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The Effects of *Oxalis corniculata* l. Extract against MPTP Induced Oxidative Stress in Mouse Model of Parkinson's Disease

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Abstract

Neurodegeneration refers to a condition of neuronal death occurring as a result of progressive disease of long term. It involves degeneration of circumscribed group of neurons that may be functionally or neuroanatomically connected. Parkinson's disease (PD) is a chronic, progressive neurological disorder. Recent studies have indicated that a part of active compounds extracted from herbal medicines, herbal extracts and herbal formulations have effects on Parkinson disease models *in vitro* and *in vivo*. The present study deals with the effects of *Oxalis corniculata* L. extract against MPTP induced oxidative stress in mouse model of Parkinson's disease. *O. corniculata* extract at doses of 250 and 500 mg/kg along with MPTP administration significantly restored the peroxides and antioxidant levels to near normal in the brains of the test animals. The standard drugs L-dopa + carbidopa combination also significantly restored the peroxides and antioxidant levels to near normal.

Key Words: antioxidant, catalase, MPTP, *Oxalis corniculata*

INTRODUCTION

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease caused when a small group of brain cells that control body movements die. Oxidative stress appears to play an important role in the sporadic forms of Parkinson's disease. Reactive oxygen species (ROS) are produced during several intracellular pathways, and they induce oxidative stress. There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants [1].

Recent studies have indicated that a part of active compounds extracted from herbal medicines, herbal extracts and herbal formulations have effects on Parkinson disease models *in vitro* and *in vivo*. One plant that has been used in mental conditions and illnesses is *Oxalis corniculata* L. It is commonly known as Indian sorrel (Puliiyarai in Tamil), and belongs to the family Oxalidaceae. It has antipsychotic, CNS-stimulant and antiepileptic properties [2]. The leaves of *Oxalis corniculata* are used traditional Indian medicine to treat epilepsy [3].

The neurotoxin, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is well studied for its ability to induce oxidative damage and mitochondrial dysfunction in the nigrostriatal dopaminergic system in animal models, resembling the idiopathic PD in humans [4]. MPTP-induction has been well reported for the development of cognitive and behavioral deficits in both non-human primates and mice models [5, 6]. Therefore, MPTP-induced neurotoxicity in mice is considered as the most useful functional model of Parkinsonism (Schneider, 2006). In the present study, the effect of *Oxalis corniculata* alcoholic extract on the antioxidant enzyme status in MPTP-induced mouse brain tissues was evaluated to correlate its neuroprotective effect.

MATERIALS AND METHODS

Chemicals and reagents

MPTP hydrochloride was purchased from Sigma Chemical Co. All other chemicals used were of analytical grade. Stock solutions of all chemicals were prepared in distilled water and the dilutions were made fresh on the day of the experiment.

Plant extract

The medicinal plant *Oxalis corniculata* was collected from Tirunelveli District, Tamil Nadu, India. Mature and healthy plants were collected naturally from different locations after the rainy season (February, March and April). The specimens were identified referring to the Flora of Presidency of Madras [7] and Flora of Tamil Nadu Carnatic [8]. The specimens were shade-dried at room temperature (18-20^{o}C) for a period of 3 weeks to 8 weeks. The completely dried materials were made in to coarse powder by mechanical grinder and the powder was passed through a 40-mesh sieve, to get a uniform particle size and then used for extraction purpose. A weighed quantity of powder was subjected to continuous hot percolation in soxhlet apparatus with ethanol at 65-70^{o}C. The extracts were evaporated under reduced pressure using rota flash evaporator until all the solvent had been removed. The yield of the extract was 12% w/w. when compared to the dried starting material, which was then stored at -20°C until required.

Animals

C57 Black male mice, weighing 25-30 gm were used. All animals were obtained from the Animal house, K M C H College of Pharmacy, Coimbatore, Tamil Nadu. They were

allowed food and water *ad libitum* up to the experimentation period. Prior to use, the mice were housed in polypropylene cages in group of six to eight animals under natural light-dark cycle. Each animal was used only once under standard laboratory conditions. All the observations were made at room temperature in a noiseless diffusely illuminated room and were made between 9.00 to 17.00 h in the experimental room. All the experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) (KMCRET/PhD/ /2014-15), New Delhi, India.

