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THE EFFECT OF THE TRAMADOL ACCUMULATED IN RAT LIVER ON THE DEVELOPMENT OF THE IMMATURE STAGES OF THE FLESH FLY *SARCOPHAGA ARGYROSTOMA* (ROBINEAU-DESVOIDY, 1830) (DIPTERA: SARCOPHAGIDAE)

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Abstract

The present study investigated the effects of the accumulation of tramadol on the development rates of the larvae and pupae. Larvae of the flesh fly *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830) were reared on three groups of Wister albino rat livers. One group was administered the recommended or normal dose (D1) and the second group was injected with higher over or double dose (D2) of tramadol. Tramadol was administered by stomach tube once a day, for 3 months. The third group was injected with normal saline solution as the control. Using GC-Ms analysis, Rat livers contained 0.72 and 1.62mg/g of tramadol, in case of D1 and D2, respectively. Larvae of *S. argyrostoma* fed on D1 and D2 rat livers contained (0.11 & 0.18mg/g), respectively. The corresponding tramadol concentrations persisted in the produced pupae of *S. argyrostoma* were (0.07 & 0.09mg/g), respectively.

The accumulation of tramadol in tissues of *S. argyrostoma* larvae reduced the durations of larval stadia from (7.0408±1.0198 days) in case of the control to (6.6383±0.4857 days) when fed on D1 liver and (6.3438±0.4826 days) when fed on D2 livers. The pupal durations was altered to (14.3750±0.4919 days) for D2 and (14.9574±0.6580 days) for D1, compared to (13.9167±1.0071 days), in case of the control. The average weight of the 3rd day larva of *S. argyrostoma* decreased from 18.1807±1.4949mg, in case of the control to 14.7279±1.3366mg and 14.9560±3.8210mg, when fed on D1 and D2 tramadol treated livers. In contrast, corresponding weight produced pupae increased from 14.1750±0.4667mg (control) to 15.2449±0.302mg & 15.9062±0.2888mg, in case of D1 and D2, respectively.

Key words: Sarcophagidae, Larvae, development, Tramadol, Flesh flies, Rat liver

Introduction

Tramadol is a centrally acting analgesic medicament, prescribed for the treatment of moderate to severe pains (Musshoff and Madea, 2001). Postmortem concentrations of tramadol due to overdose was reported in several studies (Lusthof and Zweipfenning, 1998; Mitchuad *et al*, 1999; Moore *et al*, 1999; Klingmann *et al*, 2000; Musshoff and Madea, 2001; Loughrey *et al*, 2003; Bynum *et al*, 2005; De Decker *et al*, 2008).

After death, the cadaver became infested with different dipteraous larvae. Entomological techniques are the most trustworthy to determine the minimum post mortem interval (PMI). PMI describes the time between the discovery of the corpus and its infestation by the insects (Bourel *et al*, 1999). Two ways could be used, the first is the pattern of the insect succession and the second is to use the insect growth rates (Bourel *et al*, 1999). Maggots collected from decomposed corpus, can serve as alternate samples reflecting the evidence or absence of the drug in the body (Beyer *et al*, 1980; Wilson *et al*, 1993; Kintz *et al*, 1994; Sadler *et al*, 1995; Hedouin *et al*, 1999; De-Letter *et al*, 2000). In some instances where the whole cadaver tissues and fluids were completely decomposed, collected fly larvae can give clear evidence if the corpus was drug abused or not (Beyer *et al*, 1980; Kintz *et al*, 1990a). Analysis of the maggot tissues (Hedouin *et al*, 1999; Pounder, 1991; Introna *et et al*, 1990) and puparia (Introna *et al*, 1996; Wilson *et al*, 1993) using different techniques such as TLC or liquid chromatoprapghy determined the drugs amount present in mag-

gots' body and served to deduce or expect of accumulated drugs amount within abused or overdosed corpuses (Kentz *et al*, 1990b,c; Introna *et al*, 1990). Also, when certain toxic materials such as drugs are present within the corpse tissues, it can affect the larval growth rates, leading to inaccurate determination of the minimum PMI. Previous studies investigated the effect of the drugs on the growth rates of the blowflies, in case of morphine (George *et al*, 2009; Bourel *et al*, 1996l 1999); paracetamole (O'Brien and Terner, 2004); codein (Kharbouche *et al*, 2008); tramadol (Lamia *et al*, 2011) and diazepam (Carvalho *et al*, 2001).

