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Neuroprotective Effect of Tannic Acid against Rotenone Induced Rat Model of Parkinson's Disease

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Abstract:

Tannic acid is ubiquitously occurring plant polyphenol which is found in most of the beverages such as wine, beer, black and green tea and several food stuffs such as grapes, bananas, peas, sorghum, chocolates and lentis. It is reported to have antioxidants. Anti-mutagenic, anti-cancer and neuroprotective effects. The present study is designed to evaluate the anti-parkinsonic role of TA against rotenone induced rat model of PD by analysing the levels of dopamine, oxidants and activities of antioxidants. Animals were randomized and divided into 4 groups, control, rotenone treated, rotenone+TA treated and TA alone treated. Rotenone injection, enhanced the levels of DA and TBARS, diminished the levels of GSH and activities of SOD, catalase and GPx. Oral administration of TA attenuated the rotenone induced toxicity by its neuroprotective and antioxidant properties.

INTODUCTION

Parkinson disease (PD) is a neurological disease that is reported to cause motor and non-motor dysfunctions mainly in the elder populations. PD is mostly considered as motor disease due to the presence of movement related symptoms such as tremor at rest, rigidity, akinesia/bradykinesia and postural instabilities. The main pathology involves a progressive degeneration of nigral dopaminergic neurons and accumulation of intraneuronal inclusions (Lewy bodies) [1]. Current therapeutic strategy engage drugs that imitate function of dopamine, induce dopamine receptors and inhibit the destruction of dopamine. But nothing is capable of protecting the neuro-degeneration. So there is an urgent need of a novel treatment approach for stopping or halting the progression of disease.

Although various pathological condition such as genetic mutations, ubiquitome dysfunction and unfolded protein response, inflammation, stimulation of autophagic pathways, neuroinflammation and stimulation of immune response are linked with the etiology and progression of PD, oxidative stress and its related pathologies are considered as a key factors involved in the loss of dopaminergic neurons [2]. Previous studies indicated that the phytochemical and synthetic compounds with potent antioxidant effects showed neuroprotective effects against experimental models of PD [3,4]. As the relationship between the oxidative stress and pathologies of PD such as inflammation, mitochondrial dysfunction and apoptosis were well explored; more studies are needed to identify the new compounds that prevent the dopaminergenic neuronal loss via targeting these pathways.

Tannic acid, a ubiquitously occurring plant polyphenol consisting of a glucose molecule attached to a or more galloyl residues, is found in various beverages such as wine, beer, black and green tea and several food stuffs such as grapes, bananas, peas, sorghum, chocolates and lenthils [5]. It is reported to have antioxidant, anti-mutagenic, anticancer and nephroprotective properties. Chung et al., [5] indicated that the antioxidant

properties of tannic acid and gallic acid relies on the respective functional groups found on them . This experiment is planned to evaluate the antioxidative and neuroprotective effect of TA against rotenone induced experimental model of PD, which is not investigated so far.

Materials and Methods

Animals

Male Albino Wistar rats (225–250 g) were obtained, kept under standard conditions at Central Animal House, RMMC&H, Annamalai University and fed with standard pellet diet and water ad libitum. The experimental protocols met with the National Guidelines on the proper care and use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, 2000) and were approved by the Animal Ethics Committee of the Institute (IAEC Proposal.No.AU-IAEC/1208/4/18 approved on 27.4.2018)

Chemicals

Rotenone, tannic acid, thio barbituric acid (TBA), glutathione, NADH, NBT, 5,5-dithiobis[2-nitrobenzoic acid] (DTNB) were purchased from Sigma Chemical Company, Bangalore, India. All other reagents used were of analytical grade and procured from Himedia company.

Experimental Design

Experiment I

The 36 rats were grouped (n=6) into control rats (received 0.5 ml of sunflower oil i.p. for 45 days), rotenone (2.5 mg/kg/day i.p in sunflower oil for 45 days) alone treated [3], rotenone (as group II) and low dose of TA (5 mg/kg in saline was administered p.o. after 1 h of rotenone treatment and continued up to 45 days) treated, rotenone and middle dose of TA (10 mg/kg in saline p.o. for 45 days) treated, rotenone and high dose of TA (20 mg/kg in saline p.o. for 45 days) treated and TA (20 mg/kg/day p.o.for 45 days) alone treated. After the end of

the experimental period, behavior analysis (akinesia and catalepsy) were carried out. The ST and SN from control and experimental rats were dissected for the estimation of neurochemical and biochemical indices.

