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Impact of ante-mortem fluoxetine administration on estimation of post-mortem interval and insect activity in rabbit carcasses

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Abstract

Background Increasing the number of drug-related deaths has affected medico-legal death investigations. Drugs within a corpse have a great impact on the insects' development rate which in turn will affect the rate of post-mortem decomposition and the estimation of the post-mortem interval. This explains the importance of the application of forensic entomotoxicology, which studies the impact of drugs and toxins on the development and succession patterns of insects. The current study aimed to determine the impact of fluoxetine, one of the selective serotonin reuptake inhibitors (SSRIs), on post-mortem decomposition, insects' attraction, and its pattern of succession on carcasses. Sixteen healthy male and female *Oryctolagus cuniculus* rabbits were chosen to be included in this study. They were divided into a treated group of eight rabbits received oral fluoxetine for 28 days and a control group of eight rabbits received oral distilled water for 28 days. After oral administration of 10 mg/kg/day of fluoxetine and distilled water for 28 days, rabbits were sacrificed, and carcasses were transmitted to the roof of Research and Training Centre on Vectors of Diseases at faculty of Sciences, Ain Shams University, for following up the post-mortem decomposition process and insect's attraction to carcasses for 60 days. Carcasses were put in two outdoor sites that differ in temperature to assess the effect of temperature on decomposition process.

Results Fluoxetine administration has accelerated the rate of post-mortem decomposition in the treated carcasses by 3–9 days compared to the control ones and affected the numbers and species of attracted insects, while it had no effect on the insects' succession patterns. Exposure of carcasses to direct sunlight has accelerated the rate of decomposition in comparison to that of carcasses put in shade in the overall period of decomposition by about 14 to 16 days.

Conclusions Fluoxetine has an important and effective role in post-mortem decomposition and estimation of post-mortem interval (PMI) and has a great impact on attracted insects to the treated carcasses. Temperature has a great effect on the rate of decomposition of carcasses. Higher temperature accelerates the rate of post-mortem decomposition.

Keywords Entomotoxicology, Fluoxetine, Post-mortem interval, Decomposition process, Insect succession

Background

Insects have a significant role in criminal investigations, whether on land or in water, as studying the biology of insects found on corpses and understanding their behavior and distribution can help forensic investigators provide important data about the conditions and location of the dead person or where and when the crime had been committed (Gennard 2012). Forensic entomology

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is the study of insects' behavior towards cadavers during the period of post-mortem decomposition (Amendt et al. 2004). It can give a relative estimation for the minimal post-mortem interval (PMImin) by calculating the period since the adult insect is attracted to the cadaver soon after death. The minimal PMI is approximated by estimating the age of the immature stages of insects emerging from the dead body and examining the species characteristics for specific decomposition stages and durations (Thummel et al. 2023).

The rate of decomposition and the pattern of succession of insects attracted to corpses vary according to seasons, temperature, habitats, location of the cadavers, whether indoors or outdoors, and countries, but the basic pattern remains constant worldwide (Zar and Huang 2018). The insects that have relevance to forensic investigations include flesh flies, blowflies, and skin beetles. Some of these families have both juvenile and adult stages of carrion feeders (necrophages),however, in some other families, only the juvenile stages are carrion feeders. Other insect families are attracted to the carrion solely to feed on the necrophagous species present (Gennard 2012).

The most important step in the application of insects in forensic approaches is to identify the forensically important species. The estimation of the post-mortem interval (PMI) will be incorrect if the insect identification is improper (Wang et al. 2021). PMI is the period that passed between the occurrence of death and the discovery of the dead body. Estimation of PMI is considered a main goal in entomological studies nowadays (Gennard 2012),Abd El-Gawad et al., 2019). In relation to forensic investigations, the necrophages and predator groups, including mainly species from the orders Diptera (flies) and Coleoptera (beetles), are the most important (Amendt et al. 2004). When death occurs, the body starts decomposition, the combined actions of autolysis and putrefaction (Martin and Verheggen 2023). Autolysis is the self-destruction of cells triggered by hydrolytic enzymes found within the cells (Teo et al. 2014). The decomposition process begins in the gastrointestinal tract, where bacterial action produces liquids and gasses that destroy the soft tissue and escape outside the dead body. These gasses attract insects (Jaf et al. 2021).

Based on physical putrefactive changes, the decomposition process goes through a few stages. Immediately upon death, there is the "fresh stage," which lasts for about 24 to 48 h on average (Almulhim and Menezes 2023). The stage of body inflation, which is referred to as the "bloating stage" (Martin and Verheggen 2023). It takes about 18 to 36 h to be apparent externally on the body (Shedge et al. 2023). The rupture of the corpse declares the beginning of the "active decay stage." As the decomposition process continues, an "advanced decay stage" develops where the general appearance of the corpse deteriorates. By the appearance of the bones, "the skeletonized stage" will continue, leaving behind certain more durable tissues, including cartilage, hair, and dried tissue. (Teo et al. 2014).

Drugs within a corpse have a great impact on the insects' attraction to corpses and their development rate (Verma and Paul 2013). With increasing cases of drug-related deaths worldwide, forensic entomotoxicology has been developed as a branch of the forensic sciences. It includes toxicological and entomological techniques (Dayananda and Kiran 2013),Lei et al. 2018). Forensic entomotoxicology investigates the impact of various drugs and toxins on the behavior, development, and succession patterns of insects. It uses insects as alternative samples for investigating the presence of drugs and for quantifying different drug doses as a reflection of the drug concentration in the cadaver. This occurs especially in cases where there is advanced decomposition or even skeletonization of the body and there are no available samples for drug analysis (Introna et al. 2001).

