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ResearchArticle

Evaluation of Neuroprotective effect of medicinal plants in *Drosophila melanogaster* model

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Abstract

In this study, we investigated the neuroprotective effects of ethanolic extract of *Bombax ceiba* (EEBC) and ethanolic extract of *Gymnemasylvestre* (EEGS) against the toxicity induced by rotenone (ROT) in *Drosophila melanogaster*. Materials and Methods:Adult wild-type flies were concomitantly exposed to ROT (500 µM), EEBC (0.05% w/v and 0.1% w/v) and EEGS (0.05% w/v and 0.1% w/v) in the culture medium for 7 days. Results: ROT treated flies produced marked decreased in locomotor performance (i.e., climbing capability) in the negative geotaxis assay when compared to control group. EEBC and EEGS flies after treatment offered protection (24-42%) against the ROT-induced locomotor impairment in the negative geotaxis assay suggesting attenuation of ROT-induced locomotor deficits. Conclusion: The results of this study suggest that EEBC and EEGS were effective in reducing the toxicity induced by ROT in *D. melanogaster* as well as it confirms the significance of this model to explore possible therapeutic approaches in Parkinson's disease (PD).

Introduction

PD is a second most common neurodegenerative disease after Alzheimer's disease. It is characterized by loss of dopaminergic neurons in the substantianigra in the mid brain resulting in depletion of dopamine, formation of filamentous intraneuronal inclusions known as Lewy bodies on the surviving dopaminergic neurons and an extrapyramidal movement disorder[1,2]. However, free radical overproduction and abnormalities in mitochondria function have emerged as critical mediators of the dopaminergic neuronal damage[3].PD is clinically characterized by locomotor symptoms such as muscle rigidity, bradykinesia, postural instability and tremor [4].

ROT a commonly used natural pesticide is a classic high affinity specific inhibitor of mitochondrial complex I which is capable of causing mitochondrial perturbations, dose-dependent ATP depletion, oxidative damage, and early mortality that mimics PD. Exposure of adult flies to sub lethal doses of ROT in the diet over 7 days causes a concentrationdependentlocomotor deficits, specific dopaminergic neuronal loss and reduction in the dopamine levels in adult flies [5].

Bombax ceiba belongs to the family Bombaceae,is one of the important medicinal plant.It contains various phytoconstituents such as vitexin, isovitexin, chlorogenic acid, rutin, hentriacontanol, sitosterol, isomangiferin, protoatechuic acid and kaempferol etc. A paste of leaves and flowers is used as external application for skin trouble. Seeds are applied on the skin in small pox and chicken pox. Leaves are used as laxative and hematinic. Bark is used for wound healing.It has various pharmacological activities like Anti-inflammatory, Anti-diabetic, Anti-obesity, Cytotoxicity, Aphrodisiac, Hypotensive, Antioxidant, Antiangiogenic, Antimicrobial, and Antipyretic[6].

Gymnema sylvestre belongs to the family Asclepiadaceae, popularly known as "gurmar" is a reputed herb in the Ayurvedic system of medicine used for treatment in conditions ranging from diabetes,malaria,to snakebites [7].The leaves contain major constituents like gymnemic acids and gymnema saponins are members of oleanane type of saponins while gymnemasides are dammarane saponins[8,9]. Other constituents present in it are anthraquinones, hentriacontane, flavones, phytin, pentatriacontane, formic acid, butyric acid, tartaric acid, lupeol, resins,-amyrin related glycosides, calcium oxalate and stigmasterol[10].It is used as naturopathic treatment for diabetes[11,12]. It has also demonstrated promising effects in

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the treatment of obesity, arthritis, hyperlipidemia, and hypercholesterolemia [13-15]. Furthermore, the bioactive phytocompounds have antimicrobial, antiinflammatory, and anticancer properties. The leaves are used for the treatment of obesity [16], dental caries [17], antibiotic, in stomach ache, blood purifier, and in rheumatism [18].

In the present study,the possible neuroprotective properties of EEBC and EEGS against ROT-induced oxidative stress and neurotoxicity were explored. Flies chronically exposed to ROT (500μM) developed selective loss of dopaminergic neurons in the brain and severe locomotor dysfunctions [19]. Dietary exposure of adult flies to EEBC (0.05% w/v and 0.1% w/v) & EEGS (0.05% w/v and 0.1% w/v) along with ROT (500μM) for 7 days, thereafter its ability to modulate locomotor behaviour was determined by negative geotaxis assay.

Chemicals: Rotenone (Sigma Aldrich)

Gymnemasylvestre (Family:Asclepiadaceae) leaves powder was procured from Konark Herbal and Health Care, Daman, India.

Bombaxceiba (Family:Bombaceae)bark powder wasprocured from the Rajesh chemicals Mumbai.

D.melanogaster wild-type, Canton special strain was obtained from the Indian Institute of Science and Education Research, Pune, India.

The flies were grown in length 12 cm; diameter 2 cm vials containing standard medium (8.3%, w/v maize flour; 5%, w/v glucose; 2.5%, w/v sucrose; 1.5%, w/v agar; 2%, w/v yeast powder; 0.04%, v/w propionic acid; 0.6%, v/w orthophosphoric acid; 0.7%, v/w methyl paraben) at constant temperature and humidity (23°C±1°C; 60% relative humidity, respectively) under 12 h dark-light cycle. All experiments were performed with the same strain.

The powders were macerated for 8 hours using the solvent petroleum ether for defatting of the material. Crude extracts were obtained by placing 20-30 gm of powder in the soxhlet extractor using ethanol at 40°C. The crude extract that is EEBC and EEGS were further evaporated in the Rotavac evaporator and were dried to get the free flowing powder. The powders were stored in airtight container.

