Protective Effect of Quercetin against Oxidative Stress and Mitochondrial Bioenergetic Deficiency Caused by Lambda- Cyhalothrin

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ABSTRACT

Mitochondria are a convenient model to understand the oxidative damage induced by various xenobiotic-prooxidants. This study was designed to investigate (1) the possibility of lambda-cyhalothrin (LCT), a type II pyrethroid, to induce oxidative stress response in rabbit liver mitochondria *in vitro* and its effect on selected parameters and (2) the role of quercetin in alleviating the cytotoxic effects of LCT. Mitochondria were divided into two groups. The first group, mitochondria were incubated for 30 min at 37°C with different concentrations (0, 5, 10, 15 and 20uM) of LCT. In the second group, mitochondria were pre-incubated with (10µM) quercetin for 30 min and followed by LCT incubation for 30 min at 37°C. Following *in vitro* exposure, LCT caused a significant induction of oxidative damage in mitochondria at all tested concentrations as evidenced by increased superoxide dismutase (SOD) activity and reduced glutathione (GSH) level. However, a significant decrease in the activities of NADH dehydrogenase and ATP synthase (ATPase) was obtained. While the quercetin treated mitochondria showed significant enhancement in all tested parameters except GSH content, there was no significant change. Quercetin showed a significant protection against the cytotoxic effects induced by LCT on the studied parameters. In conclusion, antioxidant quercetin could be able to ameliorate LCT-induced oxidative stress by altering antioxidant defense system and recovered the bioenergetic activity of mitochondria.

Keywords: ***Mitochondria, Lambda cyhalothrin, Qurcetin, Bioenergetic deficincey and oxidative stress.***

INTRODUCTION

Pesticides are occasionally used in large amounts causing environmental pollution and health problems. Synthetic pyrethroids now account for more than 30% of insecticide due to their high efficacy, easy biodegradability, and low toxicity to birds and mammals. Lambda cyhalothrin (LCT) is a type II synthetic pyrethroid insecticide is widely used in Egypt for cotton, cereals and vegetables as well as in public health application against insect, ticks and flies (Abdel Aziz and Abdel Rahem, 2010). Residues of LCT have been reported in vegetables and fruits, milk and blood of dairy cows, and also in cattle meat (Muhammad *et al*., 2010; Turgut *et al*., 2011). Placental transfer of LCT has been observed in goats (Oliveira *et al*., 2000). Consistent with its lipophilic nature, LCT has been found to accumulate in biological membranes leading to oxidative damage (Michelangeli *et al.,* 1990; El-Demerdash, 2007; Fetoui *et al*., 2008 and 2010). However, in many cases the metabolism of xenobiotic substances can give rise to toxic metabolites or to reactive oxygen species (ROS) that can harm the cell further. Alterations in biochemical systems are often more sensitive indicators than those at higher levels of biological organization. Indeed, changes at the molecular level will underlie the effects at higher levels of organization. Pesticides are known to induce oxidative stress by inducing ROS production as byproducts of detoxifying metabolism, alternating the mitochondrial respiration or by their own redox (reduction/oxidation) cycling properties (Tebourbi *et al*., 2011).

Mitochondria are responsible for converting the energy released by electron transport and stored as the binding energy molecule ATP. NADH dehydrogenase, is the most complicated redox enzyme in mitochondria, it couples electron transport to proton translocation across the inner mitochondrial membrane. Consequently, in the process of oxidative phosphorylation, electrons generated from the oxidation of fuel molecules by oxygen are passed along a transport chain leading to the generation of ATP from ADP and inorganic phosphate through the master enzyme, ATP synthase (Hatefi 1985).

Dysfunction of mitochondrial energy metabolism leads to reduce ATP production, and generation of ROS such as superoxide anions, hydroxyl radicals and hydrogen peroxide (Cassarino and Bennett, 1999). ROS are increasingly recognized as playing an important role in many diseases because of their ability to cause oxidative stress and consequently damage cellular contents. Mitochondria constitute the major source of superoxide (O_2 $\cdot^-$) and other ROS within cells, generating approximately 85% of total cellular (O_2 $\cdot^-$), via aberrant (O_2) reactions (Droge, 2002). Mammalian mitochondria possess a multi-leveled ROS defense network of enzymes and nonenzymatic antioxidants. Manganese containing superoxide dismutase (MnSOD) protect cells from (O_2 $\cdot^-$) attack by facilitating its dismutation into H_2O_2. Glutathione is considered the

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major antioxdiant molecule inside the cell. Approximately ~90% of glutathione is in its reduced form, GSH (γ-glutamylcysteinylglycine). GSH serves as a scavenger of hydroxyl radical thereby preventing free radical chain reaction (Ji, 1999) .Toxicity by oxygen radicals has been suggested as a major cause of cancer, aging, heart disease and cellular injury in hepatic and extrahepatic organs (Gram *et al.,* 1986). Studies describing the oxidative stress mechanisms in pyrethroids-induced toxicity in mammalian mitochondria are limited. Few recent reports (Lim *et al.*, 2009; Castanha Zanoli *et al.,* 2012; Abdel-Razik and Hamed 2015) have demonstrated the induction of oxidative stress by other pesticides and its effect on mitochondria.