Little studies dealt with the effect of drugs on the developmental rates of the flesh flies, for example, in case of cocaine, Goff *et al.* (1989); Goff *et al.* (1991) in case of heroin; methamphetamine, (Goff *et al*, 1992); amitriptyline, (Goff *et al*, 1993); phencyclidine (Goff *et al*, 1994) and methenedioxymethamphetamine, (Goff *et al*, 1997).

The main goals of the present study were: 1- To investigate qualitatively and quantitatively the amount of tramadol or its metabolites consumed by the sarcophagid larvae and pupae from tramadol treated rat livers. 2- To determine the effect of accumulated tramadol inside rat livers on the duration and the development, of the larvae and pupae of certain sarcophagid flesh fly *S. argyrostoma*.

Materials and Methods

Animal model: The laboratory rat, *Rattus norvegicus* (Wistar albino strain), was used in this study. With an average weight of 200±12.9g, 90 rats were used and were kept at the animal house of Zoology Department, under controlled temperature (25-27°C) and relative humidity (30-40%). The Committee of Zoology Department, Faculty of Science, Fayoum University, on Animal Research and Ethics (FU-CARE) permitted the use of the experimental rats; approved and monitored the method of euthanizing the animals (permission # I25-013). FU-CARE follows the CITES no. 123 of 18 March 1986 and 2005 revision of the European convention for the protection of vertebrate animal used for experimental and other scientific purposes (http://conventions.coe.int/Treaty/EN/Reports/HTML/123.htm) and the Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (C (2007) 2525: http://ec.europa.eu/transparency/regdoc/rep/3/2007/EN/3-2007- 2525-EN-1-0.Pdf).

The stock colony of the flesh fly *Sarcophaga argyrostoma*: AbouZied (2016) recorded that adults and larvae of *S. argyrostoma*, were the most attracted and abundant species among fly fauna towards addicted rat carcasses, in the campus of Fayoum University. Therefore, the colony was established from a single wild female catch, since 2014. Genitalia of the third generation males were dissected under stereomicroscope, cleared in potash and examined by El-Hawagry (Professor of Insect Taxonomy, Entomology Department, Cairo University). Genitailia were also sent to Thomas Pape (Professor of Taxonomy, Danish Natural History Museum, Copenhagen) for justifying our identification. The colony was reared inside the insectary of Faculty of Science, Fayoum University. The laboratory temperature ranged from 23 - 26°C and 45-50% relative humidity. The photoperiod ranged from (16:8 L/D).

Dose determination: Tramadol hydrochloride tablets (200 mg) (Zydol® SR Tabs; Grünenth- al Ltd, UK), were used during the progress of this study. Each tablet (weight 0.35±0.03g) was ground in a dry-sterilized porcelain mortar. According to Paget and Barnes (1964), the corresponding dosage for the albino rats was calculated as equal to 6.3 mg/200g rat. Two concentrations of tramadol were used, the recommended dose (D1) and an overdose (D2). In case of the recommended dose (D1), 6.3mg of the tramadol powder was dissolved in 0.2 ml distilled water. The over dose (D2), 12.6 mg was dissolved in 0.2 ml distilled water. Each rat was administered once a day, using stomach tube.

Rats were arranged into 3 groups (30 rats each). G1 was injected with the recommended dose (D1). G2 30 was injected with the over dose (D2). G3 was injected with 0.9 saline solutions as control. After 3 months of tramadol administration (n= 90 doses), rats were killed by cervical dislocation (Cressey 2013), then dissected in isotonic saline solution. Livers were removed from each rat, preserved in plastic bags, labeled and stored in freezer until using to nourish the flesh fly larvae. One gram from each liver was cut off by sharp knife blade, and then labeled either as control, (D1) liver or (D2) liver, frozen at (-20^{o}C) for extraction and further GCMs analysis.

Reagents and chemicals: HPLC grade solvents, of tert-butyl methyl ether, n-hexane, acetone were purchased from Redel and Fluka (Germany) and were used in all procedures without further purification. Borax was obtained from Sigma-Aldrich (Germany). High purity water was obtained through a Milli-Q water purification system (Millipore, Bedford, MA, USA) and was used in all procedures.

