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Histopathological Effects of Carbaryl on Digestive System of Snake-eyed Lizard, *Ophisops elegans*

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Abstract We examined the effects of carbaryl in the digestive system of *Ophisops elegans*. Lizards were exposed once to different concentrations of carbaryl (2.5, 25 and 250 μg/g). After 96 h, findings related to the esophagus in all treatment groups were not conspicuous. The most important histological defects were observed in the stomach. In the small intestine, collapse of villi was prominent at high-dose. In the large intestine, disintegration in epithelial cells and scattered secretory granules of goblet cells were observed at high dose.

Keywords *Ophisops elegans* · Lizard · Carbaryl · Digestive system

Carbaryl (1-naphthyl-*N*-methylcarbamate) is a broad spectrum pesticide in the carbamate family which is widely used in gardening, commercial agriculture, and forestry as well as being used to control the crustacean predators of shellfish and aquatic weeds in bays and estuaries (Pfeiffer et al. 1997; Kiely et al. 2004). Due to having high efficiency and low persistence in the environment, carbamates which are systemic and contact pesticides are used as substitutes for organochlorine insecticides (Ribera et al. 2001). Carbaryl has a wide range of use in Turkey (Delen et al. 2005).

Carbamate insecticides are reversible inhibitors of the enzyme AChE (Gruber and Munn 1998; Mora et al. 2000). Some studies support that carbaryl causes various adverse effects such as neurotoxicity, developmental toxicity, mutagenicity, oncogenicity and immunotoxicology (Cranmer 1986).

A large number of studies examining the adverse effects of pesticides have been conducted on birds and fish. However, Hopkins (2000) stated that relatively few studies have been carried out on herpetofauna. Recently, some studies related to the effects of AChE-inhibiting pesticides on amphibians have been conducted (Boone and Bridges 2003; Rohr et al. 2003; Boone et al. 2004; Metts et al. 2005; Relyea 2005), also the adverse effects of carbaryl and carbamates on amphibians' health have been examined (Bridges 1997, 2000; Relyea and Mills 2001; Relyea 2003; Davidson et al. 2007; Bacchetta et al. 2008; Kang et al. 2010).

From this point of view, the aim of the study was to investigate histopathologic effects of carbaryl on the digestive system of the snake-eyed lizard, *Ophisops elegans*, which is a model laboratory organism for reptile toxicity studies (Cakici and Akat 2012).

Materials and Methods

The protocol was approved by the animal ethical committee of Ege University, Faculty of Medicine (2011-048). The study was carried out on adult lizards (*O. elegans*), widely distributed in Turkey. They were caught by hand around Ozdere, İzmir/Turkey (N 38°03′ and E 27°02′). There were no important variations in size, age and weight (3.17 g ± 0.04) among the lizards. They were maintained in the Reptile Biology and Ecology Research Laboratory at Ege University Campus, Bornova-İzmir. After 15 days of acclimation, the lizards were randomly assigned to either the carbaryl-treated groups (low dose: 2.5 μg/g, medium dose: 25 μg/g and high dose: 250 μg/g) or to the control

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group, each consisting of 8 lizards (4 male/4 female). Each group was housed in a terrarium (60 × 45 × 60 cm) with glass sides and a plastic floor being covered a 40 mm layer of sand and small stones. For each treatment were made duplicates, so the "n" number represents eight lizard for each duplicate (n = 16).

Lizards were maintained under laboratory conditions at a 12-h dark/light cycle, 22 ± 3°C temperature and 45%–50% relative humidity. Control and treated lizards were fed once a day mainly on two or three ants (*Messor structor*)/flies (*Musca domestica*). Carbaryl (purity 98%) was supplied by AgroBest Grup (İzmir, Turkey).

Carbaryl concentrations were administered once by oral gavage to examine histologic alterations at the end of the 96 h. The chosen concentrations of carbaryl were designed according to DuRant et al. (2007). They estimated that 1 g of prey could ingest dose concentrations ranging between 3.9 and 78.5 mg/g up to 2 days following carbaryl application and factoring the short half-life of carbaryl into their estimates, they selected three doses that encompass the range of concentrations that they believed lizards could encounter in the environment.

Ninety-six-hours following exposure to carbaryl, the lizards were euthanized by decapitation with a guillotine and their digestive tracts were quickly removed. Tissue samples were fixed in Bouin's fixative (Strobel et al. 1981) for 24 h, dehydrated in ethanol, cleared in xylol, and embedded in paraffin. Serial sections at 5 μm were stained with Harris Haematoxylin and Eosin (Humason 1979) and examined by light microscopy. They were photographed with Olympus CX31-Altra 20 Soft Imaging System and Leica DM 4000B-Olympus DP 71 Imaging System.