Plant-derived products are playing an essential role in the primary health care of about 80–85% of the world's population. Quercetin (3, 5, 7, 3′, 4′-pentahydroxyflavanone) is a plant-derived flavonoid present in foods (onions, citrus fruits, vegetables and grains) and beverages (tea and red wine) (Ikizler *et al.,* 2007), inhibits oxidative stress induced by organophosphorous pesticides in experimental animals (Kalender *et al.,* 2012). This flavonoid exhibits a wide range of biological activities including anticarcinogenic, anti-inflammatory, antiviral and antibacterial activities (Adewole *et al.,* 2007). Many researchers have studied the *in vitro* cytotoxicity of different insecticides (Pardini *et al*., 1980; Pereira *et al*., 2009; Castanha Zanoli *et al*., 2012), while there is no enough data on the cytotoxic effects of lambda-cyhalothrin on mitochondria, therefore the present study aimed to investigate the *In vitro* effects of Lambda-cyhalothrin on the bioenergetics and the oxidative status of mitochondria isolated from rabbit liver.

MATERIALS AND METHODS

1- Chemicals

Lambda-cyhalothrin technical grade (98 %) was obtained from Agromen Chemicals Co., Ltd, while Quercetin, 98 % were obtained from Sigma Chemical Co. All other chemicals used in this study were of the highest purified grades purchased from Simga-Aldrich and Merck Chemical Companies.

2 - Animals and care

Male New Zealand White rabbits (age of seven months and initial weight of 2.873 ± 0.0617 kg) were used. Animals were individually housed in stainless steel cages at room temperature (25 ± 2^{o}C) with a relative humidity of 50–60% and on a 12 h light–darkness cycle. The animals had free access to commercial pellet diet and water *ad libitum*. All maintenance and care were in accordance with the animal welfare guidelines established at the university.

3- Preparation of mitochondria

Rabbit liver mitochondria were isolated using a slightly modified protocol of Krause *et al*., (2005). The liver tissues were minced by a scalpel before disruption of the cells by a loose-fit glass teflon homogenizer with 4–5 strokes in 10 volumes of homogenization buffer (250 mM sucrose, 50 mM Tris–HCl, 5 mM EDTA, and 0.5 mM phenylmethanesulphonyl fluoride (PMSF), pH 7.4). The homogenates were centrifuged at 800g (10 min, 4^{o}C). The supernatants were layered on top of 350 mM sucrose, 50 mM Tris–HCl, 5 mM EDTA, and 0.5 mM PMSF, pH 7.4, and centrifuged at 7000g (10 min, 4^{o}C). The mitochondria (top layer) were washed twice with homogenization buffer and resuspended in 250 mM sucrose and 0.5 mM PMSF. Isolated mitochondria was stored at -20^{o}C.

4- Estimation of protein

The protein content of the mitochondrial preparations was estimated by a method of Lowry *et al* (1951).

5 - Treatment of mitochondria

Mitochondria were either incubated for 30 min at 37°C with different concentrations of LCT (0, 5, 10, 15, 20 and 25 uM), or pretreated with 10 uM quercetin for 30 min and followed by incubation with the same concentrations of LCT for 30 min at 37°C. Controls contained either mitochondria only or quercetin.

6- Biochemical analysis

6.1 NADH deydrogenase activity

NADH deydrogenase activity was determind by the method of Galante and Hatefi (1978). Liver mitochondria (30 µg protein) were mixed with a mixture containing 40 mM phosphate buffer, pH 7.4, 0.1% sod-cholate, 1.5 mM potassium cyanide and 1.3 mM potassium ferricyanide and incubated for 1 min at 30 °C then 0.14 mM NADH was added. The decrease in absorption was followed spectrophotometercally at 340 nm for 1-3 minutes. Specific activity was expressed as nmol NADH oxidized / min / mg protein.