SSRIs are a widely used category in treating major depressive disorders. This category includes hundreds of drugs; one of the most widely used is fluoxetine (Micheli et al. 2018). Fluoxetine shares the same primary mechanism of action as the rest of SSRIs, which is acting by blocking the reuptake of serotonin into presynaptic serotonin neurons by blocking the reuptake transporter protein located in the presynaptic terminal, which in turn leads to increased 5-hydroxytryptamine (5-HT) levels in various areas of the brain (Peng et al. 2014). It also exerts a neurogenesis-independent antidepressant effect by influencing the plasticity of the generated new neurons (Micheli et al. 2018).

There have been several entomotoxicological studies done in Egypt. Egyptian researchers studied the effects of different drugs and toxins on the decomposition process and insect infestation, such as tramadol, heavy metals (Aly et al. 2023), snake venom (Khalil et al. 2023), morphine (El-Samad et al. 2020), organophosphorus compounds (Bakr et al. 2018), and others. This current study is considered the first one in Egypt that reported the impact of fluoxetine administration on insects' behaviors and post-mortem decomposition. In fact, there has been one previous study done to discover the entomotoxicological effect of fluoxetine. The study was done to outline the ability to detect and quantify fluoxetine in larvae and pupae reared from treated carcasses, but it did not study the effect of fluoxetine on larval and pupal growth (Zanneti et al. 2019). There have been no studies done to discover the effect of fluoxetine on the decomposition process or insects' attraction to corpses. Hence, we

thought that the current study may be helpful to outline that ante-mortem fluoxetine administration may have a great effect on the rate of decomposition, development, and the pattern of insect attraction to carcasses.

Methods

The present study aimed to determine the impact of fluoxetine, one of selective serotonin reuptake inhibitors (SSRIs) on post-mortem decomposition, insects' attraction, and its pattern of succession on carcasses.

This was an experimental interventional study that was held from January 2022 to April 2022, from winter to mid-spring. The study period was divided into an ante-mortem period of 42 days and a 60-day post-mortem interval. The ante-mortem period of 42 days was held in the Medical Ain Shams Research Institute (MASRI) at the Faculty of Medicine at Ain Shams University, which included 14 days of quarantine and acclimatization of the animals to the housing and 28 days of oral fluoxetine administration. The post-mortem period of 60 days was conducted on the roof of the Research and Training Center on Vector Diseases (RTC) at the Faculty of Science at Ain Shams University to observe the process of animal carcass decomposition.

Study animals

Sixteen adult, healthy male and female (*Oryctolagus cuniculus* Linnaeus, 1758) rabbits were chosen as they are good surrogate model mimicking decomposition in human cadavers, and the *Oryctolagus cuniculus* strain was chosen as it is the most common trait to be used as experimental rabbits (Sengupta and Dutta 2020). Study sampling was chosen according to the sampling method and sample size that were done in the department of community medicine at the Faculty of Medicine at Ain Shams University. Using the resource equation method (Mead 1988), with two groups and a maximum error term of 20, they stated that a sample of at least 8 rabbits per group is satisfactory. The chosen rabbits, aged 8 to 10 weeks old and weighing 1.5 to 2 kg, were chosen according to Zhu et al. (Zhu et al. 2019) and to be suitable for group housing, according to Mullins and Munroe (Mullins and Munroe 2003). Rabbits were grouped to be one rabbit in each cage during the housing period.

The animal housing was settled in MASRI for 42 days. The rabbits in the animal house were observed for 14 days and examined for scabies and other contagious diseases (Mapara et al. 2012). This period of 14 days also helped in rabbit acclimatization to the animal housing and diet. The next 28 days were for oral fluoxetine administration. The rabbits were grouped into a control group of eight rabbits that received distilled water for 28 days and a treated group of eight rabbits that received oral fluoxetine solution for 28 days.

Rabbits were maintained in mesh cages ($0.9 \times 0.6 \times 0.45$ m) that were suspended above the ground, allowing the waste to fall into collecting tray sets. The cages permit all-in, all-out approaches, cleansing, and disinfectant measures. Cages were equipped with a feeder and a nipple drinker. At the beginning of the drug administration period, the rabbits were grouped to be one rabbit per cage. The indoor housing temperature was measured daily by using a fixed thermometer; the average temperature was between 15 °C and 20 °C with a 12 light to 12 dark cycles in a well-ventilated room (Krunt et al. 2022). This light and dark cycle is necessary for the rabbit circadian rhythms. Rabbits were regularly monitored for dietary habits and waste features.

A nutritionally adequate diet was provided for rabbits. According to Mapara et al. (Mapara et al. 2012), the rabbit's diet is comprised of fresh grass, fresh veggies, and water. Food was spread out to encourage rabbits to forage. This manner of food distribution encourages the rabbits to search for it, which minimizes the time it takes for rabbits to fight with each other. Fouled or uneaten food was removed daily. The water was changed every day.

Fluoxetine administration and dosage

The treated group of rabbits received a dose of 10 mg/kg/day of philozac® capsules supplied by Amoun Pharmaceutical Company as a source of fluoxetine ($C_{17}H_{18}F_3NO$) that was administered for 28 days as a solution administered orally by a special oropharyngeal tube, a separate tube for each rabbit, according to Marcussen et al. (Marcussen et al. 2008). Fluoxetine oral solution was prepared as 20 mg of fluoxetine in 5 mL of distilled water according to the method of Sohel et al. (2022) and 10 mg/2.5 mL of distilled water per kilogram, so that a rabbit weighing 2 kg received 5 mL of the oral solution.

Animal necropsy

After the 42-day housing period, rabbits of all groups were sacrificed in a merciful manner using carbon monoxide (CO) according to the method of Makowska and Weary (Makowska and Weary 2009). The rabbits were placed inside an acrylic-covered container with a centered gas inlet and a 1.8-cm in diameter air outlet, which is enclosed in a mesh at the closest end to the tube. Nine percent of CO was pumped per minute to the container by cylinders filled with compressed gas, and the gas flow was controlled by an air flow meter for 2–3 min in a closed laboratory room. Rabbits from both groups—the control and treated ones—were transferred to the roof of the RTC.

