Impact of Captopril on *Ephestia kuehniella*: Ovarian Nucleic Acid Amounts and Protein Analysis

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ABSTRACT

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Ephestia kuehniella is a serious stored product pest, especially in whole and milled grains. Knowing the mechanisms that control the reproduction and development of these pests is therefore of fundamental and economic interest. Captopril, an inhibitor of angiotensin converting enzyme, was tested in vivo by topical application on reproduction of *E. kuehniella*. The drug was dissolved in acetone and topically applied (10 µg/pupa) on newly molted pupae. In follow-up experiment, the adults that survived from treated pupae were investigated for different reproductive event parameters. Captopril significantly reduced the ovarian contents of proteins and nucleic acids. The electrophoretic separation of proteins on sodium dodecyl sulfate polyacrylamide slab gels showed differences in the number of protein fractions between control and treated series. We noted the absence of three protein fractions in treated series.

Keywords: Biochemistry, captopril, electrophoresis, *Ephestia kuehniella,* ovaries, reproduction

Little is known about the role of the invertebrate angiotensin-converting enzyme (ECA). The vertebrate counterpart (ACE, EC 3.4.15.1) plays an important role in the rennin angiotensin system. It regulates blood pressure and water by converting angiotensin I into the water by converting angiotensin I into the vasoconstrictive peptide angiotensin II and deactivating the vasodilator, bradykinin I (Corval et al. 2004). ACE is therefore crucially involved on the homeostatic regulation of blood pressure and electrolyte balance and is strongly linked with a number of cardiovascular and renal diseases (Bernstein et al. 2005; Shen et al. 2008b). More recently, insect cell extracts were found to exhibit in vivo antihypertensive activity without any extra digestion requirement (Staljanssens et al. 2011). Since the discovery of an ACE in *Musca domestica*, ACE-like activity has been detected in several insect species from different orders (Fernandez et al. 2001; Lemeire et al. 2007; Wijffels et al. 1996; Williams et al. 1996). Therefore, inhibiting ACE activity is expected to interfere with the peptidergic endocrine system and to have detrimental effects on growth,

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development and reproduction of insects (Issac et al. 2007).

The Mediterranean flour moth, *Ephestia kuehniella*, is a serious worldwide pest of flourmills (Caspari and Gottlieb 1975). Recently, *E. kuehniella* has been used as a lepidopteron model to investigate normal development (El Ouar et al. 2010; Yezli-Touiker and Soltani-Mazouni 2011). Therefore, these data provide a strong basis to investigate the effect of various xenobiotics on development and reproduction of pest insects, particularly Lepidoptera. In *Tenebrio molitor*, we have shown that captopril affected the morphometric measurements of ovaries and no significant effect was observed on both thickness and fine structure of chorion (Soltani-Mazouni et al. 2007). Kirane-Amrani and Soltani-Mazouni (2012) tested three ACE inhibitors (namely captopril, enalapril, and lisinopril) in *E. kuehniella* pupae and showed that only lisinopril significantly reduced the ecdysteroid contents in whole body extracts at day 3. When captopril and fosinopril, two ACE inhibitors, were added to the rearing water of mosquitoes, high levels of larval mortality were observed of *Aedes aegypti* and *Anopheles gambiae* (Abu Hasan et al. 2017). More recently, we found that captopril affects the fecundity and the egg viability in *E. kuehniella*. This compound also caused a decrease in ovarian protein, lipid and carbohydrate amounts, suggesting interference with the vitellogenesis process (Yezli-Touiker et al. 2016). In order to complete these findings, experimentations on the effects of captopril on reproduction were performed with special attention to the mechanism by which this decrease of fecundity occurs. Thus, the separation of ovarian proteins by polyacrylamide gel electrophoresis (PAGE) was examined. Since the decrease in fecundity following treatment with some xenobiotics, like insect growth disruptors, may be due to the inhibition of ovarian DNA synthesis (Mitlin et al.1977; Soltani-Mazouni and Soltani 1994), the DNA and RNA amounts were also determined in ovaries of surviving adults from treated pupae.

MATERIALS AND METHODS

Insects.

