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Q1 (–) Epigallocatechin-3-gallate attenuates reserpine-induced orofacial  
2 dyskinesia and oxidative stress in rat striatum

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## ABSTRACT

Reserpine-induced orofacial dyskinesia (OD) has been used for decades as an animal model for human tardive 28  
dyskinesia (TD) because both of them have pathophysiology strongly associated with striatal oxidative stress. 29  
Green tea catechins, especially (–) epigallocatechin-3-gallate (EGCG), have potent antioxidative effects and 30  
are able to protect against various oxidative injuries. In this study, we examined the potential protective effects 31  
of EGCG on reserpine-induced behavioral and neurochemical dysfunction in rats. Reserpine treatment 32  
(1 mg/kg s.c. one injection every other day, three injections total) induced significant increases ( $p < 0.001$ ) in 33  
the frequency of vacuous chewing movement (VCM) and tongue protrusion (TP) as well as the duration of facial 34  
twitching (FT). EGCG treatment (100 mg/kg i.p. for 11 days, starting 7 days before the reserpine injections) was 35  
able to prevent most of the reserpine-induced OD. Also, EGCG treatment was able to reduce the reserpine- 36  
induced lipid peroxidation (LPO) production, and enhances the antioxidation power in the striatum of 37  
reserpine-treated rats. The above results indicate that EGCG has a protective role against reserpine-induced 38  
OD, probably via its powerful antioxidative properties. Thus, EGCG may possible have a clinically relevant thera- 39  
peutic effect in preventing, delaying or even treating TD. 40

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## 46 1. Introduction

47 Reserpine, an indole alkaloid, was used clinically to relieve psychotic 58  
48 symptoms and control blood pressure several decades ago (Davies and 59  
49 Shepherd, 1955; Durlach, 1956). More recent reports have associated re- 60  
50 serpine with the development of tardive dyskinesia (TD) and behavioral 61  
51 symptoms of Parkinson disease (PD) by depleting catecholamines 62  
Q4 (Fernandes et al., 2012; Ishibashi and Ohno, 2004; Neisewander et al., 63  
Q5 1994; Santos et al., 2013; Skalisz et al., 2002). Rats treated with this 64  
54 monoamine-depleting compound develop orofacial dyskinesia (OD) 65  
55 which is characterized by increased vacuous chewing movement, 66  
56 tongue protrusion, orofacial burst, and cataleptic behavior 67  
Q6 (Neisewander et al., 1994; Patil et al., 2012a,b). In this reserpine- 68

69 treated animal model, striatum had an increased production of lipid 70  
71 peroxidation (LPO) byproducts as well as a decreased antioxidative 72  
73 enzyme activity. In animal models it has been suggested that the antiox- 74  
75 idant treatment may be essential in treating TD (Burger et al., 2003; 76  
Nade et al., 2013; Patil et al., 2012a,b). Q7

Green tea is one of the most common beverages in the world, and its 63  
main active compounds are catechins, including (–) epigallocatechin- 64  
3-gallate (EGCG), (–) epigallocatechin (EGC) and (–) epicatechin gal- 65  
late (ECG) (Graham, 1992). Tea catechins are primarily absorbed in the 66  
small intestine and are then transported to other organs, one of which is 67  
the brain (Nakagawa and Miyazawa, 1997). Owing to their unique Q8  
chemical structure, catechins have varied pharmacological properties 69  
such as sedative, hypotensive, lipid-lowering and antitumorigenesis 70  
effects (Chang et al., 2009; Lin, 2002; Negishi et al., 2004). Besides 71  
being able to scavenge reactive radicals and reduce peroxidative status 72  
(Łuczaj and Skrzydlewska, 2005; Skrzydlewska et al., 2002; Vignes 73  
et al., 2006), EGCG, the main tea catechin, has been suggested to have 74  
neuro-protective effects to treat behavioral impairments induced by is- 75  
chemia, toxins, stress, and hypertension (Chen et al., 2010; Lee et al., 76

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2000; Levites et al., 2001; Wang et al., 2012). However, the reports regarding its protective effect on reserpine-induced OD are still limited. Given the antioxidative properties of EGCG, we hypothesize that EGCG is able to counteract the increased oxidative damage caused by reserpine, and sequentially disrupt the development of OD.

In this study, we examined the potential therapeutic effects of EGCG to prevent the reserpine-induced OD in rats. OD is defined by the increases in vacuous chewing movement (VCM) frequency, tongue protrusion (TP) frequency, and the facial twitching (FT) duration. In addition, we examined the oxidative status and antioxidation power of striatum; the levels of LPO byproducts, glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were measured in both control and reserpine-treated rats with or without EGCG treatment.

## 2. Materials and methods

### 2.1. Animals

All the experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. All experimental protocols were also approved by the Institutional Animal Care and Use Committee (IACUC) of National Taipei University of Technology. Male Wistar rats weighing 270–320 g (about 3 months old) were used for study. A group of three animals was kept in Plexiglas cages with free access to food and water in a room with controlled temperature ( $22 \pm 3^\circ\text{C}$ ) and in 12-h light/dark cycle with lights on at 7:00 a.m. The rats were randomly assigned into six groups with sample size of eight rats in each group: control group (C), reserpine treatment group (R), (–) epigallocatechin-3-gallate (EGCG) 30 mg/kg treatment group (E30), EGCG 100 mg/kg treatment group (E100), EGCG 30 mg/kg + reserpine treatment group (E30 + R) and EGCG 100 mg/kg + reserpine treatment group (E100 + R). The current choice of EGCG dosage was based on our previous lab findings (Wang et al., 2012). EGCG was started from the low dose of 1 mg/kg body weight. Owing to no significant effect of the low-dosage application; the EGCG dosage was increased gradually and eventually up to 30–100 mg/kg body weight to induce apparent response.