Post-mortem observation of the decomposition process and insect succession

The study site

The study was constructed on the top of the Faculty of Science, Ain Shams University in Abbassyia, Cairo Governorate, Egypt, from February 20 to April 20, 2022 (winter to spring). The research site has an elevation of 18 m (59.1 ft.) above ground, 48.50 m above sea level, about 159.12 ft., and a covered area of approximately 53 square meters.

Environmental data

To monitor the impact of temperature, humidity, and rainfall on carrion decomposition and insect activity, daily measurements were taken in the research area. Weather data was obtained from the Egyptian meteorological authority located 943.6 m (about 3095.8 ft.) away from the study location. Temperature maximums ranged from 33 to 40 °C, with minimums of 8 to 23 °C and relative humidity of 9 to 77%. It rained 3 days during the entire study period.

Experimental design

The rabbit carcasses were kept in eight wooden framed cages of 50 cm^3 to allow daily observation of the decomposition process of the rabbit carcasses and to be away from crowding to reduce the undesirable view of decomposed carcasses and their released odor. Each cage includes two rabbit carcasses. The cages were covered by stainless steel wire mesh about 1×1 cm to be guarded against scavengers while allowing the insect access. The cages were opened on top to facilitate inspection and taking out the rabbit carcasses. In order to study the effect of direct sunlight and shade on the process of decomposition and insect succession, the cages were grouped according to their position on the roof into: a group of four cages put in direct sunlight, two cages for the control group, and the other two cages for the fluoxetine-treated group put in two parallel lines at a distance of 10 m to provide an isolated source for insects inhabiting the carcasses. A group of four cages put in shade included two cages for the control group and two cages for the fluoxetine-treated group placed also, in two parallel lines at a distance of 10 m. Each cage of the same group was placed about 1 m apart from one another, according to Velásquez (Velásquez 2008). Sand was placed under each cage to create a safe environment for the pupation of the developing larvae.

The process of decomposition and insect succession was daily supervised by the research team to record the decomposition stage and collect insects. Daily follow-ups were conducted to gather all life stages of each significant species that inhabits rabbit carcasses.

Collection and preservation of insect samples

Live adult insects were collected from the soil around the carcasses using bit traps and meshwork. The collected adults were transferred into a collecting jar containing ethyl acetate. Ethyl acetate is usually used as it kills the insects very slowly; hence, if alive insects were needed for experimental purposes in the laboratory, they could be reared in glass jars containing ethyl acetate. It is also widely used as it delays the hardening of insects and preserves the integrity of the insect's body for proper identification and quantification (Kumar et al. 2022). Samples were properly labeled, showing the type of specimen, the date, the time, the place of collection, and the temperature (Erskine 2018). Specimens were then conveyed to the laboratory for analysis and identification using the keys of Fan et al. (Fan et al. 1997), Morgulis and Freidberg (Morgulis and Freidberg 2014), and Rochefort et al. (Rochefort et al. 2015).

Larvae and pupae of various species were collected from dry places around carcasses using forceps, killed and preserved in specific jars containing 80% ethyl alcohol, according to Richards et al. (Richards et al. 2013), and transferred to the laboratory for analysis.

Statistical analysis

Recorded data were analyzed using the Statistical Package for Social Sciences, version 23.0 (released in 2015), IBM SPSS Statistics for Windows (Armonk, New York: IBM Corporation). Quantitative data were expressed as mean ± standard error (SE) and range, then compared with an independent *t*-test, and the analysis of the data was done using the chi-square test. The confidence interval was set to 95%, and the margin of error accepted was set to 5%. So, the *P*-value was considered significant as follows:

- *P*-value > 0.05 was considered non-significant (NS).
- *P*-value < 0.05 was considered significant (S).
- *P*-value < 0.01 was considered as highly significant (HS).

Results

Effect of fluoxetine administration on the decomposition stages and post-mortem interval

The decomposition process and post-mortem interval

For 60 days post-mortem, five stages of the carcasses' decomposition were noticed: the fresh stage, the bloating stage, the active decay stage, the advanced decay stage, and the dry to skeletonized stage.

Table 1 Comparison of decomposition process rate and post-mortem interval between control carcasses and treated carcasses put in direct sunlight using independent two-sample (*t*) test

Stages of decomposition	Control group, sunlight (*N* = 4) Mean ± SE (days)	Treated group, sunlight (*N* = 4) Mean ± SE (days)	*T*-test value	*P*-value
Fresh stage	1.63 ± 0.48	1.63 ± 0.48	0.000	1.000 **NS**
Bloating stage	10.25 ± 0.96	7.25 ± 0.50	8.485	0.000 **HS**
Active decay stage	10.63 ± 0.48	9.63 ± 0.48	4.513	0.000 **HS**
Advanced decay stage	10.25 ± 0.96	6.75 ± 0.50	9.899	0.000 **HS**
Dry to skeleton	27.25 ± 2.87	34.75 ± 1.50	7.071	0.000 **HS**
Duration from fresh stage to beginning of dry stage	32.75 ± 2.87	25.25 ± 1.50	7.071	0.000 **HS**

N Number, *SE* Standard error, *NS* Non-significant, *HS* Highly significant

Data in Tables 1 and 2 illustrate that for the group of carcasses put in direct sunlight as well as in shade, there is no statistically significant difference in the duration of the fresh stage between the control carcasses and the fluoxetine-treated carcasses, while there were statistically significant differences in the rate of decomposition from the bloating stage duration to the dry stage duration between the control group and the treated group of carcasses put in direct sunlight (Table 1) and a statistically significant difference in the rate of decomposition in the bloating stage duration and dry stage duration between the control group and the treated group put in shade (Table 2). Fluoxetine has significantly accelerated the rate of decomposition of the treated carcasses by 3–9 days more than the rate of decomposition of the control group. Hence, fluoxetine administration has decreased the overall post-mortem interval in the treated group compared to the control group.