Last instars larvae of *E. kuehniella* were collected from flour infested, separated according to their sex and placed in plastic boxes. These last were then put in a drying oven at a temperature of 27°C and a relative humidityof 80% as reported before (Soltani-Mazouni et al. 2012). Newly molted female pupae (< 8 h old) were collected every day and treated immediately. The surviving adults from treated pupae were collected. Paired ovaries from the newly emerged adult females (< 8 h old) were dissected from control and treated series.

Chemical and treatment.

Captopril (D-3mercapto-2-methyl-propionyl-L-proline) used was purchased from Sigma Co. (98%, Bornem, Belgium). A stock solution of ACE inhibitor captopril was prepared in acetone (5 mg/ml). Newly molted pupae (< 8 h old) were topically treated with a dose of 10 µg/pupae according to previous studies (Soltani-Mazouni et al. 2007; Yezli-Touikeret al. 2016). The drug was easily diluted in acetone, allowing more effective diffusion throughout the cuticle (Soltani et al. 1983; Soltani-Mazouni et al. 2012). In the control groups, pupae were treated with 2 µl of acetone alone.

Extraction and determination of nucleic acid amounts.

Six pairs of ovaries were taken from newly emerged adult females from control and treated series. Nucleic acid extraction from each pair of ovaries was performed according to Shibko et al. (1966). The assay was performed by a colorimetric method using as reagents diphenylamine for DNA (Schneider 1957), and orcinol for RNA (Burton 1956).

Protein quantification.

The surviving adults from treated pupae were collected from control and treated series and ovaries were dissected from newly emerged adult females. Each pair of ovaries was individually extracted following the procedure of Shibko et al. (1966). In brief, each sample consisting one pair of ovaries was homogenized in 1 ml of trichloroacetic acid (20%), and then centrifuged (5.000 g for 10 min). The pellet added with a mixture of ether and chloroform (1/1 v/v) was subjected to a second centrifugation (5.000 g for 10 min). The second resulting pellet added with NaOH (0.1N) served for total protein quantification in accordance with (Bradford 1976) assay using Coomassie blue brilliant (G 250, Merck) as a reagent and bovine serum albumin (Sigma) as standard. Absorbance was read at 595 nm wavelength. Protein contents were expressed as µg/mg of wet weight of tissues.

Electrophoresis.

For electrophoretic analysis of ovarian proteins, 12.5% sodium dodecyl sulfate-polyacrylamide slab gels (SDS-PAGE) were prepared following the procedure of Laemmli (1970) under native conditions. Gonads were dissected at appropriate times (< 8 h old) from females. Each pooled sample (each containing 12 ovaries per series) was placed in phenyl methyl sulfonylfuoride (PMSF, 45 mg/ml ethanol) at 0.1% (500 µl) and stored at -20°C until analysis. Proteins were extracted from samples as previously described by Soltani et al. (2002).

Statistical analyses.

Results were expressed as mean ± standard deviation (SD). Comparison of mean values were made by Student's *t*-test. All statistical analyses were performed using the MINITAB Software (Version 16, PA State College, USA) and $P < 0.05$ was considered to be a statistically significant difference.

RESULTS

Effect on ovarian nucleic acid amounts.

The effect of captopril applied topically on newly molted pupae was examined on the nucleic acid amounts in each ovary collected from newly emerged adult females. The compound caused a significant decrease in both amounts of DNA ($P = 0.02$) and RNA ($P \leq 0.05$) (Table 1).

Effect on ovarian protein amounts.

Results presented in Table 2 showed that captopril significantly ($P < 0.001$) reduced the ovarian amounts of proteins when compared to control series.

SDS-PAGE separation of ovarian proteins shown that there were 11 bands in control females (Fig.1, Table 3). Table 3 presents the different protein bands with their respective molecular weights. As compared to the protein pattern from controls, captopril caused the absence of three bands (the fourth, the fifth and the tenth ones).

Table 1. Effect of captopril (10 µg/pupa) applied topically on newly molted pupae of *Ephestia kuehniella* on nucleic acid amounts in ovaries from newly emerged adults

Treatment	DNA (µg/mg tissue)	RNA(µg/mg tissue)
Control	4.85 ± 0.30 a	12.76 ± 0.38 a
Captopril	3.69 ± 0.19 b	8.91 ± 0.27 b

Values are presented as means ± SD; n = 6 females. Within each column, values followed by the different letter are significantly different based on Student's t-test at $P < 0.05$).