6.2 ATP synthase (ATPase) activity

The basic idea of this method is to measure the amount of inorganic phosphate produced from the hydrolytic reaction of ATP by the ATPase. Mitochondria (1 mg protein/ml) were added to a medium containing, 20 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$ and 5 mM ATP. Then the mixture was incubated for 5 min. at 37°C in a shaking water bath. The reaction was stopped by the addition of 5% trichloroacetic acid (TCA), and the inorganic phosphate (Pi) was determined

colorimetrically as described by Taussky and Shorr (1953). The intensity of the color was measured spectrophotometrically at 740 nm. The concentration of Pi was calculated graphically from a standard curve and the specific activity was computed as μmole Pi/mg protein/min.

6.3 Superoxide dismutase (SOD) activity

SOD as enzymatic antioxidant was measured spectrophotometrically at 25 °C by the method of Marklund and Marklund (1974), with some modifications. The assay medium in a total volume of 1.0 ml containing 50 mM Tris – HCl buffer (pH 8.0) and 0.24 mM pyrogallol. Autoxidation of pyrogallol was monitored at 420 nm for 3 min in the absence and presence of the enzyme. At least three concentrations of the enzyme which produced between 30 to 60 % inhibition of pyrogallol autoxidation were used. One unit of the enzyme activity is defined as the amount which produced 50% inhibition of pyrogallol autoxidation under the standard assay conditions. Mitochondrial SOD activities were expressed as Units/ mg protein.

6.4 Reduced glutathione (GSH) content

GSH content as a nonenzymatic antioxidant was determined according to the method of Beutler *et al.* (1963). The method based on the reduction of 5, 5' dithiobis (2- nitrobenzoic acid) (DTNB) with glutathione to produce a yellow complex measured at 405 nm. The reduced chromogen is directly proportional to GSH concentration. The amount of GSH was expressed as μmole glutathione oxidized/mg protein.

Statistical analysis

Data obtained from the experiments were expressed as mean ± standard deviation (SD). Significant differences of measurement traits were analyzed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls Test. The criterion for statistical significance was set at $p<0.05$. These test were performed using a computer software CoStat program.

RESULTS and DISCUSSION

1- Bioenergetics parameters

Mitochondria are responsible for converting the energy released by electron transport and stored as the binding energy molecule ATP through the oxidative phosphorylation (Hatefi 1985). Xenobiotics that interfere with its synthesis or utilization can be acutely or chronically toxic. Mitochondria carry out a variety of biochemical processes, but their main function is to produce a majority (>90%) of cellular ATP. Pesticides have been reported as able to interfere with mitochondrial function, either by inhibiting the respiratory chain or by uncoupling oxidative phosphorylation, and alter the mitochondrial bioenergetics which involved in many diseases (Gomez *et al.,* 2007).

1.1 NADH deydrogenase (Complex I) activity

The electron transport chain (ETC) of the mitochondria is the means by which electrons are removed from the reduced carrier NADH and transferred to oxygen to yield H_2O, begins with NADH deydrogenase. Complex I catalyze the first step of oxidative phosphorylation and, hence, it is the key for the efficient ATP production.

As shown in (Fig .1), liver mitochondria treated with LCT at different concentrations (5-20μM) exhibited significant reduction ($p < 0.05$) in complex I activity in an inversely manner. Mitochondria pretreated with (10μM) quercetin reduced that inhibition and almost keep the enzyme activity to the control value, while quercetin treatment alone induced significant activation ($p < 0.05$). In agreement with the present results, Rei-ming and Yu-gu (1998) reported that Isophenphos-I markedly inhibited complex I activity in rat liver mitochondria. Sherer *et al. (*2007) examined the *in vitro* toxicity and mechanism of action of several putative complex I inhibitors that are commonly used as pesticides. So, the protective effect of quercetin was observed against the toxicated effect of LCT.

Fato *et al.* (2009) investigated the production of ROS by complex I in isolated open bovine heart submitochondrial membrane fragments during forward electron transfer in presence of NADH. ROS production by complex I is strictly related to its inhibited state. So the inhibition in complex I activity in our results may resulting in decreased oxygen consumption and attributed to increase ROS which alterate the oxidative status of the mitochondria. Complex I is inhibited by more than 60 different families of compounds starting from Rotenone, the prototype of this series, to a number of synthetic insecticides/acaricides (Degli, 1998).

1.2 ATP synthase (ATPase) activity

Results represented in (Fig.2) revealed that, ATPase activity was significantly ($P < 0.05$) decreased in the LCT treated mitochondria in all tested concentrations in a concentration dependant manner (inversely proportion), while the quercetin alone significantly increased the activity. The pretreated mitochondria with quercetin relatively preserved the activity of the enzyme. The same results were obtained with other pesticides (Desaiah and Mehendale 1977; Ruder *et al.,* 1991; Dugravot *et al.,* 2003; Castanha Zanoli *et al.,* 2012).