The effect of temperature on the rate of decomposition and the post-mortem interval

The decomposition rate in the fluoxetine-treated group exposed to direct sunlight was more rapid compared to the fluoxetine-treated group kept in the shade as shown in Table 3. Similarly, the decomposition rate in the control group exposed to direct sunlight was more rapid than in the control group kept in the shade.

Table 2 Comparison of decomposition process and post-mortem interval between control carcasses and treated carcasses put in shade using independent two-sample (*t*) test

Stages of decomposition	Control group, shade (no = 4) Mean ± SE (days)	Treated group, shade (no = 4) Mean ± SE (days)	*T*-test value	*P*-value
Fresh stage	2.38 ± 0.75	1.75 ± 0.29	2.376	0.032 **S**
Bloating stage	13.50 ± 1.91	8.63 ± 0.48	7.546	0.000 **HS**
Active decay stage	9.63 ± 0.95	9.75 ± 1.50	0.215	0.833 **NS**
Advanced decay stage	22.25 ± 1.89	20.88 ± 1.31	1.823	0.090 **NS**
Dry to skeleton	12.25 ± 2.87	19.00 ± 3.56	4.509	0.000 **HS**
Duration from fresh stage to beginning of dry stage	47.75 ± 2.87	41.00 ± 3.56	4.509	0.000 **HS**

N Number, *SE* Standard error, *NS* Non-significant, *HS* Highly significant, *S* Significant

Table 3 Statistical comparison between the post-mortem interval of the decomposition of the treated rabbits exposed to direct sunlight and the treated rabbits placed in shade using independent two-sample (*t*) test

Stages of decomposition	Treated group in sunlight Mean ± SE (days)	Treated group in shade Mean ± SE (days)	*T*-test value	*P*-value
Fresh stage	1.63 ± 0.48	1.75 ± 0.29	0.683	0.506 **NS**
Bloating stage	7.25 ± 0.50	8.63 ± 0.48	6.068	0.000 **HS**
Active decay stage	9.63 ± 0.48	9.75 ± 1.50	0.243	0.812 **NS**
Advanced decay stage	6.75 ± 0.50	20.88 ± 1.31	30.674	0.000 **HS**
Dry to skeleton	34.75 ± 1.50	19.00 ± 3.56	12.458	0.000 **HS**

N Number, *SE* Standard error, *NS* Non-significant, *HS* Highly significant, *S* Significant

Table 4 Time duration of the immature stages (larvae and pupae) of all insects collected during the study duration in days (first day and last day of infestation) and their relation to the decomposition stages

Rabbit carcasses	Larvae and pupae	Decomposition stage
Control group in sunlight	**Days 13 to day 41 (29 days)**	**Full bloat–dry**
Control group in shade	**Days 16 to day 48 (38 days)**	**Active–advanced**
Treated group in sunlight	**Days 5 to day 39 (35 days)**	**Bloating–dry**
Treated group in shade	**Days 9 to day 46 (38 days)**	**Bloating–dry**

The effect of fluoxetine administration on the appearance of immatures (larvae and pupae) of different insect orders

The first appearance of the larval stage in the control group put in direct sunlight was on the 13th day post-mortem, and it took 29 days for the 1st larval instar stage to emerge into the stage of splitting the pupae, from end of bloating stage till the dry stage. So, the life cycle of different insects from the stage of the larval instar to the pupal stage was 29 days. While the first appearance of the larval stage in the fluoxetine-treated group put in direct sunlight was on the 5th day post-mortem, during the bloating stage and continued till the dry stage, it took 35 days for the first larval instar to emerge into the pupal stage. Hence, fluoxetine administration has decelerated the period of life cycle of various insects and has lengthened the insect life cycle. For the group of carcasses put in shade, the first appearance of the larval stage in the control group put in shade was on the 16th day post-mortem, and it took 38 days for the 1st larval instar stage to emerge into the stage of splitting the pupae, during active decay and advanced decay stages. While the first appearance of the larval stage in the fluoxetine-treated group put in shade was on the 9th day post-mortem, it took 38 days for the first larval instar to emerge into the pupal stage, from bloating stage and continued till the dry stage. The temperature has an inverse effect on the length of the insect life cycle, as the life cycle of the insects attracted to the carcasses put in sunlight is shorter than the life cycle of the insects attracted to the carcasses put in shade by about 9 to 10 days (Table 4).

The effect of fluoxetine administration on the numbers and the pattern of succession of the adult insects of different orders

There was no statistically significant difference in the numbers of insects from the Dipteran order attracted to both groups of carcasses: the control group or the fluoxetine-treated group. While there were statistically significant differences in the numbers of adult insects from Coleopteran and other various orders of insects attracted to the control carcasses and the fluoxetine-treated carcasses, the numbers of Coleopteran insects attracted to the control carcasses were less than those attracted to the treated carcasses, while the numbers of insects from other orders attracted to the control carcasses were more than those attracted to the treated carcasses (Tables 5 and 6).

