Protective Role of Turmeric in Manganese-Induced Oxidative Alterations in Rat Brain

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Turmeric powder obtained from the rhizomes of Curcuma longa Linn., has been traditionally recognized for treatment of several diseases. Overexposure to manganese (Mn) results in a neurological disorder, termed manganism which shares a similar phenotype to Parkinson's disease. The present study explores the protective effect of turmeric against the toxicity of manganese (Mn) in adult albino male rat brain. Rats were divided into four groups. Group I rats served as control. Group II rats received turmeric (1g/kg body weight/day, orally) for 45 days. Group III rats were received Mn as $MnCl_2$ (8mg/Kg body weight/day), intraperitoneally for 15 days. Group IV rats were orally received turmeric for 45 days. Besides turmeric, group IV rats were also received Mn as $MnCl_2$ (8mg/Kg body weight/day, intraperitoneally) for last 15 days. Levels of reactive oxygen species, lipid peroxidation potential, conjugated dienes, blood-brain barrier permeability, glutathione reductase and glutathione peroxidase were significantly increased, however lower levels of superoxide dismutase, reduced glutathione and membrane fluidity were observed in brain of group III. There was no change found in the activity of catalase in brain of any of the experimental groups. These changes were ameliorated in group IV. The study suggests that turmeric exhibits neuroprotection against free radical-mediated neurotoxicity of Mn.

Keywords: Turmeric, Manganese, Parkinson's disease, Blood-brain barrier, Neuroprotection.

1. INTRODUCTION

Reduced expression or activity of antioxidant proteins lead to oxidative stress, rapid aging and neurodegeneration. Manganese (Mn), an element found in many foods is an essential nutrient for proper health and maintenance. Mn is an essential metal of toxicologic concern because of risk of overexposure through occupational and environmental sources. Various findings indicate that exposure to high Mn levels result in the onset of a neurological disorder, called manganism similar to, but distinct from, Parkinson's disease (PD) [1,2]. Mn may cause cellular damage through free radical-mediated pathway by peroxidising unsaturated lipids and enhances production of reactive oxygen species (ROS) and interfere with the antioxidant defense system of the body [3]. There is increasing interest in dietary compounds that can inhibit or retard the steps leading to neurodegeneration in PD.

Turmeric is a known natural antioxidant derived from the plant Curcuma longa which has been traditionally used in India for food and medicinal purposes. Turmeric is a source of

numerous aromatic compounds present as volatile oil, the curcuma oil, semi-solid oleoresins and non-volatile compounds such as curcumin. It shows a variety of pharmacological activities such as neuroprotective, anti-inflammatory, anti-carcinogenic and antioxidant properties [4,5,6]. Antioxidant properties have been associated with turmeric powder and its active components, curcumin, a lipid soluble peptide and the aqueous extract, turmerin which constitute upto 5% and 0.1% of turmeric, respectively [7]. Turmeric and its ingredients offer protection by inhibiting lipid peroxidation and scavenging free radical which play major role in the pathogenesis of various organs [4,7]. The present study was carried out to study the protective role of turmeric against manganese-induced neurotoxicity.

2. MATERIALS AND METHODOLOGY

2.1. Chemicals

Turmeric tubers were purchased from the local market and finely powdered. $MnCl_2$ and other chemicals were procured from SISCO Research laboratory, Mumbai, India and Sigma Chemical Co., St. Louis, USA.

2.2. Experimental Design

Male albino rats of Druckrey strain weighed 90$\pm$10 gram were divided into four groups (15 animals per group). Each group of rats kept in separate polypropylene cages in an air-conditioned animal house maintained on 12 hour light/dark cycles. Rats had free access to drinking water and standard rodent pellet diet. Group I rats served as control, group II rats were given turmeric powder suspended in water at a dose of 1g/Kg body weight/day through oral intubation for 45 days. Group III rats were treated with Mn as $MnCl_2$ prepared in physiological saline at a dose of 8mg/Kg body weight/day, intraperitoneally (i.p.) for 15 days. Group IV rats were treated with turmeric, orally at the same dose as group II one month prior and simultaneously with Mn (8mg/Kg/d, i.p.) for 15 days. Animals were sacrificed on 46^{th} day and brains were dissected out in cold condition. All animal experiments were performed according to the ethical guidelines suggested by the Purpose of Control and Supervision of Experiments on Animals, Government of India. Membrane fluidity was assessed in freshly prepared synaptosomal membrane. Whole brain slices were prepared in Mc Evan tissue slicer and slices were taken randomly for the estimation of reactive oxygen species and whole homogenate of whole brain was prepared in 0.15M KCl for other biochemical analyses.