Table 2. Effect of captopril (10 µg/pupa) applied topically on newly molted pupae of *Ephestia kuehniella* on amounts of proteins in ovaries from newly emerged adults

Treatment	Protein amount (µg/mg tissue)
Control	28.72 ± 1.06 a
Captopril	10.02 ± 0.66 b

Values are presented as means ± SD; n = 6 females. Values followed by the different letter are significantly different based on Student's t-test at $P < 0.05$).

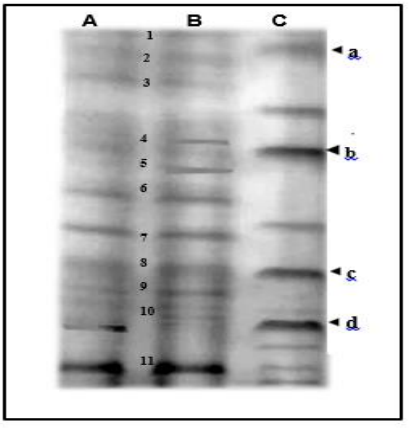

Fig. 1. SDS-PAGE patterns of ovarian proteins from newly emerged adults of *Ephestia kuehniella*in control (**A**) and captopril-treated (**B**) series as compared to protein markers (**C**). **a**: myosin 200 kDa, **b**: phosphorylase 100 kDa, **c**: albumin 75 kDa, **d**: ovalbumin 50 kDa; Numbers**1 to 11**: correspond to the numbers of protein fractions.

Table 3. Electrophoretic separation of ovarian proteins: number and molecular weight (MW) of fractions in control and treated series of *Ephestia kuehniella*

Fraction		Control	Captopril
N°	MW (kDa)		
1	206.06	+	+
2	181.55	+	+
3	150.31	+	+
4	124.45	+	-
5	96.60	+	-
6	83.52	+	+
7	80.50	+	+
8	76.03	+	+
9	63.57	+	+
10	53.96	+	-
11	29.04	+	+

Presence +; -: Absence.

DISCUSSION

In this study, we evaluated the effect of captopril an ACE inhibitor applied topically on reproduction of *E. kuehniella*. Results revealed that this drug reduced significantly ($P < 0.05$) the ovarian amounts of both RNA, DNA and proteins recorded at the end of vitellogenesis process (i.e. at adult emergence). Previous data obtained in a coleopteran species *T. molitor* reported that captopril affects the morphometric measurements of ovary but no significant effect was observed on both thickness and fine structure chorion (Soltani-Mazouni et al. 2007). Bensalem et al. (2013) showed that enalapril and lisinopril applied on newly molted *E. kuehniella* pupae exerted an inhibitory effect on both the amount of testicular proteins and a significant increase on testicular contents of DNA and RNA.

In lepidopteron species, such as *E. kuehniella*, the process of vitellogenesis takes place during the pupal stage and the oocyte accumulates and organizes yolk from precursors imported from hemolymph, while the cells of follicular epithelium synthesize additional components of the yolk as well as the vitellin envelope and chorion (Telfer 2009). The intermediary metabolism is directly involved in several physiological processes (growth, immunity, molt, reproduction and sexual maturation) which require a quantitative and qualitative contribution of various metabolites namely proteins, carbohydrates, lipids, and glycogen (Telfer 2009). In addition, proteins play an important role in the formation of gametes (Borsa and Millet 1992).

Several studies suggest a physiological role for the enzyme in insect reproduction. When *Anopheles*

stephensi females were fed with a blood meal containing either captopril or lisinopril, a reduction in fecundity in a dose-dependent manner was observed (Ekbote et al. 2003). Captopril was found to decrease the duration of the oviposition period and the morphometric measurements of ovaries, the fecundity and the egg viability in *E. kuehniella*. Moreover, biochemical analyzes revealed that treatment reduced total protein, lipid and carbohydrate amounts of ovaries, respectively. Lastly, enzyme immunoassay measurements of ovarian ecdysteroids indicated that captopril increased the amounts of both total and free ecdysteroids, and decreased that of conjugated (Yezli-Touiker et al. 2016). The reduction of reproductive capacity was probably due to an interference of captopril with the vitellogenesis process and/or ecdysteroid biosynthesis. This is supported by the reduction of vitellogenin titers in *Neobellieria bullata* (Vandingenen et al. 2001) and altered oviposition in *Spodoptera littoralis* (Vercruysse et al. 2004). In the current experiments, we noted that captopril caused significant reduction of protein amount in the ovaries. Moreover, PAGE of ovarian proteins revealed that three protein bands of 124.45, 96.60 and 53.96 kDa, respectively, were missing. Our results agree with data obtained after topical treatment of captopril on *T. molitor* adults (Soltani-Mazouni et al. 2007). Indeed, captopril caused a significant reduction in both the weight of ovaries, the number of oocytes per paired ovaries, and the size of basal oocytes as compared to controls. Captopril reduced the number of eggs per female in *E. kuehniella* (Yezli-Touiker et al. 2016) probably via the protein accumulation process and consequently the formation of eggs. The lack of three bands in the ovaries from treated series supports this hypothesis. ACE should be considered as a potential target for the development of new insect growth regulators (Issac et al. 2007).