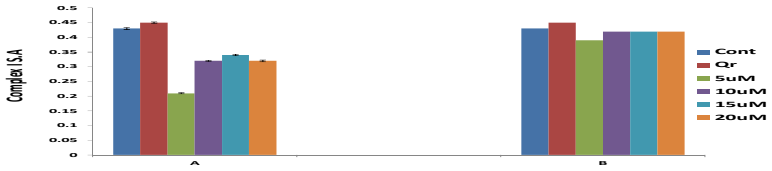

Fig. 1. Change in Complex I specific activity (S.A) of rabbit liver mitochondria treated with lambda cyhalothrin (LCT) at different concentrations (A) and the pretreated mitochondria with 10uM qurcetin (Qr) followed by LCT (B). The values are expressed as means ± SE. n-Values = 5

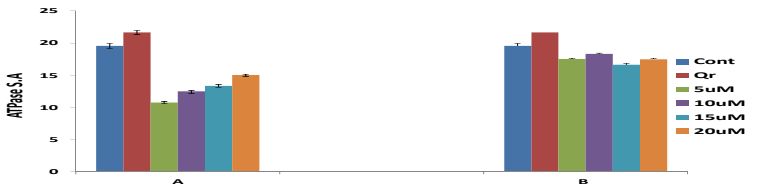

Fig. 2. Change in ATP ase specific activity (S.A) of rabbit liver mitochondria treated with lambda cyhalothrin (LCT) at different concentrations (A) and the pretreated mitochondria with 10uM qurcetin (Qr) followed by LCT (B). The values are expressed as means ± SE. n-Values = 5

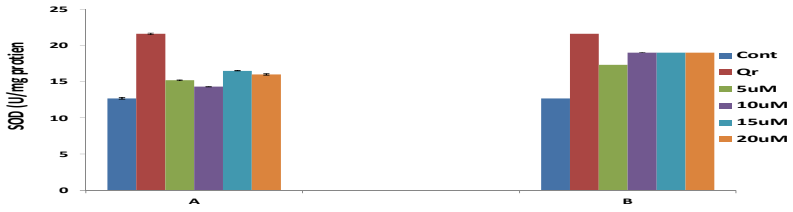

Fig. 3. Change in SOD activity of rabbit liver mitochondria treated with lambda cyhalothrin (LCT) at different concentrations (A) and the pretreated mitochondria with 10uM qurcetin (Qr) followed by LCT (B). The values are expressed as means ± SE. n-Values = 5

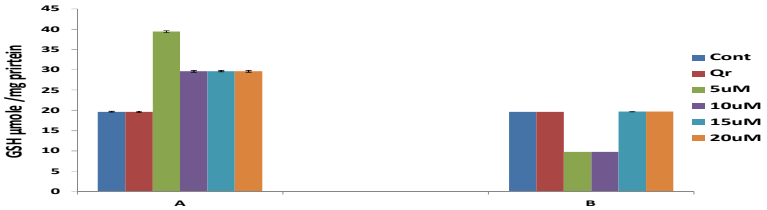

Fig 4. Change in GSH content of rabbit liver mitochondria treated with lambda cyhalothrin (LCT) at different concentrations (A) and the pretreated mitochondria with 10uM qurcetin (Qr) followed by LCT (B). The values are expressed as means ± SE. n-Values = 5

2- Antioxidants Parameters

Mitochondria are the primary intracellular site of oxygen consumption and the major source of ROS, most of them originating from the ETC. In accordance with this, it has been estimated that the steady state concentration of superoxide in the mitochondrial matrix is 5-to10 fold higher than in the cytosol (Cadenas and Davies 2000). Associated with this constant flow of ROS generation, mitochondria are also a target for the damaging effects of oxygen radicals (Fernandez-Checa and Kaplowitz 2005; Kaelin 2005; Orrenius *et al.,* 2007). Although ROS generated under physiological conditions are not harmful, and likely play a signaling role, toxic or pathological conditions that lead to an impairment of mitochondrial function can increase the release of ROS. Mitochondria efficiently reduce free radicals under normal conditions predominantly through antioxidant mechanisms, including SOD as well as GSH (Wheeler *et al.,* 2001).

