L-DOPA AND FERROUS IRON INCREASE DNA STRAND-BREAKS IN THE DESERT LOCUST *SCHISTOCERCA GREGARIA* (ORTHOPTERA: ACRIDADE)

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ABSTRACT

Interahemocoelic injection of L-DOPA and Fe^{2+} ($FeSO_4$) into the 5th instar *Schitocerca gregaria* caused oxidative damage to DNA in the form of strand breaks. DNA strand breaks were measured by the comet assay (single-cell gel electrophoresis) as tail moment values (tail length ×% DNA in tail). Post injection of L-DOPA and Fe^{2+} ($FeSO_4$), the values of the tail moment and % severed cells were multiplied several times in both thoracic muscles and midgut cells. The obtained data were discussed emphasizing autoxidation and redox-cycling properties of L-DOPA and its pro-oxidant and antioxidant behaviors, as well as participation of the redox-active metal iron in production of reactive oxygen species.

Keywords: Oxidative stress; DNA damage; L-DOPA; Fe^{2+}; *Schistocerca*.

INTRODUCTION

Oxidative stress is generated in aerobic living organisms as a result of formed reactive oxygen species (ROS). These species are produced continuously during reactions of, for example, the mitochondrial electron transpooort system, NADPH oxidase, xanthine oxidase, monoamine oxidase, cyclooxygenase, lipooxygenase, cytochrome P450 oxidase. The formed superoxide anion radical ($O^{\bullet}_2^-$) can spawn other ROS, the non-radical hydrogen peroxide (H_2O_2) and the hydroxyl radical •OH (Gilbert, 2000; Pardini, 1995; Evans and Halliwell, 2001; Fang et al., 2002; Halliwell and Whiteman, 2004; Korsloot et al., 2004).

At the normal physiological condition ROS can be produced at pictogram levels (Pardini, 1995; Fang et al., 2002; Korsloot et al., 2004). Under these physiological conditions, ROS production is balanced by antioxidant systems (Felton, 1995; Felton and Summers, 1995; Malanga et al., 1997; Aguilera et al., 2002). However, under stressful conditions of exogenous and pathological niche, ROS are produced at higher levels (Pardini, 1995; Fang et al., 2002; Korsloot et al., 2004; Birben et al., 2012). When a state of homeostatic imbalance between prooxidants and antioxidants systems (in favor of oxidants), oxidative stress and subsequently oxidative damage to the cell macromolecules DNA, proteins, and lipids will take place (Kohen and Nyska, 2002; Birben et al., 2012).

In herbivorous insects, ingestion of food with oxidizable allelochemicals such as phenolics can exacerbate the oxidative stress (Ahmad, 1992; Felton and Summers, 1995; Paradini, 1995). Heavy metals also can induce production of ROS via a Fenton-reaction mechanism (Miller et al., 1990). Iron ions are an important component of free radical biological oxidation; this may involve a Fenton-type and an iron-catalyzed Haber-Weiss reaction to produce •OH. Also, iron may participate via another mechanism including other species known as iron-dioxygen complexes such as perferryl and ferryl ions (Qian and Buettner, 1999; Schafer et al., 2000).

Therefore, the present work was conducted to determine the possible oxidative damage to DNA, as DNA-strand breaks in the 5th instar *Schistocerca geregaria* post-intrahemocoelic injection with the catechol derivative L-DOPA, a redox-cycling chemical, and with Fe^{2+} ($FeSO_4$) a redox active metal. This may help exploring an increased vulnerability of this agronomic pest to some control measure under generated oxidative stress conditions.

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MATERIALS AND METHODS

Insects

Desert locust, *S. gregaria* (Forskål), was from a well-established laboratory colony at the Entomology Department, Faculty of Science, Cairo University, Egypt. The insects were reared in wooden cages (60cm ×60cm ×40cm) at 30±2˚C, 60±5 RH, and 16:8 h (L:D) photoperiod. Locusts were fed on fresh alfalfa, *Medicago sativa* (Papillioacea). Mature females deposited their eggs in pots filled with slightly moistened, sterile sand. 5th instar individuals 5-7-day old were used in all experiments. Detailed description of the colony and rearing methodologies are described by Hinks and Erlandson (1994).