Analysis of DA and their metabolites

The levels of striatal DA were quantified by the high performance liquid chromatography (HPLC) by the protocol used of Muralikrishnan and Mohanakumar, [6]. Striatum was homgenised in perchloric acid containing ethylene diamine tetraacetic acid (EDTA). Then it was centrifuged (10,000 g for 10 min) and the supernatant fraction was separated and injected (10 µl) into the HPLC with Electrochemical detector. The mixture of acetonitrile, citric acid, monobasic phosphate sodium, methanol, octane sulfonic acid, EDTA, Kcl, heptanes sulfonic acid, o-phosphoric acid and diethylamine was used as the mobile phase. Results were expressed in ng/mg weight of brain tissue.

Biochemical Analysis

Quantification of Thiobarbituric Acid Reactive Substances (TBARS)

The method adapted by Utley et al. [7] is used for the estimation of TBARS. The dissected SN region was first homogenized in phosphate buffer and mixed with TBA and TCA reagents. Then it was centrifuged and supernatant was collected and boiled. Optical density were read at 535 nm after cooling. The levels of lipid peroxidation was expressed in the terms of nmol of TBARS formed/mg protein.

Estimation of Reduced Glutathione (GSH)

The method of Jollow et al. [8] was used for the estimation of GSH. The sulfosalicylic acid was added to homogenize tissue and kept at cooling temperature for 1 h. Then the assay mixture was centrifuged (12000 g) for 15 min at 4 ˚C. Then the supernatant was collected and added with sodium phosphate buffer and DTNB. The OD was read at 412 nm on a spectrophotometer.

Assay of Glutathione Peroxidase (GPx) Activity

GPx activity was quantified by Mohandas et al. [9]. The tissue homogenate was treated with sodium phosphate buffer, EDTA, sodium azide, glutathione reductase, GSH, NADPH, H_2O_2 and PMS. NADPH oxidation was measured at 340 nm and expressed as nmol NADPH oxidized min/mg protein.

Assay of Catalase Activity

The activity of catalase was quantified by the protocol followed by Claiborne, [10]. The tissue homogenate was mixed with phosphate buffer, H_2O_2 and PMS. The OD was measured at 240 nm and expressed as nmol H_2O_2 consumed min/mg protein.

Assay of Superoxide Dismutase Activity

The tissue homogenate was treated with sodium carbonate buffer, xanthine, NBT, EDTA and xanthine oxidase. The OD was measured at 560 nm and expressed as units/min/mg protein[11].

Statistical Analysis

Statistical analysis was calculated with the help of one-way analysis of variance using DMRT in Statistical Package for the Social Science (SPSS) - 15.0. All data are expressed as mean ± SEM for six rats in each group. Results were considered statistically significant at $p<0.05$.

Results

The striatal levels of DA and its metabolites and nigral levels of TBARS and non-enzymatic antioxidants like GSH and the activities of enzymatic antioxidants such as SOD, catalase, and GPx were quantified in control, rotenone and/or TA treated rats to assess the neuroprotective and antioxidant effect of TA.

3.1 Protective role of TA against rotenone mediated depletion of DA and its metabolites

In the rotenone injected rats, the levels of DA, DOPAC and HVA were diminished significantly as compared to control animals. The co-administration of TA to rotenone injected rats significantly enhanced the levels of DA and its metabolites as compared to rotenone alone injected rats. There is no significant alterations found in the levels of neurotransmitters between the TA alone administered and control rats (Fig 1).

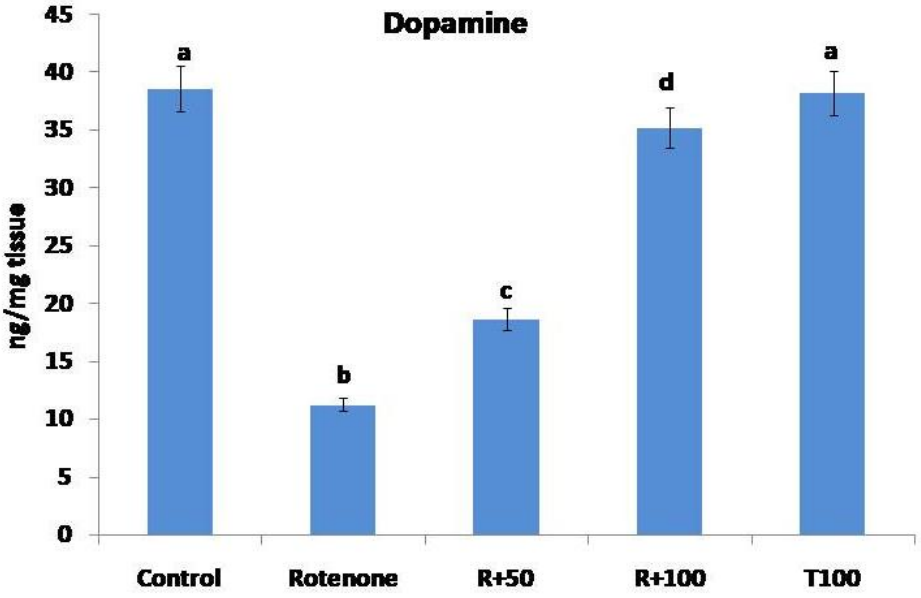

Fig. 1 demonstrates the changes in the levels of DOPAMINE in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