Captopril was found to affect the ovarian contents recorded at the end of vitellogenesis process of both RNA, DNA and proteins of *E. kuehniella*. Mitlin et al. (1977) suggested that a decrease in sexual activity among *Anthonamus grandis* results in part from inhibition of DNA synthesis in female adults by diflubenzuron an inhibitor of chitin biosynthesis. This compound also caused a decrease in ovarian protein content in *Cydia pomonella* (Soltani and Soltani-Mazouni 1992), suggesting interference with the vitellogenesis process. The decrease in fecundity observed in several insect species may be due to the inhibition of ovarian DNA synthesis (Soltani-Mazouni and Soltani 1994).

Although, the exact mode of action of captopril is still unknown, our findings suggest an interference with the reproductive events. Further experiments are needed to determine the action mechanisms of captopril more in detail, and the functions of ACE in insects.

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RESUME

Yezli-Touiker S., Taffar A., Meskache R. et Soltani N. 2018. Impact du captopril sur *Ephestia kuehniella*: Quantités des acides nucléiques ovariens et analyse des protéines. Tunisian Journal of Plant Protection 13 (si): 77-85.

Ephestia kuehniella est un lépidoptère ravageur des denrées stockées qui provoque des dégâts principalement sur la farine. Le captopril est un inhibiteur de l'enzyme de conversion de l'angiotensine. Il a été testé *in vivo* par application topique sur la reproduction d'*E. kuehniella*. Ce médicament a été dilué dans l'acétone et administré par application topique chez les chrysalides femelles nouvellement exuviées à la dose de 10 µg/chrysalide. Les adultes qui ont survécu au traitement des pupes ont été étudiés pour différents paramètres de reproduction. Le captopril a réduit significativement le taux des acides nucléiques et protéines ovariens. La séparation électro-phorétique des protéines sur des gels polyacrylamide additionnés de dodécylsulfate de sodium a montré des différences dans le nombre de fractions protéiques entre les témoins et les traitées. Le profil électro-phorétique a révélé l'absence de trois fractions chez les séries traitées.

Mots clés: Biochimie, captopril, électrophorèse, *Ephestia kuehniella*, ovaires, reproduction

ملخص

يزلي تويقر، سميرة وأسماء طفار ورانية مسقاش ونور الدين سلطاني. 2018. تأثير كابتوبريل على حشرة *Ephestia kuehniella* : مقادير الأحماض النووية المبيضية وتحليل البروتينات.

Tunisian Journal of Plant Protection 13 (si): 77-85.

تعتبر الحشرة *Ephestia kuehniella* إحدى الآفات المضرة بالمواد المخزنة. كبتوبريل هو مانع أنزيم تحويل الأنجيوتنسين. أجريت التجارب المخبرية عن طريق المعاملة السطحية. تم تخفيف هذا الدواء في الأسيتون واستعمل عن طريق الدمج الموضعي لعذارى الإناث بجرعة (10 مكم/عذراء) . تمت دراسة البالغين الذين نجوا من المعاملة السطحية للعذارى اعتمادا على معايير التكاثر. خفض كبتوبريل من كمية البروتينات والأحماض الأمينية في الرحم. بين الرحلان الكهربائي SDS-Page على خارطة الكسور البروتينية المتأثرة بكبتوبريل اختفاء ثلاثة كسور عند الإناث المعاملة.

كلمات مفتاحية: بيوكيمياء، تكاثر، رحلان كهربائي، كبتوبريل، مبيض، *Ephestia kuhniella*.

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