Sample preparation: Half gram of each sample (rat liver, larvae and pupae) was accurately weighed and transferred to glass beaker. Borax (0.5 ml) was added to adjust pH at 9. The suspension was homogenized and mixed well with a glass rod, and allowed to stand for 30 minutes. Then 2 ml of tert-butyl methyl ether was added. The test tube was firmly capped, shaken vigorously for 5 minutes and the tube was left for 10 minutes. For phase separation, the upper phase was filtered using fine syringe filter (0.2µm) and the filtrate was transferred to GC vials for analysis.

Gas chromatography: Chromatographic analysis of tramadol was carried out using gas chromatography Agilent 6890N equipped with a 7683B automated injector, flame ionization detector (FID) and 5975 inert XL mass selective detector. The chromatographic separation was achieved with a non-polar column, DB-5MS (60m, ID 0.25mm & film thickness 0.25µm) from J&W Scientific (USA). Helium was used as carrier gas at constant flow 1.0 ml/min. Split less injection mode was used and the injection performed at 260^{o}C. Oven temperature was programmmed as initial temperature (100^{o}C), then increased by 10^{o}C/min to 310^{o}C and held isothermal for 20 minutes at this temperature. The mass spectrometer was scanned from m/z 50 to 500. The ion source, quadruple and interface temperatures were 230, 150 & 310^{o}C, respectively. The chromatographic data was analyzed using Agilent Chemstation Rev. B.02.01-SR1 (260) and MSD Chemstation D.02.00.275. The compounds under study were identified by their retention times and confirmation of identity was performed by mass selective spectrometer (GC-MS).

Estimation of tramadol consumed by larvae of *S. argyrostoma*: One day after female larviposition, 3 groups of larvae (60 larvae each) were picked up under stereomicroscope. Each group was supplied with 60 gm of the corresponding liver. One group fed on liver free tramadol (control), the second group fed on (D1) livers, and the third one fed on (D2) liver. After 3 days, 10 larvae from each group were killed in boiling water, weighted, labeled, and stored at (-20^{o}C) in a polystyrene test tubes. The remaining alive larvae (n=50) were reared till pupation. One week after pupation, 10 pupae from each group were picked up by fine forceps, weighted individually from each group, and then stored as described in case of the larvae. The stored samples of the livers, larvae and pupae were sent for the laboratory of the National Institute of Standards, for further tramadol extraction and chromatographic GCMs analysis.

Effect of tramadol doses accumulated in rat livers, on the developmental stages of *S. argyrostoma*: The remaining alive 50 larvae from each group were kept in plastic containers (50cm diameter x 30cm depth) half filled with moistened fine saw dust and covered with muslin and fastened with rubber

band. Containers were labeled with date and the case of study. During the 3rd day, 30 larvae were picked up randomly from each group; weighted individually using Analytical Sartorius Cubis series balance (max. 250g, 0.00001g range). After weigh-ting, larvae were returned back again to its' original group. Containers were examined daily to record, the mortality of the larvae and the date of the development into pupae. Once pupation was established, they were kept in Wooden rearing adult fly boxes. One week after pupation, all the pupae were weighted individually. Boxes were supplied with powder sugar, and glass bottle filled with moistened cotton piece as a water source for future emerging adults. The laboratory temperature ranged from 23-26°C and 45-50% relative humidity. The photoperiod ranged from (16:8 L/D).

Statistical analyses: Complex online statistical calculator was used to compare between the mean values of the weights and durations of both, the larvae and the pupae of the three cases (the control, D1 and D2 fed larvae). One way ANOVA followed with post-hoc Tukey HSD was established using test calculator which is freely available at http://astatsa.com/OneWay_Anova_with_TukeyHSD/

Results

Identification and quantitation of tramadol: At 21.08 minutes, a characteristic peak appeared in the chromatogram of treated liver samples (Fig. 1A). Compared with the chart of the control, no diagnostic peaks were found, reflecting the complete absence of any tramadol traces (Fig. 2A). Mass spectrum of tramadol treated liver samples detected a compound which has a peak corresponding to its molecular weight at *m/z* 263 in addition to ion base peak at *m/z* 58 (Fig. 3). For identification and quantitation purposes, GC-MS analyses were performed in Scan/SIM mode at two ions 58 and 263 *m/z* and the typical retention time 21.08 minutes (Fig. 1b & 2b). All these criteria were unique for tramadol.