2.3. Biochemical Analyses

The parameters studied were superoxide anion production [8], hydrogen peroxide [9], lipid peroxidation potential [10], conjugated dienes [11], membrane fluidity [12], blood-brain barrier [13], reduced glutathione [14], superoxide dismutase [15], catalase [16], glutathione peroxidase [17], glutathione reductase [18], and protein [19].

2.4. Statistical Analysis

The data were analyzed using one-way ANOVA and the significance of the difference between two groups was evaluated by student's t-test. p values less than 0.05 were considered to be significant.

3. RESULTS AND DISCUSSION

The animals were subjected to Mn exposure (Gr III) showed a significantly higher levels of superoxide anion radicals, O_2^- (25%, $p<0.01$). High values of O_2^- may be due to the lower activity of superoxide dismutase enzyme (17%, $p<0.01$) as compared control group, that converts superoxide anion into H_2O_2 as shown in Table 1. Curcumin was shown to scavenge O_2^- radicals in iron-induced oxidative stress [20]. Similarly, our results revealed that turmeric lowers O_2^- level in pre- and post-treatment with turmeric (Gr IV). Other studies showed that mitochondrial membrane potential, reactive oxygen species, peroxynitrite levels and caspase-3 activities were significantly inhibited after treatment with curcuma oil [4].

Table 1: Neuroprotective effect of Turmeric against Mn-induced toxicity on non-enzymatic and enzymatic antioxidants.

S.No.	Parameters	Control	Turmeric	Mn	Turmeric+Mn
1	Glutathione(µmol/g)	1.65±0.028	1.64±0.15	1.39±0.012*	1.68±0.015
2	Superoxide Dismutase (Units/min/mg protein)	0.64±0.013	0.61±0.013	0.529±0.019**	0.603±0.044
3	Catalase (µmol/min/mg/protein)	16.12±0.931	16.68±2.49	16.63±0.88	16.44±0.885
4	Glutathione Reductase (pmol/min/mg protein)	10.01±0.615	10.45±0.824	12.65±0.904*	10.89±0.91
5	Glutathione Peroxidase (pmol/min/mg protein)	131.38±6.44	145.51±8.86	167.71±3.95**	148.36±11.26

Values represent mean ± S.E. of six rats per group. The data were analyzed using one-way ANOVA and the significance between two groups was evaluated student's t-test.
* $p<0.05$, ** $p<0.001$ as compared with control group.

It has been suggested that curcumin induces its activity through free radicals quenching mechanism and electron hydrogen donation [21]. However, hydrogen peroxide (H_2O_2) level was remained unchanged in all the three groups as compared with control group as shown in Table 2. The normal value of H_2O_2 in Mn treated rats as compared with control rats may be due high activity of GSH-peroxidase; GPx (28%, $p<0.01$) in Mn treated rats (Gr III) which detoxifies the H_2O_2. The activity of GPx was normalized in Mn+turmeric treated group (Gr IV). However, the activity of catalase (CAT) remained unaltered in Mn

treated group (Gr III). It is thought that GSH-peroxidase has higher affinity for H_2O_2 and is more effective than catalase in removing H_2O_2 [21].

Table 2: Neuroprotective effect of Turmeric against Mn-induced toxicity on Reactive oxygen species, End products of Lipid oxidation, Membrane fluidity and Blood-brain barrier.

S. No.	Parameters	Control	Turmeric	Mn	Turmeric+Mn
1	Superoxide anion radical (nmol/g/min)	12.27±0.552	10.76±0.615	15.37±0.484**	12.7±0.508
2	Hydrogen Peroxide (nmol/g/min)	0.812±0.048	0.769±0.023	0.76±0.03	0.761±0.038
3	Lipid Peroxidation(nmol MDA/h/g)	71.43±1.68	66.89±2.61	96.22±0.99***	64.07±2.56
4	Conjugated Dienes(µmol/g)	2.44±0.099	2.27±0.084	4.02±0.289***	2.25±0.059
5	Membrane Fluidity ($Poise^{-1}$)	0.426±0.022	0.442±0.018	0.296±0.021**	0.51±0.031
6	Brain uptake Index	0.281±0.01	0.264±0.011	0.383±0.016***	0.323±0.022*

Values represent mean ± S.E. of six rats per group. The data were analyzed using one-way ANOVA and the significance between two groups was evaluated student's t-test.
*p<0.05, ** p<0.01, ***p<0.001 as compared with control group.

