phytochemical screening

The extracts were subjected to qualitative chemical investigation for identification of phytoconstituents as per standard procedure [22].

Acute toxicity

The acute toxicity study was carried out as per guideline set by Organization for Economic Cooperation and Development (OECD guideline number 425) received from CPCSEA. Mortality within 48 hours was recorded in overnight fasted albino mice. The animals were observed for a further 14 days for any signs for delayed toxicity. Both the extracts have good margin of safety did not show the lethal effects on the albino mice up to the doses of 2000mg/ kg. Hence 1/10th dose of LD_{50} of each extract (i.e. 200 mg/ kg body weight) were administered orally to rats during the experimentation.

Method

Albino rats of either sex were divided into following groups of six animals each.

Group I (control): animals received 1% w/v acacia as vehicle.
Group II (stress control): animals received 1% w/v acacia as vehicle
Group III (Test 1 and stress): animals received ethanolic extract of *Moringa oleifera* (200mg/ kg body weight)

Group IV (Test 2 and stress): animals received methanolic extract of *Moringa oleifera* (200 mg/ kg body weight)
Group V (Standard and stress): animals received Geriforte (100 mg/ kg body weight)

Vehicle, extracts and Geriforte were given to rats once daily for a period of 21 days orally one hour prior to the stress procedure. Restraint stress was accomplished by immobilizing animals in restrainers for 3 hours each day at random time to avoid habituation during the experimental period of 21 days.

Lipid peroxidation assay

Measurement of MDA was done by using the method explained by Ohkawa H et al [23]. Rat brain was promptly excised after decapitation, weighed and chilled in ice-cold 0.9% sodium chloride. After washing with 0.9% sodium chloride, tissue homogenate was prepared in a ratio of 1 gm of wet tissue to 9 ml of 1.15% of potassium chloride by using a glass homogenizer. To 0.2 ml of 10% w/v tissue homogenate were added 0.2ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5ml of 0.8% of aqueous solution of thiobarbituric acid. The mixture was made up to 4ml with distilled water and then heated in an oil bath at 95^0C for 60 minutes using a glass ball as a condenser. After cooling with tap water 1ml of distilled water and 5ml of the mixture of n-butanol and pyridine (15:1 v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes the organic layer was taken and its absorbance at 532 nm was measured. 1, 1, 3, 3-Tetramethoxy propane was used as standard for calibration of the curve and the level of lipid peroxides was expressed as nmol of MDA. Blank was measured simultaneously. The data obtained were subjected to statistical analysis.

Statistical analysis

The resulted values were expressed as mean ± SEM (n = 6). All data were analyzed by One-Way ANOVA followed by Dunnet's test for individual comparison.

RESULTS

Preliminary phytochemical screening revealed the presence of glycosides, phytosterols, tannins and amino acids. During acute toxicity studies, animals did not show significant autonomical and behavioural changes observed for 14 days, thereby showing the safety of *Moringa oleifera.* The restraint stress administered for a 21 days period, induced significant increase in lipid peroxidation level in stress group when compared to control group. Treatment with ethanolic and methanolic extract of *Moringa oleifera* for 21 days, concomitant with restraint stress in the dose of 200 mg/ kg, tended to normalise stress induced increase in lipid peroxidation activity. The extract effects were at par with that of the standard drug (table 1).

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TABLE 1: Effect of *Moringa oleifera* on chronic restraint stress induced Brain Lipid Peroxidation in rats.

Group	Treatment	Dose	Lipid peroxidation level (nmol MDA/ g tissue wet)	% protection
I (control)	Vehicle(1% w/v Acacia)	2.5ml /kg body weight	128.41 ± 7.14	-
II (stress control)	Vehicle(1% w/v Acacia)	2.5ml /kg body weight	182.74 ± 2.81 *	-
III (Test 1 and stress)	Ethanolic extract of *Moringa oleifera*	200 mg /kg body weight	142.53 ± 8.23 *	22%
IV (Test 2 and stress)	Methanolic extract of *Moringa oleifera*	200 mg /kg body weight	149.24 ± 1.63 *	18%
V (Standard and stress)	Geriforte	100 mg /kg body weight	136.32 ± 8.13 *	25%

Values are mean ± SEM, *P < 0.01 when compared with control

DISCUSSION

Oxygen free radical mediated lipid peroxidation of unsaturated fatty acids was clearly implicated in pathogenesis and progression of various diseases such as atherosclerosis, hypertension, diabetes and ischemic heart diseases [24]. Biological membranes are a rich source of polyunsaturated fatty acids that are susceptible to lipid peroxidation [25]. In polyunsaturated fatty acids (PUFA), an allylic hydrogen is abstracted by a reactive species or free radical, such as the hydroxyl radical ($HO^{\bullet}$**),** resulting in the formation of lipid peroxyl radicals ($LOO^{\bullet}$). This radical can then react with a second PUFA, forming a lipid hydroperoxide (LOOH) and the second $LOO^{\bullet}$, resulting in the propagation of the lipid oxidation. Alternatively, $LOO^{\bullet}$ can attack an intramolecular double bond and form a cyclic endoperoxide which decomposes to malondialdehyde, 4- hydroxynonenal etc [26].

Exposure of organisms to stressful situations produces oxidative damage which results into lipid peroxidation. Generation of oxygen free radical occur in various stressful condition viz. intermittent exposure to hypoxia [27], cold [28] and immobilization [29]. It has been generally accepted that active oxygen produced under stress is a detrimental factor, which causes lipid peroxidation [30]. Emotional and environmental stressors reportedly influence brain function and is known to be key factor in the genesis of neuropsychiatric disorders [1, 31]. Restraint as a stress model combines both emotional and physical components of stress, without any painful stimulation, in addition to producing robust increases in basal oxidative stress [32- 35]. The oxidative stress in the cells leads to lipid peroxidation [36]. Brain is a high level of oxygen consuming organ, containing areas that are rich in pro-oxidant iron [37] and PUFA which all make it very susceptible to oxidative damage by oxygen free radicals [38].

Chronic exposure to stress is known to alter the prooxidant- antioxidant balance, which might lead to the development of various human pathological states [39]. These pathological states may be, at least partly induced by oxidative stress [40].

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CONCLUSION

In the present study *Moringa oleifera* extract exhibited protective effect on restraint stress induced brain lipid peroxidation, which would then result in attenuation of the adverse effects of chronic stress. This is in agreement with above discussed studies. However a detailed study is required to isolate and characterize phytoconstituents responsible for this activity.

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