3.2 Protective role of TA on rotenone induced elevated levels of lipid peroxidation products

The rotenone injected rat showed increased levels of TBARS as compared to control animals. The co-administration of TA to rotenone injected rats significantly decreased the levels of TBARS as compared to rotenone alone injected rats. There is no significant alterations found in the levels of TBARS between the TA alone administered and control rats (Fig 2).

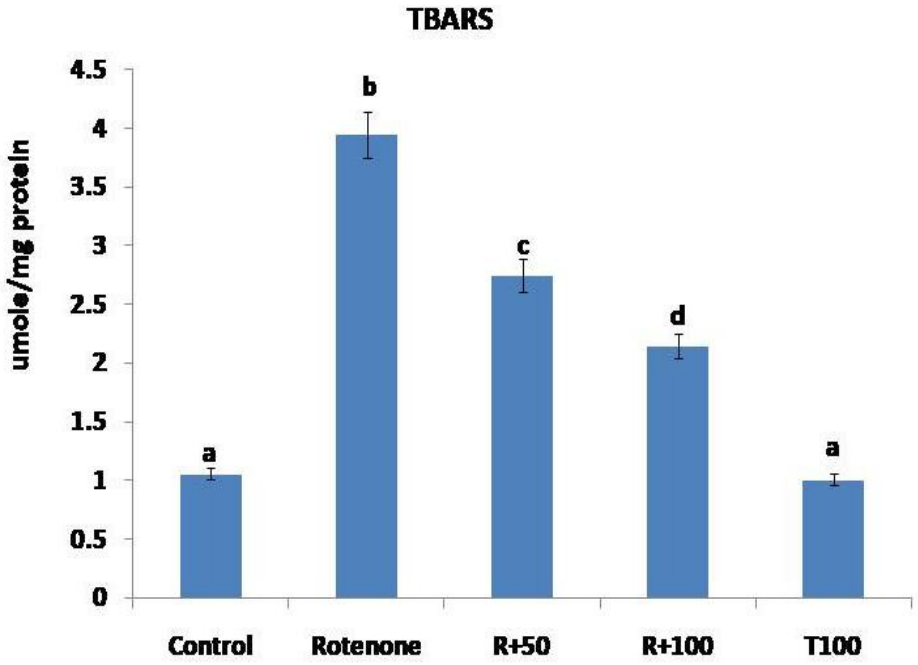

Fig. 2 demonstrates the changes in the levels of TBARS in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabetdiffered significantly ($p<0.05$) with each other.

3.3 Protective effect of TA on rotenone induced depleted levels of GSH

The rotenone injected rat showed depletion in the levels of GSH as compared to control animals. The co-administration of TA to rotenone injected rats significantly enhanced the levels of GSH as compared to rotenone alone injected rats. There is no significant alterations found in the levels of GSH between the TA alone administered and control rats (Fig 3).