C group was injected with normal saline intraperitoneally (i.p.) for 11 days and with 0.1% acetic acid solution (vehicle for reserpine) subcutaneously (s.c.) every other day on days seven, nine and eleven in total of 3 injections. The first injection of acetic acid was given on the seventh day after the administration of normal saline. R group received normal saline i.p. for 11 days and with 1 mg/kg reserpine s.c. every other day on days seven, nine and eleven in total of 3 injections. E30 or E100 group received 30 or 100 mg/kg EGCG i.p. for 11 days and with 0.1% acetic acid solution s.c. every other day on days seven, nine and eleven in total of 3 injections. E30 + R or E100 + R group was injected with 30 or 100 mg/kg EGCG i.p. for 11 days and with 1 mg/kg reserpine s.c. every other day on days seven, nine and eleven in total of 3 injections. On the seventh, ninth and eleventh days, the injection of EGCG or normal saline preceded the reserpine or acetic acid solution injection by 30 min. On the 12th day, 24 h after the third injection of reserpine or acetic acid solution, all animals were observed for the quantification of OD behavior. Animals were sacrificed about 1 h after behavioral measurements.

### 2.2. Drugs

Reserpine (methyl reserpate 3,4,5-trimethoxybenzoic acid ester; Sigma) was dissolved in glacial acetic acid and then diluted to a final concentration of 0.1% acetic acid with distilled water. Vehicle consisted of a 0.1% acetic acid solution. The above solutions were s.c. injected. However, (–) epigallocatechin-3-gallate (EGCG) ([[(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl] 3,4,5-trihydroxybenzoate; Sigma), prepared with normal saline, was i.p. administrated

during the period of housekeeping and animal nursing. All the solutions were injected in the volume of 1.0 mL/kg body weight.

### 2.3. Behavioral testing

On the 12th day, 24 h after the third injection of reserpine or acetic acid solution, animals were observed for the quantification of OD behavior (Burger et al., 2003). Animals were placed separately in the cage ( $20 \times 20 \times 19\text{ cm}$ ), equipped with mirrors underneath the floors, to permit behavioral quantification even when the animal was facing away from the observer. To quantify the occurrence of OD, the events of vacuous chewing movement (VCM) and tongue protrusion (TP), and the duration of facial twitching (FT) were recorded 15 min in each section after a period of 2 min adaptation. All behavioral experiments were conducted between 09:00 a.m. and 11:00 a.m.

### 2.4. Biochemical measurement

Rats were sacrificed 1 h after behavioral quantification. The brain was quickly removed and the part of striatum was further dissected out. The dissected out striatum tissue was rinsed with isotonic saline and weighed, and then it was homogenized with 0.1 N HCl. A 10% (w/v) tissue homogenate was prepared in a 0.1 M phosphate buffer (pH 7.4); the post-nuclear fraction for catalase assay was obtained by centrifugation of the homogenate at 1000 g for 20 min at  $4^\circ\text{C}$ . For other enzyme assays, it was centrifuged at 12,000 g for 60 min at  $4^\circ\text{C}$ .

### 2.5. Assessment of lipid peroxidative indices

Lipid peroxide concentration was measured by the thiobarbituric acid reactive substance (TBARS) assay adapted from Ohkawa et al. (1979) according to the procedures described by Hashimoto et al. (2005). The concentration was measured in nanomoles of malondialdehyde/mg protein. Malondialdehyde levels were then further normalized to a standard preparation of 1,1,3,3-tetraethoxypropane.

### 2.6. Measurement of reduced glutathione (GSH)

GSH was determined by the method of Ellman (1959). To the homogenate, 10% trichloroacetic acid was added, centrifuged followed by addition of 1.0 mL Ellman's reagent [19.8 mg of 5, 5'-dithiobisnitrobenzoic acid in 100 mL of 1.0% sodium citrate and 3 mL of phosphate buffer (pH 8.0)]. The final developed product was measured at 412 nm. The results were expressed as nanomole GSH per milligram wet tissue.

### 2.7. Measurement of superoxide dismutase activity

The assay to determine superoxide dismutase (SOD) activity was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome (Misra and Fridovich, 1972). To 0.05 mL supernatant, 2.0 mL of carbonate buffer and 0.5 mL of EDTA solution were added. The reaction was started by the addition of 0.5 mL of epinephrine, and the auto-oxidation of adrenaline ( $3 \times 10^{-4}\text{ M}$ ) to adrenochrome at pH 10.2 was measured the optical density at 480 nm. The changes in optical density every minute were measured at 480 nm normalized to a blank reagent. The results are expressed as units of SOD activity (milligram per protein). One unit of SOD activity induced approximately 50% inhibition of adrenaline. The results were expressed as nmol SOD U per mg wet tissue.

### 2.8. Measurement of catalase activity

The catalase (CAT) activity assay was adapted from Beers and Sizer (1952). The reaction mixture consisted of 2 mL phosphate buffer (pH 7.0), 0.95 mL of hydrogen peroxide (0.019 M), and 0.05 mL

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