Sarcophagidae were the most predominant family to be attracted to the carcasses of both the control and treated groups from the insects of the Dipteran order. Dermestidae were the most prominent family of the Coleopteran order to be attracted to the carcasses, while Formicidae

Table 5 Comparison between control rabbits placed in direct sunlight and treated rabbits placed in direct sunlight regarding numbers of collected adults of different orders of insects using chi-square test

Order of insects	Control group, sunlight		Treated group, sunlight		Test value	*P*-value
	Number	%	Number	%		
Diptera	622	59.7%	545	56.4%	2.367	0.124 **NS**
Coleoptera	363	34.9%	395	40.8%	7.623	0.006 **HS**
Others	56	5.4%	27	2.8%	8.469	0.004 **HS**

NS Non-significant, *HS* Highly significant, *%* Percentage

Table 6 Comparison between control rabbits placed in shade and treated rabbits placed in shade regarding numbers of collected adults of different orders of insects using chi-square test

Order of insects	Control group, shade		Treated group, shade		Test value	*P*-value
	Number	%	Number	%		
Diptera	312	59.7%	273	56.4%	1.171	0.279 **NS**
Coleoptera	182	34.8%	198	40.8%	3.890	0.049 **S**
Others	29	5.5%	14	2.8%	4.354	0.037 **S**

NS Non-significant, *S* Significant, *%* Percentage

were the most attracted family of the Hymenoptera order and from all other orders of insects attracted to carcasses.

There was no difference in the pattern of succession of all insects between the control group and the treated group. Dipteran insects were the first to be attracted to the carcasses from the fresh stage of decomposition and reached their maximum numbers during the active decay stage, then began to decrease from the advanced decay stage to the dry stage (Fig. 1). While Coleopteran insects began to appear in the bloating stage, they increased to reach maximum numbers during the advanced decay stage and decreased in the dry stage, so as the insects of other various orders (Figs. 2 and 3). So, fluoxetine had no effect on the pattern of insect succession on fluoxetine-treated carcasses.

The total numbers of insect species from different orders that attracted to both control and fluoxetine-treated carcasses are detailed in Tables 7, 8, and 9. Also, all species of different orders of insects attracted to both control and treated carcasses are detailed in Tables S1 to S2 in the supplementary files. Notably, some species were collected from control carcasses but were absent from treated ones. It is noted that the first species attracted to the carcasses of both groups—the control and fluoxetine-treated groups—were flesh flies, *Sarcophaga argyrostoma.* They reached the carcasses early during the fresh stage; however, there were no statistically significant differences between the numbers of insects from this species attracted to both groups. The number was a bit higher in the treated group than in the control group. Two hundred thirty-four adult insects were collected from the control carcasses, and 278 adult insects were collected from the fluoxetine-treated carcasses. Then, insects of blowflies Calliphoridae, *Chrysomya albiceps* and *Lucilia cuprina*, and Muscidae, *Musca domestica*, appeared. From the bloating to the active decay stage, the insects of Coleoptera began to appear. The most prominent species was *Dermestes maculatus*. Two hundred seventeen adult insects were collected from the control group, while 293 adult insects were collected from the fluoxetine-treated group. The number of coleopteran insects attracted to the fluoxetine-treated group carcasses was statistically higher than the number attracted to the control group carcasses. The species of insects of orders other than Diptera and Coleoptera were attracted to carcasses from the bloating stage to the dry stage; Hymenoptera: Formicidae: *Monomorium pharaonis* were the most prominent insects attracted to carcasses. Thirty-two adult insects were reared from the control carcasses, while 27 adult insects were reared from the fluoxetine-treated carcasses.

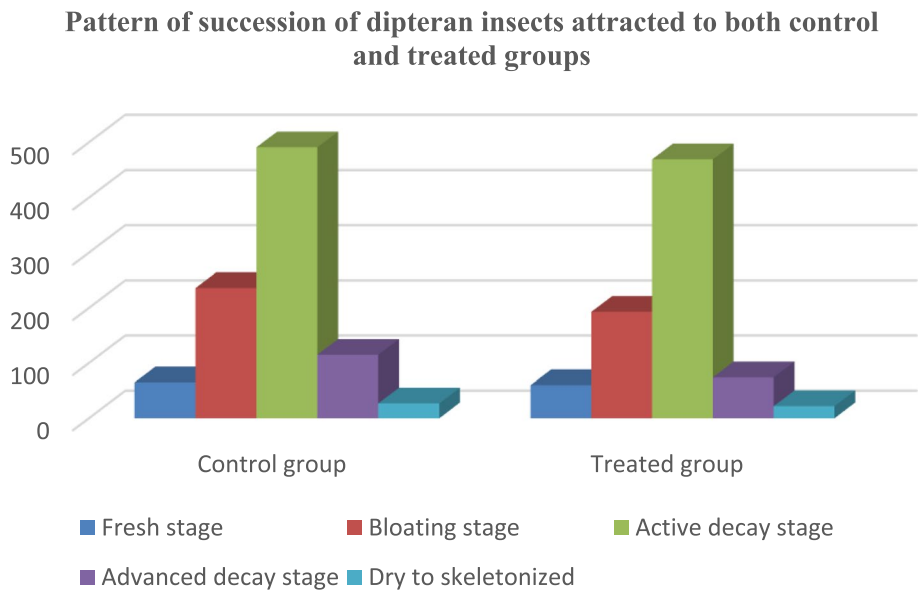

Fig. 1 Bar chart showing that there is no difference in the pattern of succession of attracted dipteran insects between the control and treated groups during the whole period of decomposition