DNA damage assay

The alkaline single-cell gel electrophoresis (comet assay) was used to assess the DNA strand breaks and carried out according to Kirilova et al., (2005), with minor modifications. Midgut and muscle tissues collected from nymphs 24 h P.I. were teased with a teasing needle in PBS buffer 1 X (8 g NaCl, 0.02 g KCl, 0.144 g Na_2HPO_4, 0.024 g KH_2PO_4 in 100 ml of distilled water, pH 7.4 adjusted by addition of 2 M HCl). After teasing for 30 s, cell homogenates were suspended in 110 µl of 1% molten low melting-point agarose a (65˚C); and put on a microscopic slide which previously covered with a layer of 0.8% regular melting-point agarose. The agarose was gelled at 4 °C and the slide was then immersed for 24 hours at 4 °C in a fresh lysis solution (164 g NaCl, 37 g of EDTA, 1 g TrisBase merged into 890 ml of distilled water, stirred before adding 8 g of NaOH; pH 10. Freshly prepared 1% TritonX-100 and 10% dimethyl sulfoxide were added). After lysis, the slides were washed two times with distilled water, and immersed for 5 min at 4°C in a freshly prepared alkaline electrophoresis buffer (30 ml of 10 N NaOH, 0.5 ml of 200 mM EDTA into 1000 ml of distilled water; pH adjusted to 13.0 with 2 M HCl). An electric field was then applied at 20V for 20 min before immersed in neutralization buffer for 15 min (TrisBase; pH adjusted to 7.5 with 2 M HCl). Slides were drained, exposed to cold absolute ethanol for 5 min, and stored under dry conditions and then, stained with 40µl of ethidium bromide solution. Analysis of DNA damage was assessed using OPTIKA B-350 fluorescent microscope with magnification of 20×20 Zoom (OPTIKA, Ponteranica, Italy) which is linked to a CCD camera to measure the length of DNA migration (tail length) and the % DNA content in the tails. The extent of DNA migration was determined by using an image analysis system (Comet Score). The parameters tail length, % DNA in the tail, tail moment, as well as % of severed cells, were recorded. For each treatment, three slides, and 50 cells per slide, were analyzed.

Statistical analysis

The differences among the treated groups and naïve control was compared by independent-*t*-test. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp.). Data were expressed as mean±SE.

RESULTS AND DISCUSSION

ROS can cause DNA oxidative damage in several forms involving strand-breaks, degradation of bases, modification of bases and sugars, deletions and/or translocation, and cross-linking with proteins (Cakmakoglu et al., 2011; Birben et al., 2012).

In the present work, oxidative damage to DNA was measured as DNA strand-breaks (expressed as tail moment and number of severed cells) post intrahemocoelic injection of the stressors L-DOPA and Fe^{2+} ($FeSO_4$) into the 5th instar *S. gregaria*. The values of tail moment, as an arbitrary expression for the quantitative estimation of DNA strand-breaks (Tice et al., 2000) were used. Also, we considered the percentage severed cells as per the recommendations of Mouron et al. (2001) and Bilbao et al. (2002). This is a feasible supplementary criterion obtained in the presented data (Fig 2d).

The results show that the tail moment values increased up to 8.5 and 10-fold that of control after 24 h P.I. (a

representative test-time) of $FeSO_4$ in thoracic muscles and midgut cells, respectively (Fig. 1, 2). Whereas, after L-DOPA treatment, the fold was increased up to 10.8 and 12.5 in thoracic muscles and midgut cells, respectively. With respect to the severed cell, the % values of these cells were multiplied by 5.0 and 7.0-fold in $FeSO_4$-treated samples and by 3.6 and 5.5 fold in L-DOPA treated ones, for thoracic muscles and midgut, respectively, in comparison to control (Fig. 2d). The determined values of DNA strand-breaks are considered as an indirect measurement to ROS and oxidative stress magnitude as reported by Halliwell and Whitman (2004). The used stressors represent a redox-cycling catechol derivative and a redox-active metal, respectively. Since L-DOPA and iron metabolism are associated with formation of ROS that generate oxidative stress (Qian and Buettner, 1999; Schafer et al., 2000), the occurring DNA strand-breaks in *S. gregaria* seems to be due to this generated stress.