Quantitative analysis revealed that larvae of *S. argyrostoma* fed on D1 liver ingested lower concentration of tramadol (0.11mg/g) compared to that content ingested from D2 liver (0.16 mg/g). Meanwhile, the amount of the tramadol persisted in pupae tissues were 0.07 and 0.09 mg/g, respectively, in cases of D1 and D2 liver. The accumulated tramadol concentrations in the livers of the two cases, D1 (0.72mg/g) and D2 (1.62mg/g) were higher than that detected in larvae and pupae samples. Effect of tramadol on larval stages development of *S. argyrostoma*: Out of 50 larvae used, only 32 larvae were alive after one day feeding on (D2) treated rat liver (mortality rate 36%). Meanwhile, larvae fed on (D1) treated liver tissue suffered lower mortality rates as 4% compared to 2% , in case of that of the control (Table 1).

Larvae fed on (D2) rat livers showed lower duration (6.3438±0.4826 days) compared to (7.0408±1.0198 days; *F= 4.1586; p= 0.0001771*) in controls. Larvae fed on (D1) treated liver showed also significantly shorter duration, compared to the control (6.6383 ±0.4857days, *F= 2.6732; p = 0.0255466*). Non-significant difference (*F= 1.7426; p=0. 2515*) was detected between the change in the durations of the larvae fed on (D1) and (D2) treated livers (Tab. 2).

During the 3rd day, larvae of the control were significantly larger, weighting (18.1807±1.4949 mg) compared to average weight of the larvae fed on D2 liver (14.9560±3.8210 mg, *F=5.3772, p= $3.7045e^{-6}$*). Also, the weight of the control larvae was significantly larger than that of the weight of the larvae fed on D1 livers (14.7279±1.3366; *F= 5.6675, p=$987e^{-7}$*).

Effect of tramadol on pupal stages development of *S. argyrostoma*: Statistical analysis revealed that pupae produced from larvae fed on (D1) treated liver acquired, significantly, longer duration (14.9574±0.6580 days) compared to control (13.9167±1.0071 days; *F= 6.5179; p= 4.8 e^{-9}*), and pupae from larvae fed on (D2) treated liver (14.3750±0.4919 days*; F= 3.2660; p=*

0.0042). Pupae produced from larvae which fed on (D2) treated livers acquired significantly (*F= 3.1404; p*=0.006), higher weight (15.9062±0.2888mg) compared to pupae produced from larvae fed on control liver (14.1750±0.4667mg). Pupae produced from larvae fed on (D1) treated liver showed insignificant (*F=2.1600*; *p=0.082*) higher weight (15.2449±0.302mg), compared to that fed on the control liver during the larval stage. Pupae resulted from larvae fed on the liver treated with (D1) and corresponding liver treated with (D2) showed insignificant change in weight (*F*= 1.2518; *p= 0.427*) (Tab. 2).

Table 1: Amount of tramadol (mg/g) detected in, rat liver, larvae and pupae of *S. argyrostoma*.

	Recommended tramadol dose (D1)	Tramadol double dose (D2)	Control
Liver	0.72	1.62	ND
Larvae	0.11	0.18	ND
Pupae	0.07	0.09	ND

Table 2: Effect of tramadol on mortalities, survival rates, durations and weights of immature stages of *S. argyrostoma*.

	Control	Normal dose (D1)	Over dose (D2)
Number (N)	50	50	50
Alive (n)	49	47	32
Mortality rate	2%	4%	36 %
Survival rate	98%	96%	46%
Larval duration ± SD (days)	7.0408 ±1.0198[a]	6.6383 ± 0.4857[b]	6.3438 ± 0.4826[b]
Pupal duration ± SD (days)	13.9167±1.0071	14.9574±0.6580[b]	14.3750±0.4919[c]
Weight of the 3rd day old larva (mg)	18.1807 ± 1.4949 [b]	14.7279 ± 1.3366 [a]	14.9560 ± 3.8210 [a]
Pupal weight (mg) ± SD	14.1750±0.4667[a]	15.2449±0.302[a,b]	15.9062±0.2888[b]

Post-hoc Bonferroni and Holm T-statistic used one way ANOVA. In rows, similar symbols referred to insignificant difference between group means (*p>0.05*), also different symbols referred to significant difference (*p<0.001, or p<0.05*).