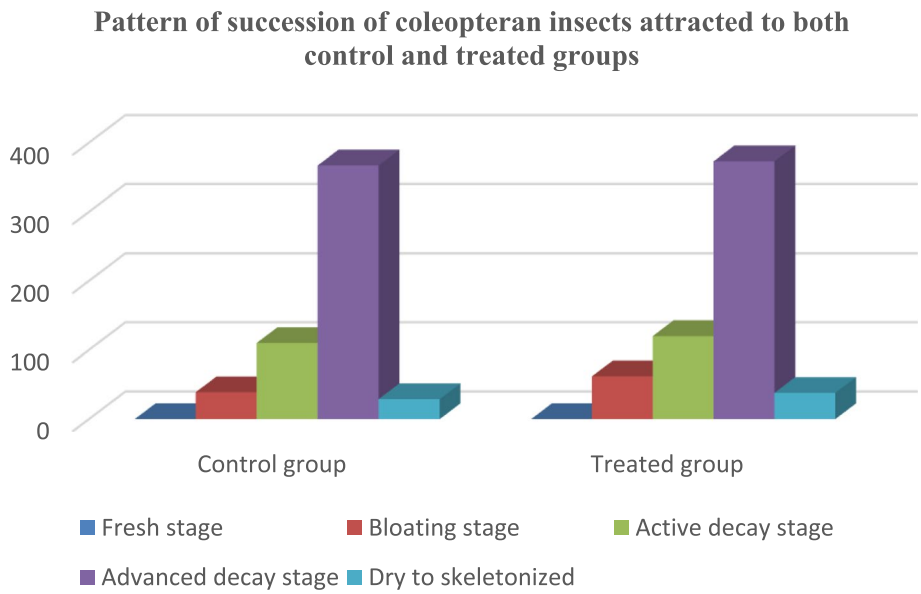

Fig. 2 Bar chart showing that there is no difference in the pattern of succession of attracted coleopteran insects between the control and treated groups during the whole period of decomposition

Discussion

There are various studies that have reported the impact of different drugs and substances on the rate of decomposition in carcasses. Drugs can alter the interior context of the body, thereby slowing or accelerating breakdown processes. Some drugs have been reported to accelerate the rate of decomposition, while others have been reported to delay the rate of decomposition. Fluoxetine, like other drugs, can alter the body's chemistry, potentially influencing the rate of decomposition. This study is considered one of the first to report the impact of fluoxetine on the rate of decomposition.

The findings of this research showed that fluoxetine administration accelerated the decomposition process of

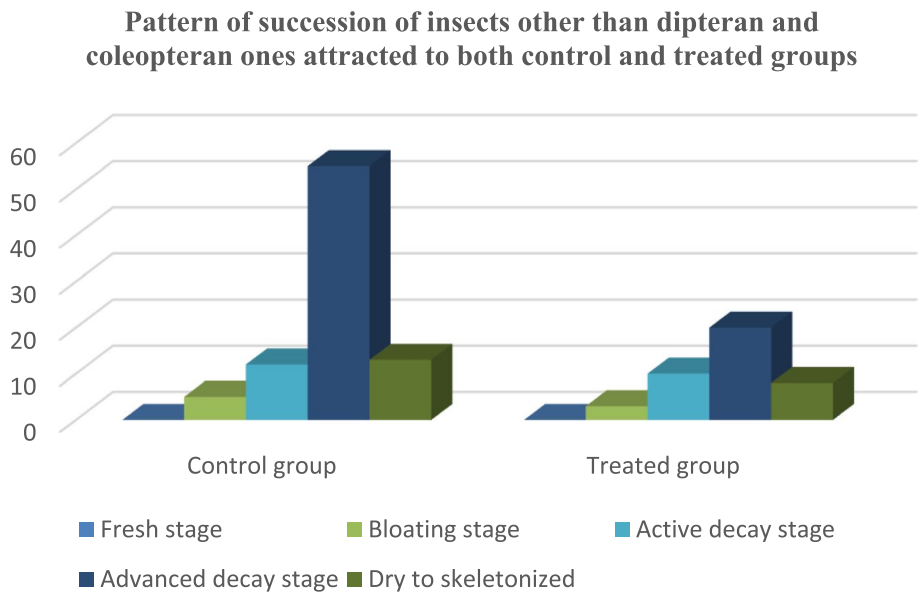

Fig. 3 Bar chart showing that there is no difference in the pattern of succession of the insects from other orders than Dipteran and Coleopteran orders that reached the control and treated groups during the whole period of decomposition

the treated carcasses by 3 to 9 days compared to the rate of decomposition of the control carcasses. As a result, fluoxetine administration decreased the overall post-mortem interval in the treated group compared to the control group. This influence of fluoxetine on the rate of decomposition may be attributed to the metabolic effects of fluoxetine on cytochrome P enzymes. Further studies should be done to assess various enzymes and biomarkers after fluoxetine administration. The results in the current study came in accordance with a study done by Khalil et al. (Khalil et al. 2023) to test the effect of snake envenomation on the rate of decomposition of rabbit carcasses and the succession rate of flies. They reported that the rate of decomposition of the envenomed carcasses was more rapid than the rate of decomposition of control carcasses by about 3 days. In a study conducted by Al-Khalifa et al. (Al-Khalifa et al. 2021), they reported that ante-mortem alcohol intake has delayed the decomposition process in the treated rabbit carcasses in comparison to the control rabbit carcasses, so alcohol intake has lengthened the post-mortem interval in the treated carcasses than in the control ones.

Environmental factors indeed have a great impact on the rate of decomposition and on insect succession, especially in the case of outdoor cadavers. Moraleda et al. (Moraleda et al. 2022) stated that high temperatures have a significant impact on both the rate of decay and the duration of each decomposition stage. These results agreed with the data reported in the current research about the effect of exposure of the carcasses to direct sunlight and the relative differences in temperature between shade and exposure to direct sunlight, and they also agreed with the results of Abd El-Gawad et al. (2019), who stated that the decomposition rate of carcasses is directly proportional to temperature. In a study done by Siddiki and Zambare (Siddiki and Zambare 2017), they studied the time duration of life stages of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) during different seasons. They concluded that high temperatures in summer have accelerated the development of the insects, while in winter and rainy weather, there is a slow rate of insect development from immature stages to adult stages.