Experimental Protocol

The following experimental procedure was followed to evaluate the Locomotor behavioral effect of *O. carniculata* (OC) on MPTP induced mice. 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP): 15 mg/kg of MPTP as a neurotoxin i.p. twice, 4 h apart *in vivo* and 20 µM *in vitro*. MPTP was purchased from Sigma, India. MPTP was dissolved in 0.9 % saline and administered i.p. Intraperitonial injection of MPTP was given to Groups II,III, IV and V. Oral dosage of Carbidopa + Levadopa (Standard drug for Parkinson's disease treatment) was given to Groups III.

The animals were divided into six groups, each consisting of six mice.

Group I served as vehicle control (Distilled water)

Group II received MPTP (20 mg/kg, i.p) (Sigma-Aldrich, Bangalore, India) four consecutive days,

Group III received MPTP + carbidopa + levodopa (100 mg/kg, p.o)

Group IV received MPTP + crude extract (250 mg/kg, p.o)

Group V received MPTP + crude extract (500 mg/kg, p.o)

Group VI received only crude extract (500 mg/kg, p.o)

Experimental Analysis

The activity of superoxide dismutase was assayed by the method of Kakkar *et al*. [9]. The catalase activity was assayed by the method of Sinha [10]. Lipid peroxidation in brain was estimated colorimetrically by the methods suggested by Fraga *et al.* [11].

RESULTS

In the MPTP treated group, the level of SOD is increased ($P< 0.001$). At the same time, the group with MPTP + 250 mg of *Oxalis* extract shows decreased level of superoxide dismutase almost equal to that of MPTP + Standard groups (Fig: 1).

In the present study, the level of Catalase is decreased in MPTP treated group and increased in the group of MPTP and 500 mg of ethanolic extract of *Oxalis corniculata* (Fig: 2). *Oxalis corniculata* extract significantly increased the level of catalase towards normal level and the results were comparable to control as shown in Fig. 2.

Furthermore, LPO levels were significantly ($p < 0.001$) increased in MPTP-induced group (Fig 3). However, OC treated groups (100, 200 and 300 mg/kg) dose dependently attenuated these changes. the value of lipid peroxidation increases much than that of the control group. But, the same is highly reduced in the group which is treated with MPTP and 500 mg of ethanolic extract of *Oxalis* (Fig: 3). *Oxalis corniculata* extract minimized the level of lipid peroxidation towards normal level dose dependently.

Fig 1: Effect of administration of *O. corniculata* ethanol extract and its combinations on antioxidant enzyme SOD

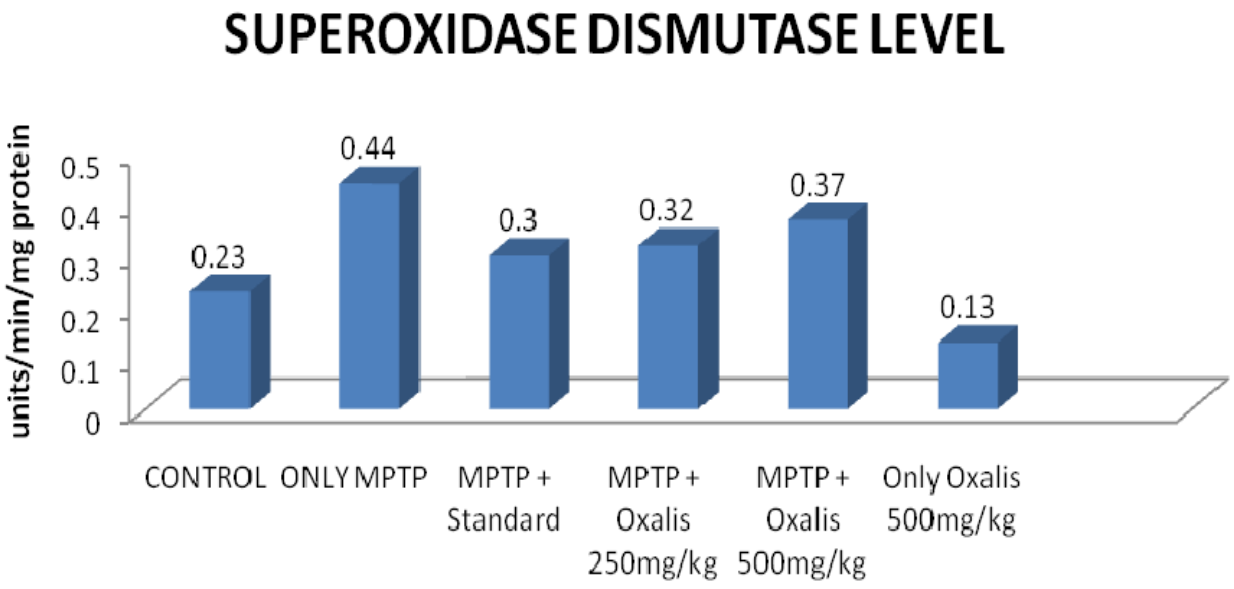


Fig 2: Effect of administration of *O. corniculata* ethanol extract and its combinations on antioxidant enzyme catalase

