Neuroprotective activity of phytochemical combination of Quercetin and Curcumin against paraquat induced oxidative stress markers in *Drosophila melanogaster.*

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Neurodegenerative diseases are progressive disorders of the nervous system that affect specific cellular populations in the central and peripheral nervous systems. Recently, *Drosophila* has emerged as a model system for studying mechanisms of neurodegeneration in relation to major human neurodegenerative diseases. *Drosophila* is also an excellent *in vivo* system for testing of the therapeutic compounds (Lu and Vagel, 2009). The process of neurodegeneration begins at the cellular level in which two main processes, oxidative stress and excitotoxicity, act relentlessly to inflict the majority of cell damage and death. Although many factors can play a direct role in the initiation of neurodegeneration, the main factors which interact at the cellular level are free radicals formed by the reactive oxygen species and reactive nitrogen species. There is evidence to show that oxidative stress contributes to tissue injury following irradiation and hyperoxia and are suspected to be the causative factors in triggering neurodegenerative diseases. Strategies aimed at limiting free radical production oxidative stress and damage may slow the progression of neurodegenerative diseases (Ravindra *et al.,* 2004).

Paraquat (1,1-dimethyl-4-4-bipyridynium dichloride), a quaternary nitrogen herbicide, a highly toxic substance for humans and animals, is used to induce oxidative stress in flies (Sittipunt, 2005). The toxicity of paraquat is a consequence of generation of superoxide anion, which can lead to the synthesis of more toxic reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxide (Suntres, 2002). Quercetin (3,3',4',5,7-pentahydroxy flavone), the most abundant dietary flavonol, is a potent antioxidant, because it has the right structural features for free radical scavenging activity (Lamson, 2000). On the other hand, Curcumin, commonly called diferuloyl methane, a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa* exhibits antioxidant activities along with anti-inflammatory, antimicrobial, and anticarcinogenic activities. Sreejayan and Rao (1996) have reported that the 4-hydroxyphenyl group confers potent antioxidant activity, which is much enhanced by one, or two, methoxy susbstituents ortho to the hydroxy group. The antioxidant potential of curcumin complexes has been investigated by several approaches. The manganese complexes of curcumin and its diacetyl derivative were found to show greater superoxide dismutase (SOD) activity (Suzuki *et al.,* 2005), hydroxyl radical scavenging activity (Sumanont *et al.,* 2004), and nitric oxide radical-scavenging activity (Barik *et al.,* 2005) than the parent molecules. The copper complex of curcumin also has been found to exhibit antioxidant, superoxide-scavenging and SOD enzyme-mimicking activities superior to those of curcumin itself (Tapiwanashe *et al.,* 2006). With this backdrop, we employed *D. melanogaster* to test the neuroprotective efficacy of phytochemical combination of Quercetin and Curcumin (PC) against paraquat induced oxidative stress. In this study we used malondialdehyde hydroperoxide (HP), reduced glutathione (GSH) as oxidative stress markers and antioxidant markers such as superoxide dismutase (SOD) and catalase (CAT).

Based on the LC_{50} values, the adult flies were fed with PC with a sublethal concentration of 250 µg/ml in 0.5% DMSO for 7 days. The homogenate of these flies was used for studying the effect of PC on levels of oxidative stress markers and antioxidants in the oxidative stress induced flies treated with 15 mM paraquat. The PC fed flies showed significant diminution in the levels of oxidative stress markers like malondialdehyde hydroperoxide, reduced glutathione with respect to control flies, and modulatory effect was observed in the levels of Superoxide dismutase and Catalase that offer protection against paraquat induced oxidative stress in flies.

In the current study, paraquat caused high mortality (45–50%) among flies during exposure period. But the low incidences of mortality among test flies clearly indicate the protective nature of PC combination. The results obtained in "paraquat resistance assay" suggest that PC prophylaxis has the propensity to protect against neurotoxicant exposure largely due to its antioxidative potential. Further, paraquat induced neurotoxicity could be evidenced by high rate of locomotor deficits as measured in the negative geotaxis assay. Synergistic activity of PC has been shown with significant changes in the oxidative stress makers compared to curcumin alone. Based on biochemical evidences, we propose that dietary feeding of PC to *Drosophila* for a short duration has the propensity to attenuate paraquat induced oxidative stress owing to its antioxidative nature and its ability to modulate the activities of antioxidant defenses such as reduced GSH. Further work is essential to know the exact mechanism of action.

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Spiroplasma in natural populations of *Drosophila melanogaster* from Ukraine.

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Abstract

We have identified endosymbiotic bacteria of the genus *Spiroplasma* in wild populations of *Drosophila melanogaster* from Ukraine. These bacteria were identified from two different locations, and this is the first finding of the bacteria in Ukraine. Flies from populations infected with *Spiroplasma* spp. did not show any signs of male killing. The results are discussed in relation to previous findings concerning spatial distribution and male-killing behavior of *Spiroplasma*. Keywords: *Spiroplasma*, *Wolbachia*, *Drosophila melanogaster*, endosymbiotic, male killing, Ukraine.