The obtained results on DNA oxidative damage (Fig. 1, 2) imply that each separate stressor is able to generate oxidative damaging stress, seemingly, as noted above, to be ROS resulting from metabolism of the injected redox-cycling L-DOPA and the redox-active metal iron. In this respect, incubation of PM2 plasmid DNA with ADP-Fe^{+3} or L-DOPA alone did not induce DNA damage, but needs to be in couple with each other to induce damage (Miura et al., 2000). This discrepancy and others were argued by Patterson et al. (2002) interpreting data obtained in this respect (Spencer et al., 1994; Husain and Hadi, 1995; Miura et al., 2000) on damage mechanisms and their conditions modulating the effects of L-DOPA and iron ions.

L-DOPA has both a prooxidant and an antioxidant behaviors according to the reaction conditions (Cadenas et al., 1989; Pardini, 1995; Riley, 1997). The resulting oxidative damage in the tested tissues of *S. gregaria* by the injected L-DOPA alone indicates that its prooxidant-behavior prevails under the used conditions. In this case, L-DOPA can undergo autooxidation (Riley, 1997), a process enhanced in presence of trace concentrations of Fe^{3+} (Patterson et al., 2002) which are found, probably attached to organic molecules as it is the case in mammalian tissues (Bakkeren et al., 1985; Weaver and Pollack, 1989). In L-DOPA redox-cycling, one-electron transfer produces L-DOPA-$SQ^{\cdot-}$ that can act by itself as an oxidative radical, or can undergo autoxidation reaction leading to production of $O^{\cdot}{}_2^{-}$ and subsequently other ROS (Eqn. 1-5) which can cause oxidative damage (Felix and Sealy, 1981; Riley, 1997; Bolton et al., 2000; Patterson et al., 2002).

$$\mathbf{DOPA} + \mathbf{Fe^{3+}} \leftrightarrow \mathbf{DOPA - SQ^{\cdot -}} + \mathbf{Fe^{2+}} \quad \mathbf{1}$$

$$\mathbf{DOPA - SQ^{\cdot -}} + \mathbf{O_2} \leftrightarrow \mathbf{DOPA - Q} + \mathbf{O_2^{\cdot -}} \quad \mathbf{2}$$

$$\mathbf{O_2^{\cdot -}} + \mathbf{HO_2^{\cdot}} \rightarrow \mathbf{OH^{\cdot}} + \mathbf{OH^{-}} \quad \mathbf{3}$$

$$\mathbf{O_2^{\cdot -}} + \mathbf{Fe^{3+}} \rightarrow \mathbf{Fe^{2+}} + \mathbf{O_2} \quad \mathbf{4}$$

$$\mathbf{H_2O_2} + \mathbf{Fe^{2+}} \rightarrow \mathbf{Fe^{3+}} + \mathbf{OH^{\cdot}} + \mathbf{OH^{-}} \quad \mathbf{5}$$

For the second stressor, injection of Fe^{2+} into *S. gregaria* caused DNA strand breaks. This damage seems to be caused by generated ROS; iron participation in this mechanism involves Fenton reaction (Eqn. 5) and iron-catalyzed Haber-Weiss reaction (Miller et al., 1990).

Haber-Weiss reaction: $\mathbf{O_2^{\cdot -}} + \mathbf{H_2O_2} \xrightarrow{\textbf{iron}} \mathbf{O_2} + \mathbf{OH^{\cdot}} + \mathbf{OH^{-}}$

Also, another oxidative mechanism was proposed (Qian and Buettner, 1999; Schafer et al., 2000). In this mechanism, iron-dioxygen complexes perferryl and ferryl irons are additional species that can initiate oxidation of the cell macromolecules (Bolton et al., 2000) including DNA.