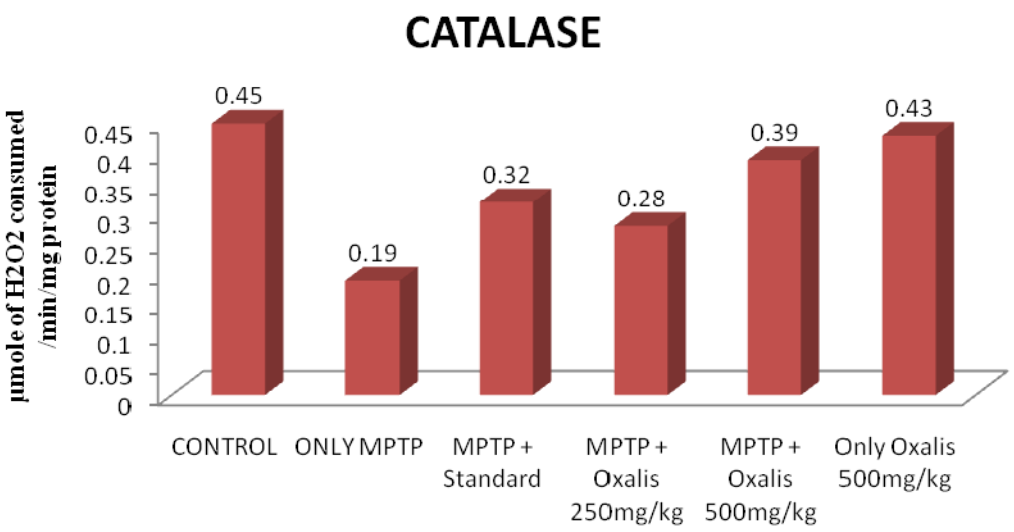
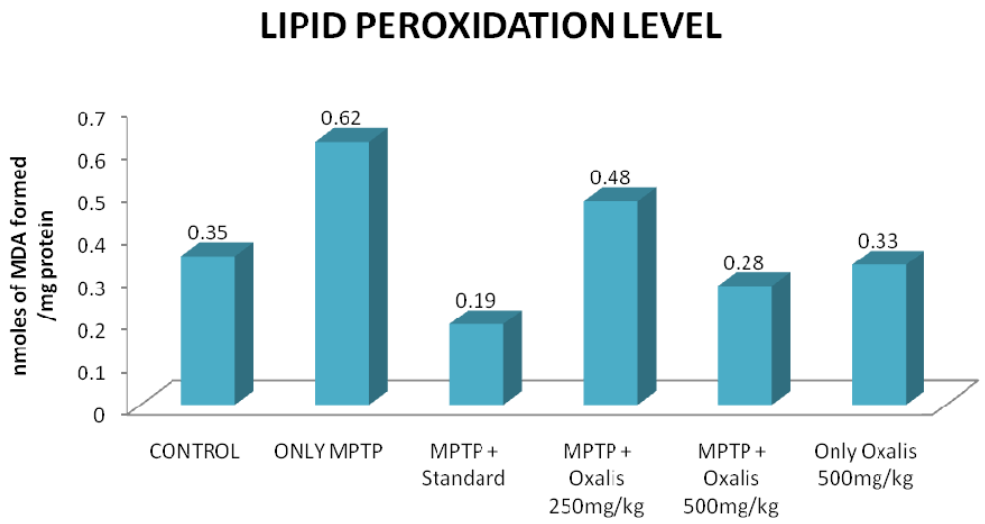


Fig 3: Effect of administration of *O. corniculata* ethanol extract and its combinations on antioxidant enzyme Lipid Peroxidation in the liver of mouse under oxidative stress