Various studies were done to expose the effect of different drugs on the larval and other immature stages of insects through their development life cycle. A study done by Al-Mekhlafi et al. (Al-Mekhlafi et al. 2022) stated that zolpidem tartrate retarded larval development and altered the estimation of the total developmental duration. Verma and Paul (Verma and Paul 2013) showed that the developmental rate of flies is highly affected by different drugs. For example, heroin delayed the rate of fly development, while cocaine and amphetamines accelerated it. These results came in accordance with the current data that fluoxetine administration has delayed the time for larvae and pupae of different orders to emerge into the adult stage of insects, thereby lengthening the development life cycle of the attracted insects. Two studies by Sohel et al. (Sohel et al. 2022) and Zanneti et al. (2019) were done to outline the ability to detect and quantify

Table 7 List of the total number of collected insects of families and species of dipteran order

Order	Family	Species	Control	Treated
Diptera	**Muscidae**	*Musca domestica* (Linnaeus, 1758)	157	165
		Musca sorbens (Wiedemann, 1830)	30	0
		Muscina stabulans (Fallén, 1817)	110	118
		Synthesiomyia nudiseta (Van Der Wulp, 1883)	23	0
	Total Muscidae		320	283
	Calliphoridae	*Calliphora vicina* (Robineau-Desvoidy, 1830)	12	0
		Chrysomya albiceps (Wiedemann, 1819)	88	75
		Chrysomya megacephala (Fabricius, 1794)	11	0
		Lucilia sericata (Meigen, 1826)	29	25
		Lucilia cuprina (Wiedemann, 1830)	52	21
	Total Calliphoridae		192	121
	Sarcophagidae	*Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830)	234	278
		Sarcophaga hertipes (Wiedemann, 1830)	103	107
	Total Sarcophagidae		337	385
	Piophilidae	*Piophila casei* (Linnaeus, 1758)	7	0
	Psychodidae	*Clogmia albipunctata* (Williston, 1893)	5	0
	Sphaeroceridae	*Coproica vagans* (Haliday, 1833)	12	0
	Hippoboscidae	*Hippobosca equina* (Linnaeus, 1758)	6	0
	Fanniidae	*Fannia canicularis* (L.,1761)	11	7
	Drosophilidae	*Drosophila replete* (Wollaston, 1858)	8	3
	Syrphidae	*Eristalis tenax* (L., 1848)	2	0
		Eupeodes corollae (Fabricius, 1794)	3	0
	Phoridae	*Megaselia scalaris* (Loew, 1866)	25	19
	Ulidiidae	*Physiphora alceae* (Preyssler, 1791)	6	0
	Total others		79	29
	Total Diptera		934	818

fluoxetine from larvae and pupae reared from treated carcasses, but they did not study the effect of fluoxetine on the larval and pupal growth. Another study done by Carvalho et al. (Carvalho et al. 2001) reported that diazepam has accelerated the developmental rate of flies reared from treated rabbit carcasses.

The reports from the current study showed that there is no difference in the pattern of succession of the insects attracted to both control and fluoxetine-treated carcasses;

Table 8 List of the total number of collected insects of families and species of Coleopteran order

Order	Family	species	Control	Treated
Coleoptera	**Dermestidae**	*Attagenus fasciatus* (Thunberg, 1795)	7	0
		Dermestes maculatus (De Geer, 1774)	217	293
		Dermestes ater (De Geer, 1774)	185	194
	Total Dermestidae		409	487
	Histeridae	*Pactolinus major* (L., 1767)	4	0
		Saprinus chalcites (Illiger, 1807)	22	18
		Saprinus furvus (Erichson, 1834)	38	37
		Saprinus caerulescens (Hoffimann, 1803)	33	29
	Total Histeridae		97	84
	Cleridae	*Necrobia rufipes* (De Geer, 1775)	24	22
	Tenebrionidae	*Mesostina puncticoli* (Solier, 1835)	3	0
		Blaps polychresta (Forskal, 1775)	2	0
	Anobiidae	*Stegobium paniceum* (L.,1758)	3	0
	Ptinidae	*Gibbium psylloides* (Gzempinski, 1778)	4	0
	Coccinellidae	*Coccinella undecimpunctata* (L., 1758)	3	0
	Total others		39	22
	Total Coleoptera		545	593

Table 9 List of the total number of collected insects of families and species of orders other than Diptera and Coleoptera

Order	Family	Species	Control	Treated
Hymenoptera	**Vespidae**	*Vespa orientalis* L., 1761	2	0
	Apidae	*Apis mellifera* L., 1758	5	0
	Evaniidae	*Evania appendigaster* (L., 1758)	3	0
	Pteromalidae	*Nasonia vitripennis* (Walker, 1836)	17	12
	Formicidae	*Monomorium pharaonis* (L., 1758) *Cataglyphis bicolor* Fabricius, 1793	32 9	27 0
	Total Hymenoptera		68	39
Dermaptera	**Forficulidae**	*Forficula auricularia* L., 1758	3	0
Zygentoma	**Lepismatidae**	*Thermobia aegyptiaca* (Lucas, 1840)	4	0
Blattodea	**Blatellidae**	*Blattella germanica* (L., 1767)	5	2
Hemiptera	**Cydnidae**	*Aethus hispidulus* (Klug, 1775)	3	0
	Rhyparochromidae	*Lethaeus lethierryi* (Puton, 1869)	2	0
Total numbers			85	41

hence, fluoxetine has no effect on the succession pattern of insects attracted to treated carcasses. These results come in accordance with several studies on various drugs. The study by Keshavarzi et al. (Keshavarzi et al. 2021) found that the overall pattern of insect succession was similar between the control and the treated rabbit carcasses. A study done by El-Samad et al. (El-Samad et al. 2021) stated that the pattern of insect succession did not differ between control carcasses and aluminum phosphide-treated carcasses,however, the succession numbers were different. While a study done by Khalil et al. (Khalil et al. 2023) revealed that snake envenomation affected the rate and pattern of succession of the insects attracted to the envenomed carcasses, the pattern of succession of insects is rarely affected by drugs, but the number of attracted insects still differs. In the present study, data revealed that there was a statistically significant difference in the number of coleopteran insects attracted to the control carcasses and the treated carcasses in favor of the treated ones, while this statistically significant difference between both groups according to insects of other orders was in favor of the control group. There is no significant difference between the control and treated groups according to the numbers of dipteran insects attracted to both of them. Other studies have various numbers and differences of insects according to the differences in seasonal factors and the type of drug used in the study.

