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Determination of fluoxetine in *Dermestes maculatus* (Coleoptera: Dermestidae) by a spectrophotometric method

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ABSTRACT

The aims of this study were to detect and quantify fluoxetine, an antidepressant, from entomological samples. Larvae, pupae and adults of *Dermestes maculatus* (Coleoptera, Dermestidae) were reared on pig muscle previously treated with fluoxetine. The concentration selected, 2000 mg/kg, emulates a fluoxetine overdose lethal to humans and laboratory animals. Thirty larvae on the fourth and fifth stages, 50 adults and several exuviae were analyzed for fluoxetine content. Detection of fluoxetine was performed by UV spectrophotometry at 270 and 277 nm. All developmental stages of *D. maculatus* and exuviae were positive for fluoxetine. We also quantified the drug and no significant differences were found either between the days or the stages in the general model, but at 277 nm a tendency of the concentration to decrease with time was observed. Concentrations of fluoxetine at 277 nm were almost equal or greater than those at 270 nm. This is the first study to detect and quantify fluoxetine from entomological samples and, in particular, from *D. maculatus* beetles.

1. Introduction

Forensic entomotoxicology is the study of insects with the purpose of qualitatively and/or quantitatively detecting toxic substances [1,2]. When a body is in an advanced stage of decomposition or the tissues or body fluids normally used (postmortem samples) are not available or not well preserved [3,4], it can be difficult to perform a toxicological exam, thus the analysis of the insects found in the scene can provide data on the cause of death and the geographical area where the cadaver had lain [5,6]. Entomotoxicology also studies the effects that drugs and other toxins can have on the development of insects and other arthropods; this underlines the influence that drugs can have in the calculation of the development rate and its importance at the moment of determining PMI [1,7–10].

Suicide is a serious global public health problem and is one of the three leading causes of death in 15 to 35 year-olds. According to the World Health Organization (WHO), the suicide rate has increased 60% in 45 years. In most countries the peak for suicides occurs in midlife for women, and for men at ages above 75 [11,12]. In Argentina specialists indicate that the suicide rate has increased in the last 10–12 years, according to studies performed in several provinces, although this can vary in the jurisdictions [11]. Laborde [13] pointed that a totally reliable statistic about suicide is difficult to obtain and there is generally an underestimation of the suicide rate in the official statistics.

Large amounts of drugs and/or alcohol are consumed by young adults and women as suicide methods [14–21], and deliberate drug overdose is the most common suicide behavior among the elderly [14,22–25]. In many countries, suicide by ingestion of prescribed or purchased medication is a serious problem [26]. In general, antidepressants are among the most widely used chemical agents in deliberate self-poisonings [11,12]. The antidepressants used in self-poisoning may differ in each country according to the substances available, however, TCAs (tricyclic antidepressants) and SSRI (selective serotonin reuptake inhibitor) antidepressants are the most common [27–32].

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and peak plasma concentrations typically occur within 6–8 h after consumption. Fluoxetine is metabolized in the body, particularly in the liver, to the active desmethyl metabolite, norfluoxetine. The half-life of fluoxetine and its metabolite have been reported to be 3–4 days and 7–15 days, respectively. Several methods have been proposed for the determination of fluoxetine in pharmaceutical formulations and biological matrices [33–40].

The aim of this study was to report, the determination and quantification of Fluoxetine from entomological samples. A spectrophotometric method was used. *Dermestes maculatus* Degeer was the species selected for this study because they have necrophagous habits, have been found in forensic cases and succession experiences, particularly in the stages of advanced decay and skeletonization [41,42] and are pests in museums

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and food industries [43,44], as well as other manufactures of animal origin [45]. Besides, there are not toxicological studies on cadaveric beetles.

2. Materials and methods

2.1. Culture

Cultures of *D. maculatus* were started in 2010 by collecting adults from decomposition and succession experiments [42]. The cultures were maintained at 24 ± 2 °C and 12/12 h light/dark photoperiod. The food substrate was pig trotters.

2.2. Preparation of artificial fodder

40–50 g of fresh pig lower limb muscle were cut into small pieces and homogenized with 15–20 ml distilled water in a blender for 2 min. Then, 100 mg of fluoxetine were added to the mixture. This concentration was selected based on a toxicity of 2000 mg/kg from reports of laboratory animal deaths [46,47]. The mixture was again homogenized for 2 min. A 1.13 g quantity of powdered agar (BRITANIA agar-agar) was dissolved in 25 ml of boiling distilled water and stirred for 2 min until it thickened. The agar solution was thoroughly mixed into the muscle preparation and then poured into plastic molds. After cooling, the fodder was stored at 4 °C.