Histopathological findings were assessed by considering 100 cells of each part of the digestive tract (esophagus, stomach, small and large intestine) in each lizard. They were randomly selected and measured in terms of epithelial cell area and its nucleus area. Data were presented as mean with standard deviation (SD). The differences were compared for statistical significance by one-way ANOVA with post hoc analysis using Tukey HSD test. Statistical evaluations were carried out via SPSS 16.0 (SPSS Inc 2007). We set the significance level at $p \leq 0.05$.

Results and Discussion

The current study was carried out to determine histopathologic alterations in the digestive system of *O. elegans* which was exposed to different carbaryl concentrations. A slowing down of the movements of the high-dose animal group was observed, but difficulty in feeding was not observed. However, death did not occur in any treated lizards during the experiment. Histopathological defects were more clearly seen in the medium (25 μg/g) and high-dose (250 μg/g) groups than in the low-dose (2.5 μg/g) group.

Our knowledge is very limited to the effects of pesticides on reptiles. Therefore, we can only compare our data with a few studies related to pesticides on other vertebrates, especially fish.

We also measured epithelial cell area and its nucleus area to determine whether there were any statistical differences in these end points of digestive system parts after carbaryl exposure. There were no statistical alterations in the esophagus related to the area of epithelial cell and area of nucleus in all experimental groups compared to control. The stomach epithelial cell area and the stomach epithelial cell nucleus area were statistically smaller in the medium and high-dose groups. In the small intestine, epithelial cell area was statistically meaningful in all carbaryl treated groups. Nucleus area of epithelial cell in the small intestine was statistically smaller in the medium and high dose groups. In the large intestine, epithelial cell area was statistically smaller in the medium and high-dose groups. Nucleus area of epithelial cells in the large intestine was only statistically meaningful in the medium-dose group (Table 1). Sastry and Sharma (1981) found that a few villi were necrosed and nuclei were reduced in volume in the intestine of *Ophiocephalus punctatus*.

Histologic examinations in this study showed that the epithelial layer of the esophagus was formed of ciliated columnar cells and mucous secreting goblet cells in the control group (Fig. 1a). In the low-dose group, a weak hemorrhage was determined in the connective tissue, but there were no significant changes in epithelial layer or connective tissue in the esophagus (Fig. 1b). Prominence of hemorrhages was found in the medium-dose group (Fig. 1c). In the high-dose group, separation of muscle layer from the connective tissue was observed in the esophagus (Fig. 1d).

The stomach was lined with mucous secreting columnar epithelium which displayed numerous invaginations and gastric pits which gave rise to gastric glands (Fig. 2a). Gastric gland cells had notable vacuolisation in the low-dose group (Fig. 2b). However, important changes in the tissue structure of the stomach in medium and especially in high-dose groups were observed. In the medium-dose group, degenerations in both epithelial layer and gastric gland cells were determined (Fig. 2c). Epithelial cells were scattered and gastric glands disappeared in the high-dose group (Fig. 2d). Sastry and Gupta (1979) studied the effects of a sublethal concentration (6.8 mg/L) of cadmium chloride in the teleost fish, *Heteropneustes fossilis.* The erosion of mucous epithelium was determined in the stomach. Similar histopathological changes were observed in the stomach of *O. elegans*, and gastric gland

Table 1 Epithelial cell area and nucleus area of epithelial cell (μm^2) in treated groups

Digestive system parts	Control	2.5 μg/g	25 μg/g	250 μg/g
Esophagus epithelial cell area	130 ± 35.4	131.3 ± 36.8	120.3 ± 17.4	121.9 ± 22.1
Esophagus nucleus area of epithelial cell	30.3 ± 5.8	31.9 ± 10.7	31.9 ± 10.7	30.4 ± 8.7
Stomach epithelial cell area	181.3 ± 39.5	165.4 ± 33	41.2 ± 10.6*	52.9 ± 12.8 *
Stomach nucleus area of epithelial cell	31.5 ± 4.19	37.8 ± 8.6	11.5 ± 2*	15.8 ± 3.2 *
Small intestine epithelial cell area	221.2 ± 19.7	137.1 ± 26.7*	91.6 ± 22.3*	87 ± 22.4*
Small intestine nucleus area of epithelial cell	51.2 ± 14.1	40.2 ± 4.1	17 ± 6.4*	24.3 ± 7.2 *
Large intestine epithelial cell area	418.7 ± 108.5	369.6 ± 91.8	198.5 ± 53.1*	151.4 ± 29*
Large intestine nucleus area of epithelial cell	50.3 ± 9.3	41.8 ± 5.1	36.6 ± 10.5*	41.7 ± 5.8