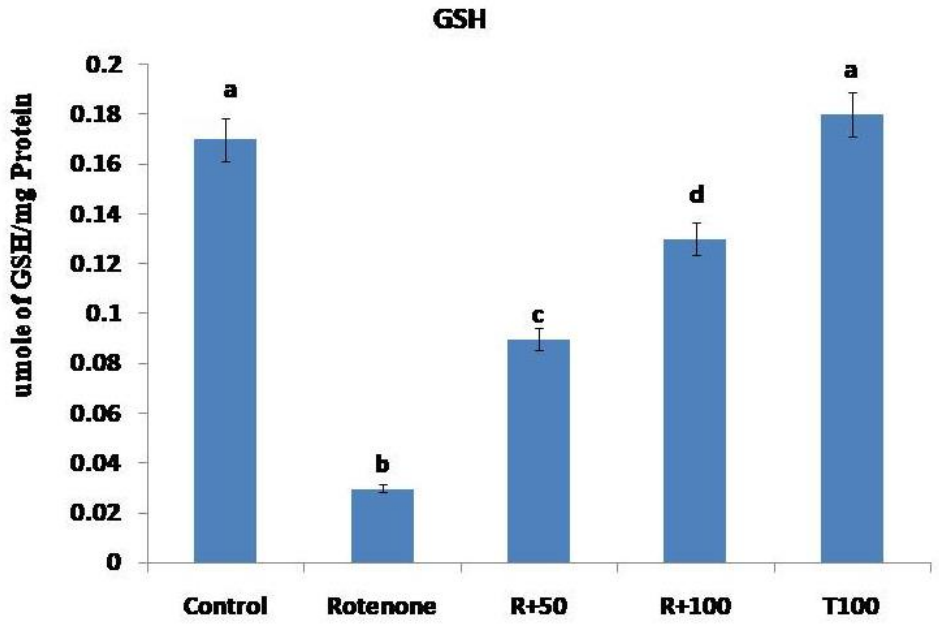

Fig. 3 illustrates the changes in the level of GSH in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

3.4 Protective role of TA on rotenone induced depleted activities of enzymatic antioxidants

In the rotenone treated rats, the activities of SOD, catalase and GPx were significantly diminished as compared to control animals. The co-administration of TA to rotenone injected rats enhanced the activity of enzymatic antioxidants as compared to rotenone alone injected rats. There is no significant alteration found in the activities of enzymatic antioxidant between the TA alone administered and control rats (Fig 4, 5 and 6).

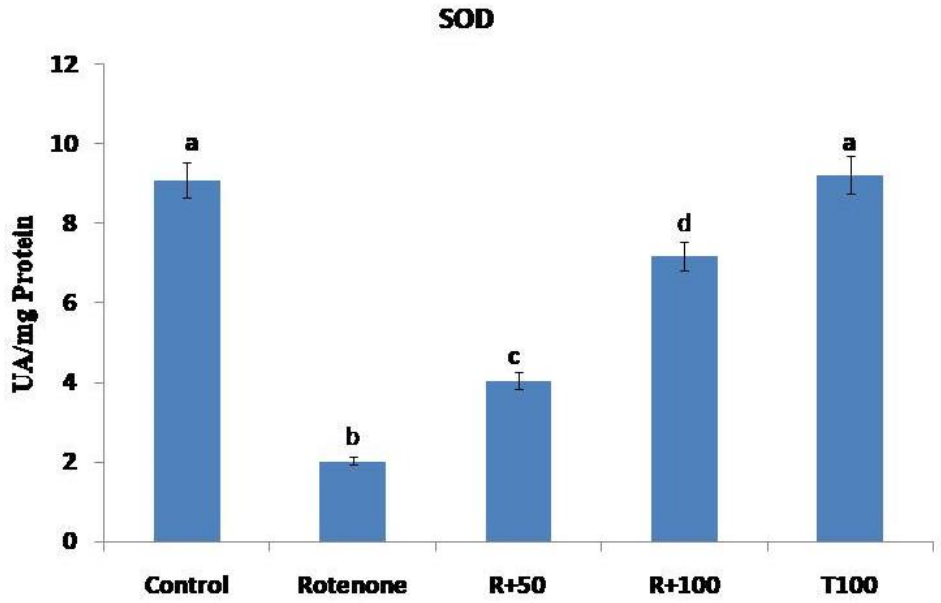

Fig. 4 indicates the changes in the activity of SOD in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

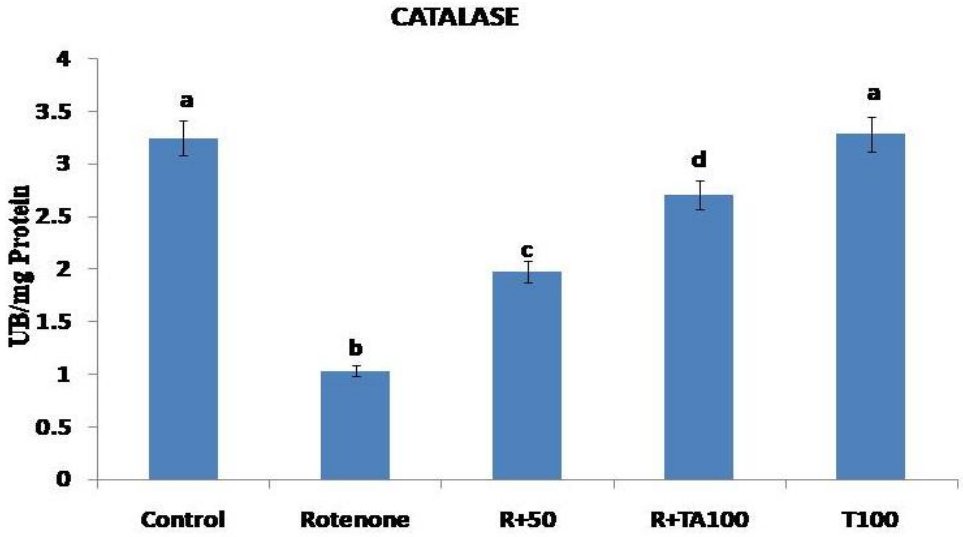

Fig. 5 shows the changes in the activity of catalase in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p < 0.05$) with each other.