In the current investigation, level of reduced glutathione (GSH) was decreased significantly by 16% ($p<0.05$) in Mn treated group (Gr III). Lipid peroxidation (LPO) potential, measured as TBARS and conjugated diene levels were significantly increased (35%, $p<0.001$, 66% $p<0.001$, respectively) in rat brain following Mn exposure as compared with control group. Brain tissue is largely composed of lipids, so it is highly vulnerable to this type of damage. An increase in LPO and decrease in GSH levels indicate the state of oxidative stress which was attenuated by pre- and simultaneous-treatment with turmeric (Gr IV). The activity of GSSG-reductase was significantly increased (26%, $p<0.05$) to maintain the intracellular GSSG/GSH ratio in Mn-treated rat brain (Gr III) which was recovered back to normal in Mn+turmeric treated group (Gr IV) in comparison to control. Pulla Reddy and Lokesh have shown that turmeric maintains the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase when rats were fed turmeric-supplemented diet [7]. These enzymes play an important role in the regulation of lipid peroxidation [22]. A study of Reddy and Lokesh has shown the inhibitory effect of whole turmeric on iron-induced LPO in rat liver [7]. Curcumin can scavenge oxygen free radicals such as superoxide anions and hydroxyl radicals, which play an important role in the initiation of lipid peroxidation [20].

Moreover, Agarwal et. al. showed an increase in MDA level and decrease in GSH level in both hippocampus and cerebral cortex in streptozotocin treated group which were

ameliorated by pre- and post-treatment of curcumin [23]. Curcumin has been reported to restore depletion of GSH levels and protects against protein oxidation during cerebral ischemia [24]. Components of turmeric (curcumin and turmerin) have been shown to inhibit the LPO of fractionated cellular components, synthetic vesicles [25,26,27] and renal epithelial (LLC-PK1) cell against H_2O_2 induced injury [5].

The blood-brain barrier (BBB) permeability was assessed fluorometrically in rats by using micromolecular tracer dye, sodium fluorescein (SF). The brain uptake index (BUI) for each group was calculated as the percentage of sodium fluorescein in the brain using SF as standard. In the present investigation BBB permeability in terms of BUI following Mn exposure was significantly elevated to 36% ($p<0.001$). Disruption of normal function of BBB to Mn is usually the result of widened inter-endothelial junctions or alterations in one of the enzyme like transport systems [28,29]. Increased LPO might be partially attributable to the increase in brain uptake index of sodium fluorescein tracer dye. However, increase in reactive oxygen species and depletion of GSH might also be contributory. Furthermore, oxidative damage to the brain could be a consequence of altered BBB integrity and function. Increase in BUI in Gr II was partially recovered (15%, $p<0.05$) when Mn treated animals were treated pre and simultaneously with turmeric (Gr IV) as compared to the control. This could be due to inhibition in the formation of end products of lipid oxidation and scavenging reactive oxygen species by turmeric. A report of Jiang et al. [30] shows that curcumin prevents BBB damage in focal cerebral ischemic rats. It has been reported that curcumin can cross the BBB to high level in vitro and inhibits tumor growth and angiogenesis in glioblastoma xenografts [31] variety of membrane functions such as permeability, membrane bound enzyme activities, hormone receptor interactions and efficacy of transport systems are affected by membrane dynamics. The membrane dynamics is controlled by membrane fluidity. Membrane lipids containing unsaturated fatty acids are particularly sensitive to oxidative stress, and peroxidation of membrane lipids leads to a disturbance in membrane integrity. Lipids play a key role in determining membrane fluidity and changes in lipid composition have been reported to alter important cellular functions [32]. In our observation, the decreased membrane fluidity (31%, $p<0.01$) in Mn treated rats could be due to enhanced lipid peroxidation. The lipid bilayer of membranes containing large number of fatty double bonds is susceptible to reactive oxygen species. The loss of instauration in the bilayer as a result lipid peroxidation has been reported to decrease membrane fluidity [33,34]. In our study, membrane fluidity recovers to the normal value as compared to control group when rats were fed with turmeric prior to and simultaneously with Mn (Gr IV). Amoruso et al. has also demonstrated the alteration of erythrocyte membrane fluidity following Mn treatment [35].

Thus, the result suggests that turmeric offers protection against manganese-induced oxidative alterations in brain by inhibiting the formation of end products of lipid oxidation, by scavenging reactive oxygen species and maintaining membrane fluidity and also BBB integrity and therefore, ameliorates neurodegenerative diseases involving oxidative stress. However, further study is needed to investigate the mechanism by which turmeric enters the BBB and maintain its composition and functions.

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