2.1 Superoxide dismutase (SOD) activity

Fig. (3) Show that SOD activity was significantly increased ($p < 0.05$) in the LCT treated mitochondria compared to control in concentration dependent manner and that activation was increased in the quercetin pretreated mitochondria.The antioxidant enzymes in mitochondria such as SOD which dismutate $O_2^{\cdot-}$, may counteract pyrethroid-induced oxidative stress. The increase in SOD activity in mitochondria after pyrethroid intoxication appears to be due to increased generation of ROS. These results in line with Lukaszewicz-Hussain and Moniuszko-Jakoniuk (2004) who indicated that chlorfenvinphos induces oxidative stress to rat liver mitochondria .The increased oxidative stress resulted in an increase in the activity of SOD (Kale *et al.,* 1999).

2.2 Reduced glutathione (GSH) content

GSH is a major mitochondrial antioxidant that protects mitochondrial DNA (mtDNA) against oxidative damage by reducing H_2O_2 via glutathione peroxidase (Mari *et al.,* 2013). The results revealed significant increase ($p < 0.05$) in GSH content in the LCT treated mitochondria, especially at the low concentration (5μM) and return to the normal value in the qurcetin pretreated mitochondria especially at 15 and 20 μM of LCT. Treatment with qurcetin alone did not affect the content of GSH (Fig. 4).The increase in reduced glutathione (GSH) content in mitochondria may probably be an initial adaptive response to the increased oxidative stress in pyrethroid intoxicated liver. GSH provide the –SH group for conjugation by glutathione-*S* transferase (GST), which detoxifies a variety of electrophilic compounds to less toxic forms. It is probable that the initial increase in mitochondrial GSH provide SH for GST activity to decrease pyrethroid toxicity (Kale *et al.* 1999).

CONCLUSION

Mitochondrial dysfunction is a fundamental pathogenic mechanism that leads to several significant toxicities in mammals, especially those associated with the liver (Szewczyk and Wojtczak, 2002; Amacher, 2005). To assess the potential involvement of mitochondria in LCT-related hepatotoxicity, we assessed its effects on the bioenergetics and the oxidative status of liver mitochondria, and the role of quercetin as a natural antioxidant to mitigate LCT injurious effect. The present study showed that LCT perturbs the mitochondrial bioenergetics and the antioxidants defence parameters, hence quercetin played a good role to protect the mitochondria. These effects constitute a potential mechanism for LCT toxicity in liver cells, which could contribute to the toxicological effects of LCT in animals and human.

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الملخص العربي

التأثير الوقائي للكيرسيتين ضد الاكسدة ونقص الطاقة البيولوجية فى الميتوكوندريا التي يسببها لامبادا سيهالوثرين

نادية على حامد

الميتوكوندريا هي نموذج ملائم لفهم الضرر التأكسدي والناتج عن المواد الغريبة بيولوجيا عن الجسم. وقد تم تصميم هذه الدراسة للتحقيق من (١) إمكانية لامبادا سيهالوثرين(LCT) وهو مبيد بيريثرويد من النوع الثاني، لتحفيز الاستجابة التاكسدية في ميتوكوندريا كبد الارنب، وتأثيره على بعض المقاييس الحيوية و(٢) دور كيرسيتين في التخفيف من الآثار السامة للخلايا نتيجة المعاملة بمبيد الـ LCT . وقد حضنت الميتوكوندريا أما لمدة ٣٠ دقيقة على ٣٧°م مع تركيزات مختلفة (٠، ٥، ١٠، ١٥ و٢٠ ميكرو مولر) منLCT، أو مع كيرسيتين(١٠ ميكرومولر) لمدة 30 دقيقة ثم التحضين مع الـ LCT لمدة ٣٠ دقيقة على ٣٧°م.

أوضحت النتائج أن LCT قد أحدث زيادة معنوية في الضرر التأكسدي للميتوكوندريا مع التركيزات المختلفة مع زيادة معنوية في نشاط انزيم السوبر اوكسيد ديسميوتيز(SOD) ومستوى الجلوتاثيون المختزل (GSH). من ناحية أخرى أحدث LCT انخفاضا معنويا في نشاط انزيم NADHديهيدروجينيزونشاط انزيم تخليق الـATP ، بينما، معاملة الميتوكوندريا بالكيرسيتين أدى الى تحفيز معنوى في كافة المعايير المختبرة باستثناء محتوى الـ GSHالذى لم يتغير معنويا. هذا وقد أظهر الكيرسيتين حماية كبيرة من الآثار السامة للخلايا الناجمة عن LCT على المعايير التي شملتها الدراسة.

ويمكن القول بان الكيرسيتين له القدرة على تحسين ضغط الاكسدة التي يسببها LCT عن طريق تغيير نظام الدفاع المضادة للأكسدة واستعادة نشاط الطاقة البيولوجية للميتوكوندريا.