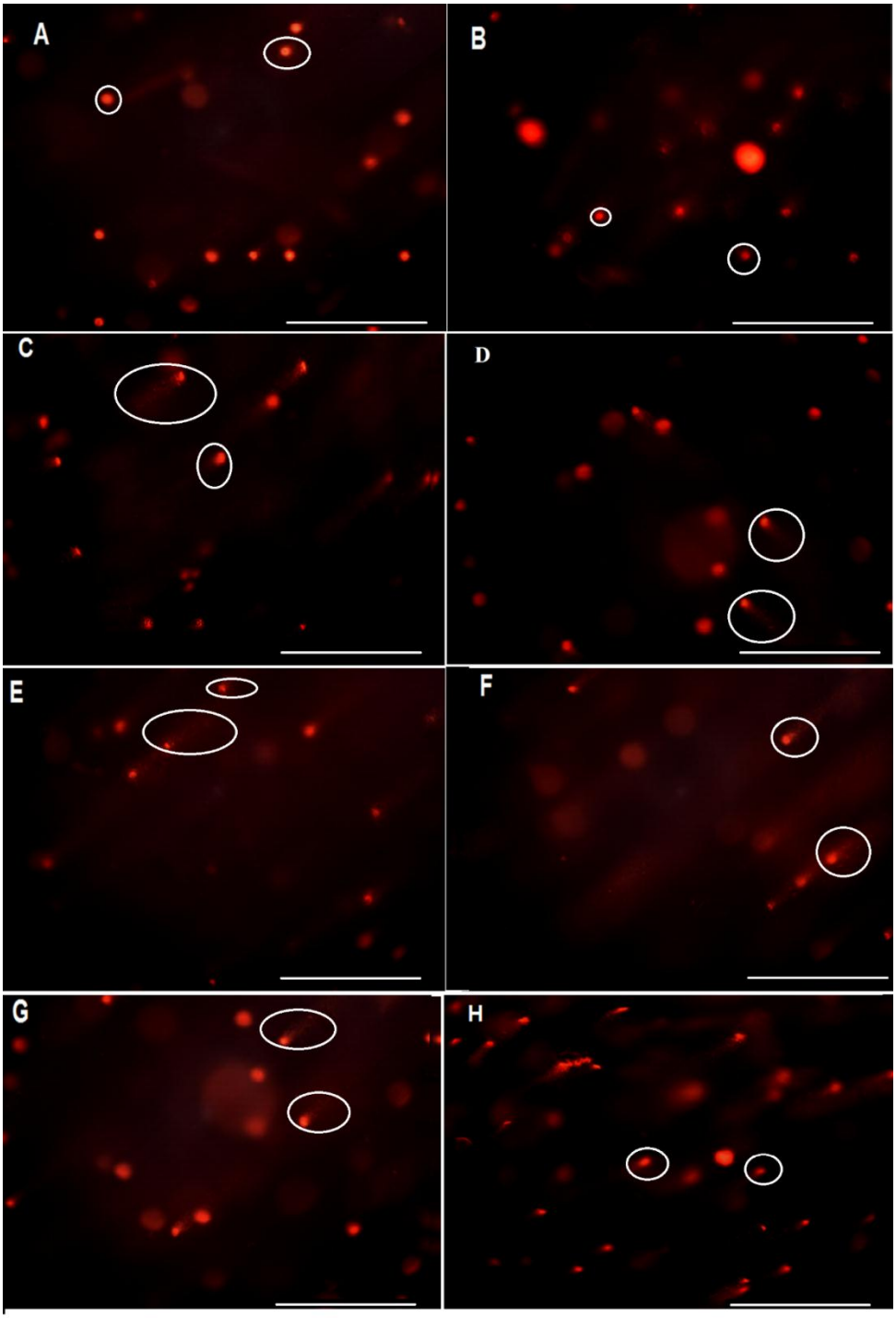

Fig. 1. A photomicrograph of DNA strand-breaks, as revealed by single-cell gel electrophoresis (the alkaline comet assay (pH≥13.0)), in thoracic muscles and whole midgut cells of 1-day starved 5th instar of *S. gregaria* assayed at 24 h P.I. (recommended time) with saline (A, B), 15 µL of 200 µM L-DOPA (C, D), 15µL of 30 µM $FeSO_4$ (E, F) per individual. 50 cells were analyzed per sample (50 cells per slide and 3 slides per treatment were assessed). Scale bar represented as 75 µm.