2.3. Rearing of larvae

Thirty fourth- and fifth-instar larvae of *D. maculatus* and 50 adults were transferred onto the fodder containing fluoxetine. The control consisted of insects of the same type and age but fed with fodder not treated with fluoxetine. Both colonies were reared in plastic containers of 19 cm × 19 cm × 11 cm in an incubator (OBSAR) at 24 ± 0.1 °C, 55.4% ± 2% humidity and 12:12 h L/D photoperiod. After 2, 7 and 12 days, 10 larvae and 17/16 adults were removed from the food source, washed with distilled water, and dried using paper towel. This process was repeated three times to reduce the possibility of surface contamination. Insects were then killed by freezing at −8 °C for 15–20 min. Exuviae were also collected for analysis following the same protocol described for larvae and adults.

2.4. Fluoxetine detection and quantification by spectrophotometry

The larvae and adults were weighed. The samples were then grounded with distilled water using mortar and pestle. After this, the mixture was filtered and then combined with 5 ml of chloroform (CICCARELLI or BIOPACK), shaken with a vortex for 10–15 min to extract the drug from the insect matrix. Then, the mixture was centrifuged at 3000 rpm for 15 min at room temperature. Following centrifugation, the organic phase was recovered and evaporated until dry. The residue was dissolved with 5 ml of chloroform. Triplicate aliquots were measured in a spectrophotometer (PG INSTRUMENTS UV-V T60) at 270 nm and 277 nm. For the calibration curve, an aliquot of fluoxetine standard solution (1 mg/1 ml) was pipetted into a 2.5 ml vial to achieve a final concentration in the 10–100 μg/μl range. The solutions were diluted to the final volume with chloroform.

Three replicates of the bioassay were made. Data were analyzed using a two-way ANOVA test using InfoStat 2012p version (FCA—Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

Fluoxetine has been detected in its pharmaceutical formulations by methods which are time-consuming, tedious, and/or designed for sophisticated and expensive analytical instruments [38]. In general, spectrophotometry is considered the most convenient analytical technique because of its inherent simplicity, high sensitivity, low cost, and availability in most quality-control laboratories [48–50]. Fregonezi-Nery et al. [37] have developed a simple, fast and economical ultraviolet spectrophotometric method for the detection of fluoxetine hydrochloride. Their results showed that the excipients did not interfere in the analysis. Moreover, Purdel et al. [39] developed a simple and accurate spectrophotometric method for the detection of fluoxetine in chloroform from biological samples. The protocol employed in this study was a combination of both methods.

To this date, most entomotoxicological studies have been conducted on Dipterans [7,9,51,52]. Beetles have mostly been used for ecological studies regarding mercury [6]. Miller et al. [53] published a toxicological analysis from exuviae and fecal pellets of dermestid beetles associated to a mummified corpse, and Bourel et al. [54] conducted studies with necrophagous beetles. In 2009, Sawaby et al. [55] evaluated the effect of organophosphorus components on two cadaveric species, one of them *D. maculatus*, proving that they have an active esterasic system in the presence of such compounds, and proposed their use to identify toxic substances in necrophagous insects.

We detected fluoxetine in larvae and adults of skin beetles removed at each day of exposure considered and, through the quantitative analysis, we found significant differences in the concentration of the drug in all samples, compared to the control, at 270 nm and 277 nm ($p < 0.05$) (Table 1). We did not find significant differences in the general model in the quantity of fluoxetine between each day of exposure in larval and adult stages at 270 nm and 277 nm ($p = 0.14$ and $p = 0.11$, respectively), but at 277 nm the concentration tended to decrease with days in both stages (Table 1; Fig. 1A and B). These results could be due to the insects' ability to metabolize and eliminate the drug. Wilson et al. [56] and Sadler et al. [57] found similar evidence for *Calliphora vicina* larvae, and Bourel et al. [54] for *D. frischi* and *T. sinuatus* larvae.

The concentrations of fluoxetine in both stages were not different at either wavelength ($p > 0.05$). This could indicate that the kinetics of detoxification or the process of feeding/excretion of larval and adult specimens are not too different. In contrast, other authors found differences between those stages of development in beetles [54], and others described differences between different stages of development [58,59]. There are still a lot of questions unanswered and discussions about drug metabolism, absorption and elimination, possible drug accumulation and localisation in insects [60,61]. Future entomological research should be carried out focusing on these subjects. We observed that in general the concentration values were greater at 277 nm than 270 nm

Table 1
Concentration of fluoxetine (μg/μl) measured in the entomological samples at 270 and 277 nm during the days of exposure.