DISCUSSION

Oxidative stress, associated with increased formation of reactive oxygen species (ROS), modifies phospholipids and proteins leading to lipid per-oxidation and oxidation of thiol groups [12, 13]. Reactive oxygen species (ROS) generated after blockade of the complex I as well as those generated due to DA oxidation could be the main cause of MPTP-induced terminal degeneration [14, 15]. Oxy free radicals are removed by superoxide dismutase in healthy organisms, but during degeneration, the lowered activity of SOD is caused by inhibition of the enzyme by excess H_2O_2. This excess H_2O_2 , besides inhibiting SOD, can cause degradation of heme rings of hemoglobin and releasing iron which is capable of free radical production via Fenton reaction [16] Superoxide radicals generate hydrogen peroxide as metabolites, which in the presence of transition metals like iron, leads to the generation of the highly toxic hydroxyl ions, known to induce lipid peroxidation. As such, an effective antioxidant agent should be capable of augmenting intracellular concentrations of not only SOD, but also Catalase in finally reducing lipid peroxidation. In the present *in vivo* study elevation in levels of end products of lipid peroxidation in liver of mouse treated with MPTP were observed. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage [17]. Treatment with extract of *Oxalis corniculata* significantly reversed these changes. Hence it may be possible that the mechanism of protection of extract is due to its antioxidant effect [17]. Elevated levels of Reactive Oxygen Species are evident from increased lipid peroxidation and DNA damage in the substantia nigra. [18]. The metabolism of dopamine by action of enzyme monoamine oxidase is accelerated in Parkinson's disease and excessive formation of hydrogen peroxide (H_2O_2) takes place [19]. According to that, here also the value of lipid peroxidation increases much than that of the control group. But, the same is highly reduced in the group which is

treated with MPTP and 500 mg of ethanolic extract of *Oxalis.*

Catalase is an enzymatic antioxidant and helps in neutralizing the toxic effects of hydrogen peroxide. Hydrogen peroxide is not reactive enough to cause a chain of lipid peroxidation reactions, but its combination with superoxide radical produces hydroxyl radical, which is highly reactive and thus initiates lipid oxidation reactions. Catalase converts hydrogen peroxide to water and nonreactive oxygen species, thus preventing generation of hydroxyl radical and protecting the cells from oxidative damage. Oxidative stress results in decrease in catalase level. A significant decrease in the level of catalase was observed in the MPTP treated animals. The level of catalase was found to be increased in carbidopa + levodopa and *O. corniculata* treated groups as compared to MPTP group (Figure 2). Thus, the MPTP group showed a significant increase in the levels of thiobarbituric acid which is an indication of extent of lipid peroxidation, decrease in the levels of CAT, SOD and GSH in the brain as compared to the control animals. All these indicate an increase in the oxidative stress in the brain of animals treated with MPTP. Thus, the MPTP group showed a significant increase in the levels of thiobarbituric acid which is an indication of extent of lipid peroxidation, decrease in the levels of CAT, SOD and GSH in the brain as compared to the control animals. All these indicate an increase in the oxidative stress in the brain of animals treated with MPTP. Pretreatment with carbidopa + levodopa and *O. corniculata* combination resulted in a decrease in TBARS level and increase in the levels of SOD, catalase and GSH, indicating its antioxidant effect in the brain of MPTP treated animals. A possible underlying mechanism of this protection can be associated with the presence of alkaloids, poly phenols and flavonoids in the extracts, which are an important source of antioxidants [20].

Oxidative processes are an important factor in the pathogenesis of several disorders, and postmortem studies have consistently implicated oxidative damage in Parkinson disease pathogenesis [21]. Then, compounds with potential antioxidant activity are notable candidates to become new therapeutic agents, since perspectives for treatment of Parkinson disease in the future could include antioxidant therapies [22]. Therefore, the results are consistent with others studies which showed protective activity by substances such as alkaloids, poly phenols and flavonoids, known by their antioxidant power in the same experimental model [23, 24]. Finally, the data revealed that *Oxalis corniculata* could be a potential therapeutic tool for neurodegenerative diseases. Active components of this extracts have to be determined. The research for substance with neuroprotective activity has increased in recent years. Since, oxidative stress produced in brain due to MPTP toxicity seems to be important in producing motor defects; therefore use of antioxidants could prove beneficial [25]. The present study which thus explored the potential of *Oxalis* extract, earlier proved to be an antioxidant, showed a promising effect in animals with Parkinsonism disease.

CONCLUSION

The findings of this study indicate that OC extract protect MPTP-induced alteration in antioxidative enzyme levels (SOD, CAT and LPO) was reversed in PD mouse brain tissues. Regulation of antioxidant defense mechanisms by OC may partly be responsible for its neuroprotective effect in MPTP-induced PD mice.

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