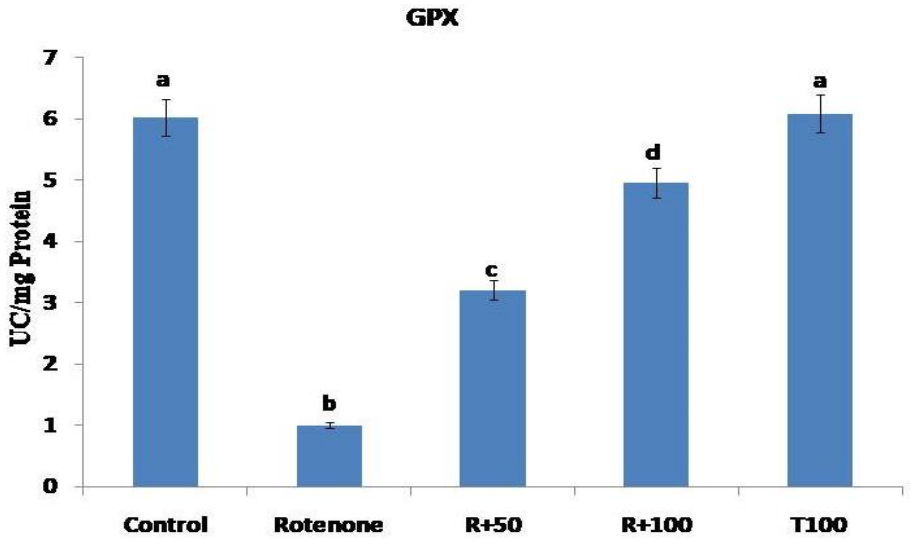

Fig. 6 demonstrates the changes in the activity of GPx in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

Discussion

The dopaminergenic neuronal loss in the SN leads to the reduction in the striatal DA output due to degeneration of nigro-striatal dopaminergenic pathway, which is mostly affected during PD. The result of the present experiment indicated that the striatal levels of dopamine and its metabolites were reduced significantly in the rats injected with rotenone, which is corroborated with our previous studies[12,13]. We also observed that the administration of TA significantly enhanced the striatal levels of DA and its metabolites which indicated the neuroprotective role of TA.

As rotenone is a potent inhibitor of complex I of the mitochondrial respiratory chain, it inhibits the flow of electrons from complex I to ubiquinone, resulting in the blockade of oxidative phosphorylation, thus the ATP production [14]. As about 20% of total body oxygen is consumed by the brain, the released electrons from mitochondria of neurons reacted with these freely available oxygen to form vigorous reactive oxygen species called superoxide anion (O_2-)[15,16]. Superoxide anion dismutated spontaneously or by the action of antioxidant enzyme SOD to form less toxic hydrogen peroxide (H_2O_2). Other antioxidant enzymes such as catalase and glutathione peroxidase decompose H_2O_2 into water and molecular oxygen. Enhanced formation of these ROS in the brain during neurodegenerative diseases is an indicator of the oxidative stress that results in the quick utilization and depletion of endogenous radical scavenging antioxidants. Increased levels of TBARS found in rotenone treated animals indicated that the enhanced formation of reactive oxygen species and lipid peroxidation processes, whereas altered activities of enzymatic antioxidants such as SOD, catalase and GPx due to their enhanced depletion. Reduced levels of GSH, the non-enzymatic antioxidant, is a cardinal marker of oxidative stress during the cause and progression of PD, indicating a concomitant rise in the levels of ROS.

Various in vitro experiments indicated that the exposure of tannic acid showed potent antioxidant activity by scavenging free radicals[17]. TA is absorbed and get metabolized into gallic acid in the intestinal tract of

mammals. Gallic acid in TA could enhance the antioxidant functions in the cells by eliminating malondialdehyde and enhancing the activities of enzymatic antioxidants like SOD, catalase and GPx[18]. As to conclude, the present experiment proves the neuroprotective role of TA against rotenone mediated neurotoxicity, which is partially due its potent antioxidant function.

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