Discussion

Entomotoxicology is defined as the analysis of toxins within the tissues of carrion feeding insects such as flies and beetles. Chromatograms revealed the presence of the tramadol inside the treated liver tissues (D1 and D2) of the rat. Tramadol metabolites were completely absent. The GCMs of the control liver tissues was free from both the tramadol and its metabolites. This agreed with Lamia *et al.* (2011), who detected tramadol by (HPLC) in various organs of experimentally injected rabbits, including the liver. But, Budd and Langford (1999) reported that tramadol was rapidly metabolized to *O*- and *N*- desmethyl tramadol, in human liver. Also, 10-30 % of the tramadol dose was excreted unchanged in the urine.

Chromatograms and mass spectrometry revealed that larvae and pupae of *S. argyrostoma* contained the tramadol. A possible explanation for the appearance of tramadol was that while feeding, larvae ingested the liver tissues together with the incubated tramadol. The tramadol persisted until the larvae reached pupation, but at very low concentrations (0.07 & 0.09 mg/g). Lamia *et al.* (2011) detected tramadol within tissues of *Lucilia sericata* larvae (Meigen). In comparison with other drugs, Beyers *et al.* (1980) analyzed the larvae of *Cochliomyia macellaria* (Fabricius) (Calliphoridae) collected from remains of the cadaver tissue. Analysis of *C. macellaria* larvae tissues revealed the presence of phenol barbital, 14 days after female cadaver suicide, when no tissues or fluids were available. Kintz *et al.* (1990A) found that calliphorid larvae contained 5 drugs (triazolam, oxazepam, phenol barbital and clomipramine) two months after corpus death. Morphine and phenol barbital were both detected in calliphorids larvae developed on chronic heroin abused cadaver (Kintz *et al*, 1990b). Physiologically, when the rate of drug absorption exceeds the rate of drug elimination by both the Malpighian tubules and the nephrocytes, the drugs appeared in maggot tissues (Chapman, 1928).

In this study, tramadol detected in the tissues of *S. argyrostoma* larvae was lower

(6.5-9 times) compared to the tramadol content in D1 and D2 rat livers. Hedouin *et al.* (1999) found that drugs in larvae were 30-100 times lower than that in tissues of the cadavers. The same was true in case of maggots of *Calliphora vicina* (R-D); fed on overdosed cadaver tissues with proxamol and amitriptyline (Wilson *et al*, 1993). Tracqui *et al.* (2004) and Campobasso *et al.* (2004) reported that the concentrations of the drugs present in the larvae were much lower than their concentrations in the cadavers. Kintz *et al.* (1990C) stated that there was a correlation between drugs in human tissues and the amount of drugs detected in maggots fed on such tissues. Introna *et al.* (1990) recorded a significant correlation (r=0.79) between the concentrations of opiates found in larvae and that in the liver tissues. However, Pounder (1991) and Hedouin *et al.* (1999) didn't record any correlation between drug concentration in maggots and that in the cadaver tissue.

Pupae of *S. argyrostoma* contained a very low concentration of tramadol as 0.07 and 0.09 mg/g, respectively, when compared with 0.72mg/g (D1) & 1.62 mg/g (D2). Introna *et al.* (1996) recorded morphine from empty puparia of *C. vicina*, which fed on morphine mixed substrates during the larval stage. However, in case of puparia and adults of *C. vicina* which were fed during their larval stage on overdosed co-proxam and amitriptyline, both were free from any drugs (Wilson *et al*, 1993).

Previous studies demonstrated that the presence of drugs and toxins can alter the developmental rates of carrion insects feeding on decomposed tissues of the cadavers (Catts and Goff, 1992; Goff *et al*, 1992; Bourel *et al*, 1999; Goff and Lord, 1994; Byrd and Castner, 2000; O'brien and Turner, 2004). In this study, the presence of the tramadol in the liver tissues of treated rats (D1 and D2), was the reason for decreasing the duration of the larvae of *S. argyrostoma*, but increased the duration of the pupal stage. AbouZied (2016) found that larvae of *S. argyrostoma* fed on tramadol treated rat carcasses pupated two days after the pupation of the control larvae. Murthy and Mohanty (2010) concluded that heroin speeded up the larval growth of the carrion insects and then decreased the development rate of the pupal stage. Verma and Paul (2013) stated that cocaine and methamphetamine accelerated the rate of the development of the flesh fly *Parasarcophaga ruficornis* (Fabricius) (Diptera: Sarcophagidae).