Days of exposure	Larva				Adult				Exuvia			
	270 nm		277 nm		270 nm		277 nm		270 nm		277 nm	
	Control (μg/μl)	Treatment (μg/μl)	Control (μg/μl)	Treatment (μg/μl)	Control (μg/μl)	Treatment (μg/μl)	Control (μg/μl)	Treatment (μg/μl)	Control (μg/μl)	Treatment (μg/μl)	Control (μg/μl)	Treatment (μg/μl)
2	0	16.1	0	42.2	0	23.25	0	43.7	0	14.9	0	25.85
7	0	21.2	0	39	0	24.2	0	34.2	0	19.6	0	27.2
12	0	23	0	31.1	0	18.4	0	22.1	0	29.15	0	41.85

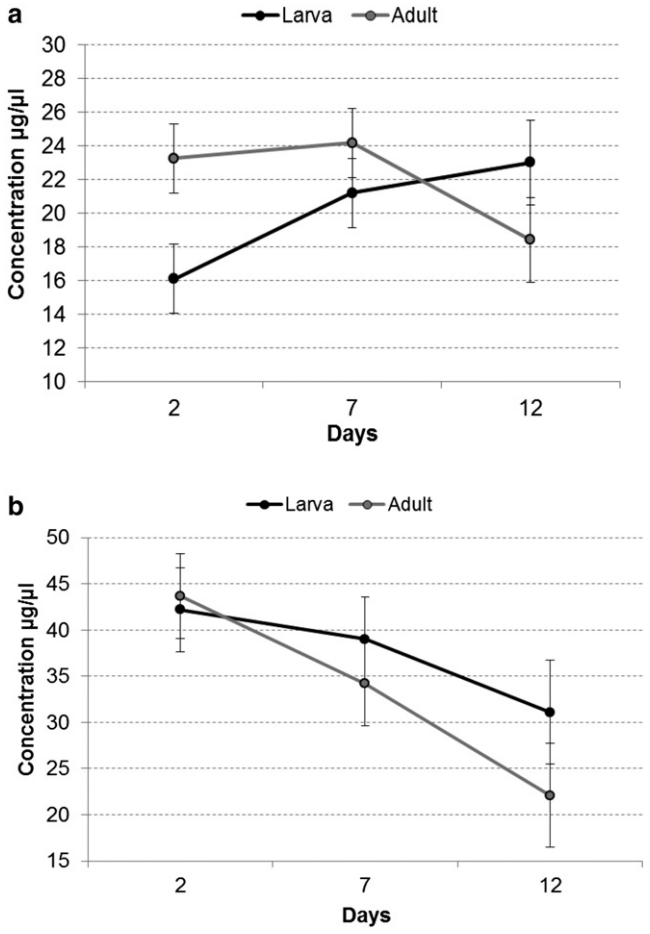

Fig. 1. Concentration of fluoxetine (µg/µl) detected in the entomological samples during the days of exposure. (a) at 270 nm, (b) at 277 nm.

at both stages. Purdel et al. [39] did not mention differences between wavelengths, stating only that both wavelengths could be used for the detection and quantification of fluoxetine.

Fluoxetine was also found in exuviae and its concentration was similar between the days of exposure tested ($p = 0.44$ at 270 nm and $p = 0.45$ at 277 nm) (only two replicates were used because the number of exuviae was small in some of the days) (Table 1; Fig. 2). This pointed out that fluoxetine was sequestered in the cuticle of the insect. Similarly, other authors have observed or suggested that the drug was included in larval tissues [62–65].

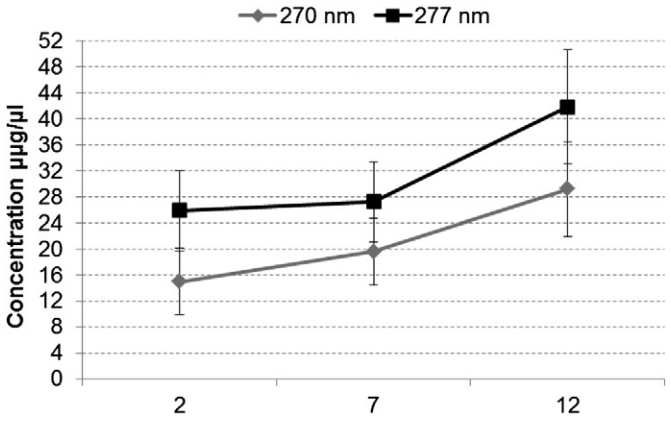

Fig. 2. Concentration of fluoxetine (µg/µl) detected in the exuviae at 270 and 277 nm during the days of exposure tested.

Novelty statement

This work provides new data on Forensic Entomotoxicology. It is the first to study the presence and quantification of fluoxetine in entomological samples and in particularly, beetles. For this purpose a spectrophotometric method was used. To this date, most entomotoxicological studies have been conducted on Dipterans, so this study reinforces the idea that beetles can be used as an alternative sample for the toxicological investigation of several classes of drugs.

Conflict of interest

None.

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