Necrophagous insects have great importance in the estimation of the post-mortem interval, can help in the localization of the body, and can provide important data about the presence of drugs and the circumstances of the occurrence of death. In the current study, we noticed that flesh flies colonized the carcasses as early as blow flies. *Sarcophaga argyrostoma* insects were the most frequent species of Sarcophagidae (flesh flies) on both control and fluoxetine-treated carcasses. Two hundred thirty-four adult insects were collected from the control carcasses, and 278 adult insects were collected from the fluoxetine-treated carcasses throughout the whole period of decomposition from the fresh stage to the dry stage. This result is related to the nature of the development of *S. argyrostoma*, which is usually attracted to outdoor carcasses in a bit warmer weather, at temperatures ± 30 °C, and its place of distribution, which includes the Middle East. This comes in accordance with the study of Gunn (Gunn 2002), who studied the colonization of Sarcophagidae on the dead remains and noticed that *S. argyrostoma* developed on the dead remains indoors and outdoors in warm, humid temperatures. Also, in studies done by Benecke (Benecke 1998) and Cherix et al. (Cherix et al. 2012), they stated that *S. argyrostoma* develops outdoors and can be considered relatively frequent on corpses. Cherix et al. (Cherix et al. 2012) noticed that they colonized the dead bodies on the same day that blowflies colonized the same dead bodies. *Musca domestica* and *Chrysomya albiceps* were frequent flesh flies that colonized both control and fluoxetine-treated carcasses. *Dermestes maculatus* insects were the most attractive coleopteran insects (beetles) that were attracted to both control and fluoxetine-treated carcasses,however, they were more frequent on the treated carcasses. They began to appear from the bloating stage to the dry stage. Two hundred seventeen adult insects were collected from the control group, while 293 adult insects were collected from the fluoxetine-treated group. As reported in the literature, the *D. maculatus* insects are among the most important insects in forensic applications, as they are frequently used to estimate post-mortem intervals. And it is well known that they grow up in dry weather and colonize the carcasses late in advanced decay and dry stages.

In this study, they were collected as early as the bloating stage, and this may return to the relatively low temperature in the first days of the study. However, their number has increased a lot during the following decomposition stages. This came in accordance with Tomberlin (Tomberlin 2009), who noticed that Dermestidae began attracted to dog carcasses early after the first 24 h of decay. A study done by Magni et al. (Magni et al. 2015) stated that *Dermestes maculatus* is the most frequent and predominant species of the family Dermestidae to be attracted to body remains. They also noticed the development of *D. maculatus* at a temperature of ± 25 °C.

Conclusions

This study was done to investigate the effect of ante-mortem fluoxetine administration on the decomposition process, post-mortem interval, and the succession pattern of insects attracted to rabbit carcasses and revealed that fluoxetine accelerated the rate of decomposition and shorten the post-mortem interval of the treated carcasses, attracted various species of insects at early stages of decomposition while retarded and lengthened the larval and pupal development into their adult stages, and it had no significant effect on the pattern of insect succession during different decomposition stages; the most prominent insect order attracted to both carcass groups was Dipteran order then Coleopteran order. Hence, fluoxetine has an important and effective role in post-mortem decomposition and estimation of PMI and has a great impact on attracted insects to the treated carcasses. A proper history of medical therapy for SSRI should be taken before proceeding with the death investigation. Temperature has an important effect on the rate of decomposition of carcasses. Higher temperatures accelerate the rate of post-mortem decomposition.

Recommendations

We recommend that more studies be done to discover the effect of fluoxetine on the decomposition process and insect attraction to cadavers in various seasons and in different geographic areas worldwide. Further studies are recommended to be constructed at enzyme and biomolecular levels to explain the effect of fluoxetine on the decomposition process. We recommend constructing more studies using different doses of fluoxetine and other SSRI drugs and a trial of the quantification of fluoxetine from different species of insects, as there are few studies about the quantification of fluoxetine from insects. Studies on different animal models, such as pigs, and on human cadavers, if possible, should compare the effect of fluoxetine on the decomposition process between various mammals.

Abbreviations

SSRIs	Selective serotonin reuptake inhibitors
RTC	Research and Training Centre on Vectors of Diseases
PMI	Post-mortem interval
PMI min	Minimum post-mortem interval
MASRI	Medical Ain Shams Research Institute
FMASU REC	Faculty of Medicine Ain Shams University Research Ethics Committee
5-HT	5-Hydroxytryptamine

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Authors' contributions

F.M.S., A.H.B., A.A., and E.A. conceived and designed the research. F.M.S, E.A., and R.M.B. conducted the experiments and collected the data. F.M.S. and A.A. analyzed and interpreted the statistical data. All authors contributed to the writing of the manuscript's final draft as well as its revision and editing. All authors read and approved the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent for participate

The approval for the current study to be done was obtained from the Faculty of Medicine, Ain Shams University Research Ethics Committee (FMASU REC) (FWA00017585, MD 237/2021).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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