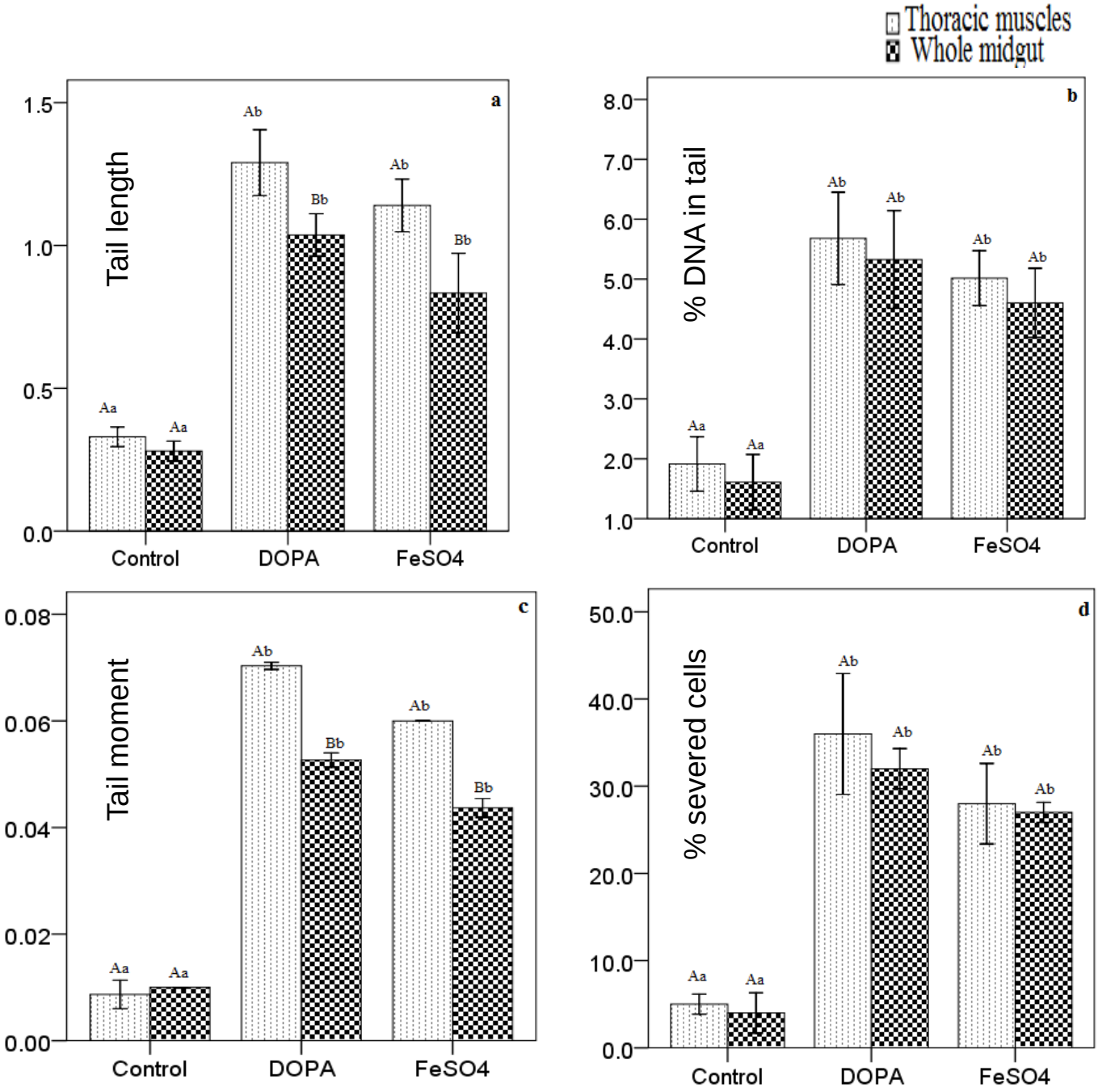

Fig. 4. Analysis of DNA strand-breaks using alkaline comet assay (pH≥13.0) in thoracic muscles and midgut cells of 1-day starved 5th instar *S. gregaria* injected with 15µL of 200µM L-DOPA or 15µL of 30µM $FeSO_4$ per individual and assayed at 24 h post injection (recommended time), (a) tail length (µm); (b) % DNA in tail; (c) tail moment; (d) % severed cell. Each replicate = 50 cells. Bars marked with different letters indicate statistical significance (p<0.05) between thoracic muscles and midgut cells (capital letters) and type of treatment (small letters) as per executed by independent *t*-test.

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