The effect of the toxins on arthropods depends on toxin concentration (Murthy and Mohanty, 2010). Thus, the over dose (D2) caused a larval mortality rate of 36%, followed by 4% in (D1) concentration. Goff *et al.* (1993) found that larval mortality rates of *P. ruficornis* were significantly higher, in colonies fed on amitriptyline liver than mortality rate of the controls. Also, the mortality rates of treated larvae were inversely correlated with the drug concentrations of (amitriptyline) in the liver (Goff *et al*, 1993).

Larvae fed on (D2) and (D1) liver fed recorded short longevity compared to larvae fed on control liver. Similarly, cocaine lethal dose caused larvae to develop rapidly, 36-76 hours after hatching (Gagliano-Candela and Aventaggiato, 2001).

Pupae resulted from larvae fed on D1 and D2 acquired longer durations compared to that corresponding case of the control. Similarly, colonies of *Boettcherisca peregrina* (Robineau-Desvoidy) fed on tissues of heroin dosed rabbit, required longer pupation time, when compared with that of the control colony (Goff *et al*, 1991). Additionally, with 600-1000mg of amitriptyline treated livers, pupae of *P. ruficornis* required a significantly longer duration, compared to the control colony (Goff *et al.* 1993). Pupation occurred earlier in larvae of *B. peregrina* (Sarcophagidae) feeding on tissues with higher concentrations of cocaine, benzoylecognine, or both (Goff *et al*, 1989). However, in case of cocaine, pupal duration showed insignificant difference among colonies of *B. peregrina* related to the concen-

trations of the cocaine or its metabolite benzoylecognine in tissues (Goff *et al*, 1989). In contrast, pupation occurred earlier when feeding on tissues containing higher concentrations of heroin (Goff *et al*, 1991).

Pupae produced from (D2) liver fed larvae had a higher weight compared to that of the control fed larvae and (D1) fed larvae. Pupae from D1 and the control showed similar weights. Similarly, lethal and twice-lethal dosages of heroin resulted in larger maggots of *B. peregrine* (Sarcophagidae) in all the treated colonies until the maximum size was attained (Goff *et al*, 1998). Also, the sarcophagid larvae *B. peregrina* fed on the control and that fed on the sub lethal dose of cocaine showed nearly the same development rate (Goff *et al*, 1998). Morphine and heroin were both believed to slow down the rate of fly development (Introna *et al*, 2001).

Generally, myiasis is a real welfare problem and many myiasis producers are zoonosis (Morsy, 2014) in Egypt *Sarcophaga* species were involved in gastrointestinal myiasis (Ahmad *et al*, 2011), wound myiasis (Abdel-Hafeez *et al*, 2015). Abroad, they were involved in diabetic foot (Demirel Kaya *et al*, 2014), ophthalmomyiasis (Giangaspero *et al*, 2017) and nosocomial myiasis (Severini *et al*, 2015).

Conclusion

The outcome data showed that larvae of *S. argyrostoma* could be used as taxa to determine the minimum PMI taking into consideration the decrease of the larval duration, in Egypt. Larvae and pupae of *S. argyrostoma* can give brief evidence about the presence or absence of any drugs, especially, when the tissues and body fluids of the cadavers are completely consumed. It was recommended to use the larvae of *S. argyrostoma* as taxa to determine the minimum PMI and prove the presence of toxins and drugs when all the tissues of the cadaver were depleted.