ROT treatedflies were exposed to low and high doses of EEBC & EEGS mixed in the culture medium. EEBC (0.05% w/v and 0.1% w/v) & EEGS (0.05% w/v and 0.1% w/v) were added in the medium at final concentrations of 5 mg/ml, 10 mg/ml. Vials of flies without test compound and ROT were used as control. Flies (male adult, 7–8 days old) were divided into six groups: (1) Control; (2) ROT (500 μM); (3) ROT plus EEBC (0.05% w/v); (4) ROT plus EEBC (0.1% w/v); (5) ROT plus EEGS (0.05% w/v) and (6) ROT plus EEGS (0.1% w/v). The total food medium contained a volume of 1% of ROT or ROT plus EEBC or EEGS. The flies were exposed to treatments during 7 days and the vials containing flies were maintained.

The motor function was assessed using a negative geotaxis assay. During their light cycle, 15 flies were transferred into a graduated flat bottom glass tube (length 12 cm; diameter 2 cm) and allowed to habituate for at least 5 min. The tube were gently tapped at the bottom and observed for 60 sec. for the climbing activity (15 flies/trial; 3 trials were done). Locomotor behaviour was expressed as percent flies escaping beyond a minimum distance of 10 cm in 60 sec[20].

All the data were expressed as mean ± standard error of the mean (SEM). Statistical significance was tested using oneway ANOVA followed by Tukey's multiple comparisons test using Prism Graph Pad version 7.0 (Graph Pad software, Inc., CA). *$p < 0.05$ was considered statistically significant.

The results of this study revealed that response of the PD induced flies were significantly lower than that of control flies. Measurement of the locomotor deficits among flies of ROT treated exhibited severe locomotor impairments as evident by the large number of flies (97.73%) staying at the bottom of the glass vial. The flies co-treated with EEBC (0.05% w/v & 0.1% w/v)& EEGS (0.05% w/v & 0.1% w/v) significantly ($p< 0.05$) improved the locomotor activity when compared with ROT treated flies (Table 1 & Figure 1). The average number of flies staying at the bottom of vial was decreased to 66.63% when co-treated with EEBC & EEGS. The flies showed negative geotaxis behaviour treated with EEBC & EEGS dose dependently; indicating its neuroprotective effect.

Table1. Effect of EEBC & EEGS on locomotor activity in *Drosophila melanogaster*

Groups	No. of flies escaped beyond 10 cm distance	% protection
Vehicle Control	11.67±0.88	77.80
Disease Control	0.33±0.33	2.27
EEBC(0.05% w/v)	3.67±0.67*	24.47
EEBC(0.1% w/v)	6.34±0.33*	42.27
EEGS(0.05% w/v)	3.67±0.67*	24.47
EEGS(0.1% w/v)	6.34±0.33*	42.27

Values are expressed as mean ± SEM; n=15, where n is the number of flies per group. *indicates there is significant difference with $p< 0.05$ when compared with disease control group by One-way ANOVA followed by Tukey's multiple comparisons test.

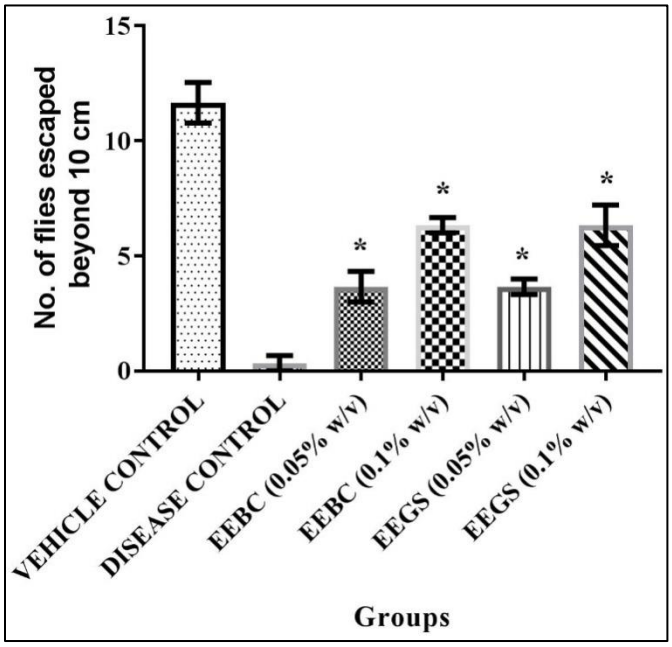

Fig. 1: Effect of EEBC & EEGS on locomotor activity in *Drosophila melanogaster* by negative geotaxis assay

In Drosophila model, ROT is commonly used for PD model. It has high affinity specific inhibitor of mitochondrial (complex I) NADH dehydrogenase. Being highly lipophilic, it crosses the blood brain barrier rapidly and accumulates in subcellular organelles like mitochondria [21]; where oxidative damage leads to neurotoxicity of dopaminergic neurons and locomotor deficits [22,23]. In ROT (500 μM) induced significant locomotor impairment in flies as indicated by large number of flies remaining at bottom of vials in disease control group. The EEBC & EEGS at doses of 0.05% w/v and 0.1% w/v showed protective effect against ROT induced locomotor impairment exhibited significant improvement in locomotor activity, thus could be proved with possible action on central nervous system indicates that EETP has an ability of free radical scavenging properties helps in neuroprotection of dopaminergic neurons in brain.

In summary, this study demonstrated that EEBC & EEGS improves locomotor performance and reduce the toxicity in ROTinduced*D. melanogaster* fly PD model. This study confirms the utility of this model to investigate therapeutic strategies that may be promising in the treatment of neurodegenerative diseases and can be used for studies in higher rodent model.

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