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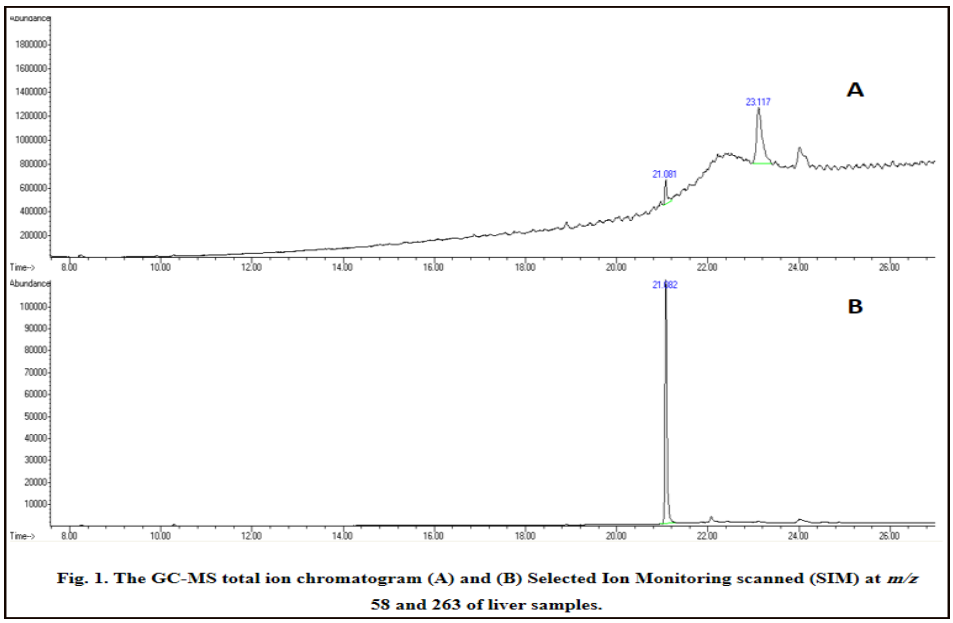

Fig. 1. The GC-MS total ion chromatogram (A) and (B) Selected Ion Monitoring scanned (SIM) at *m/z* 58 and 263 of liver samples.

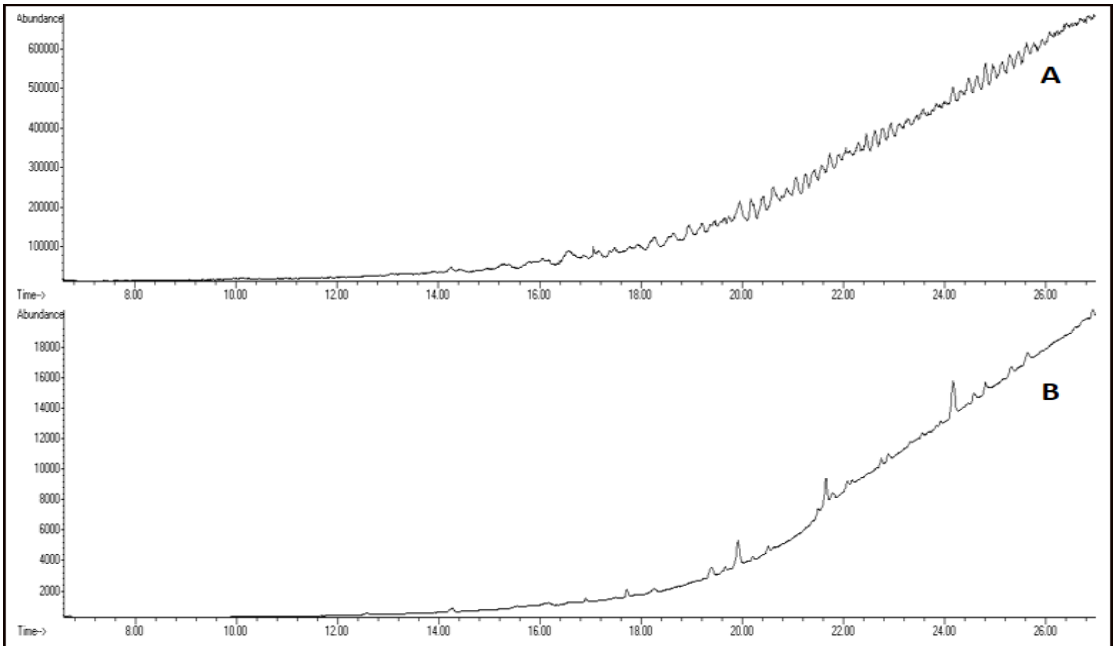

Fig. 2. The GC-MS total ion chromatograms (A) and (B) Selected Ion Monitoring (SIM) scanned at *m/z* 58 and 263 of control samples of rat liver, and larvae of *S. argyrostoma*.

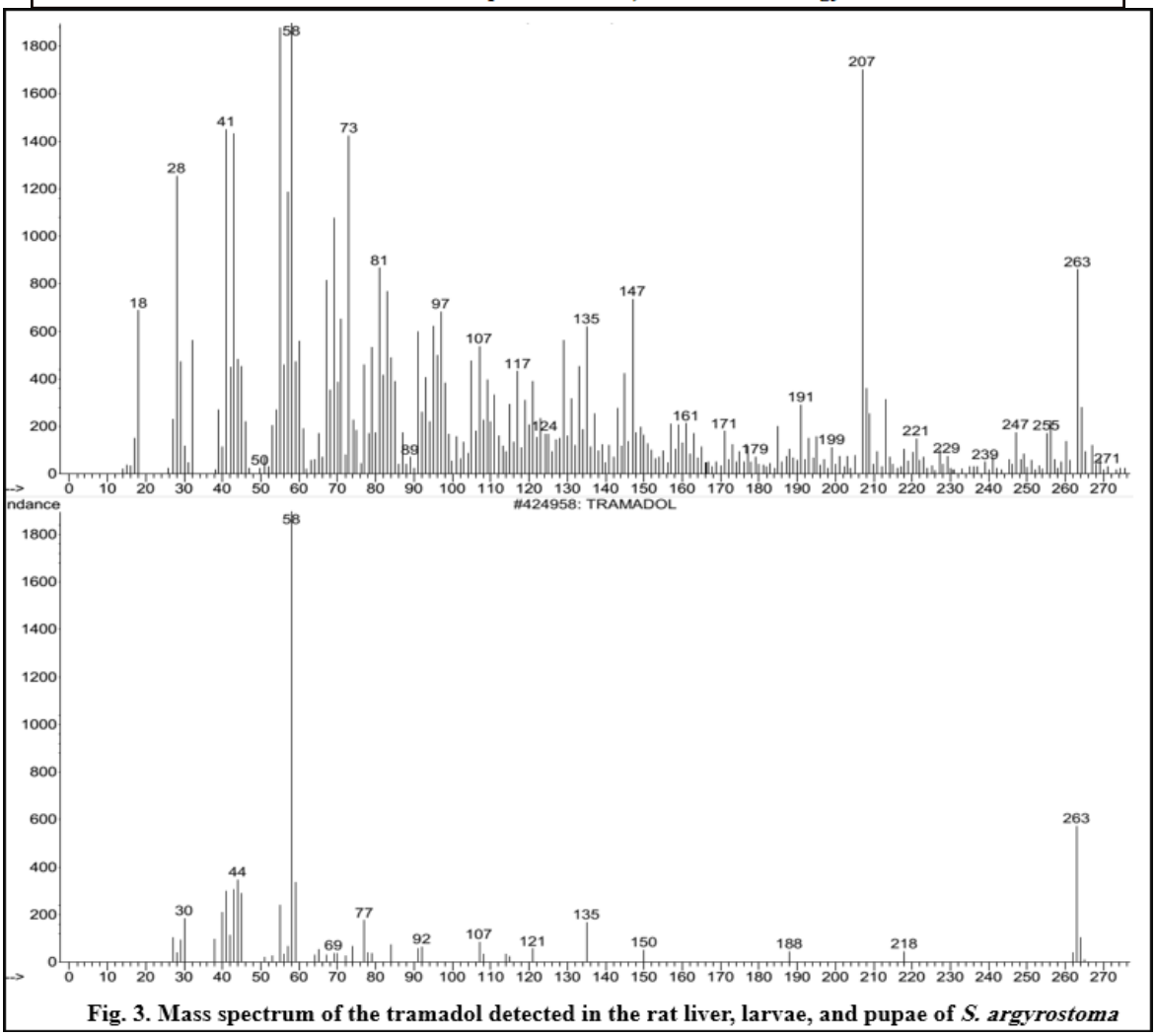

Fig. 3. Mass spectrum of the tramadol detected in the rat liver, larvae, and pupae of *S. argyrostoma*