Values are given as mean ± standard deviation (n = 8) in duplicate *$p \leq 0.05$ (statistically significant difference from control)

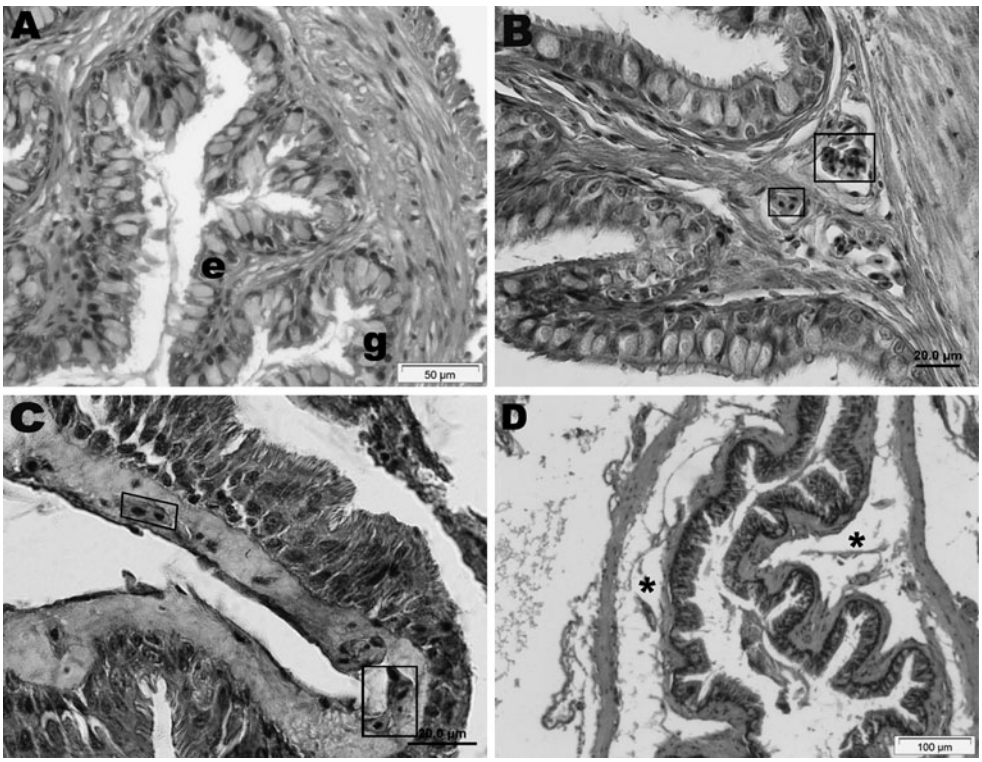

Fig. 1 **a** Normal appearance of esophagus. Epithelial cells (*e*), goblet cells (*g*), **b** hemorrhage (*rectangulated*) in connective tissue of esophagus in low dose group. **c** Prominent hemorrhage (*rectangulated*) in connective tissue of esophagus in medium dose group. **d** Separation (*asterisk*) of muscle layer from connective tissue of esophagus in high dose group

degeneration was determined. Particularly in the medium and high dose groups, prominent erosion was observed in the structure of stomach in *O. elegans*. The effects of a sublethal concentration (0.32 mg/L) of Dimecron for 20 days on the digestive system of a fresh water teleost fish, *Channa punctatus* were investigated (Sastry and Malik 1979). The mucosa and gastric glands of the stomach were disrupted like in the medium and high-dose carbaryl-treated groups in *O. elegans.*

Epithelial layer contained two types of cells in the small intestine: columnar absorptive cells and goblet cells. There were microscopic extensions in the absorptive cells; called microvilli (Fig. 3a). There were no changes in the small intestine of the low-dose group. On the other hand, degeneration of epithelial cells was first observed at medium-dose (Fig. 3b). Disruption in villi and prominent hemorrhage were noticed in the high-dose group (Fig. 3c, d). Histopathological alterations in the intestine of *C. punctatus* induced by chronic nonlethal levels of Elsan (211 ppb), mercury (16.7 ppb), and ammonia (15.64 ppm) were studied at 7 day intervals for 90 days (Banerjee and Bhattacharya 1995). Destruction of the structure of villi was prominent in *C. punctatus* like in *O. elegans.* The intestinal lesions which were noted included infiltration of eosinophils into the lamina propria and atrophy of epithelial cells in *Cirrhinus mrigala* which was exposed to sublethal concentrations (0.3 and 0.6 ppb) of lambda-cyhalothrin for 10 days

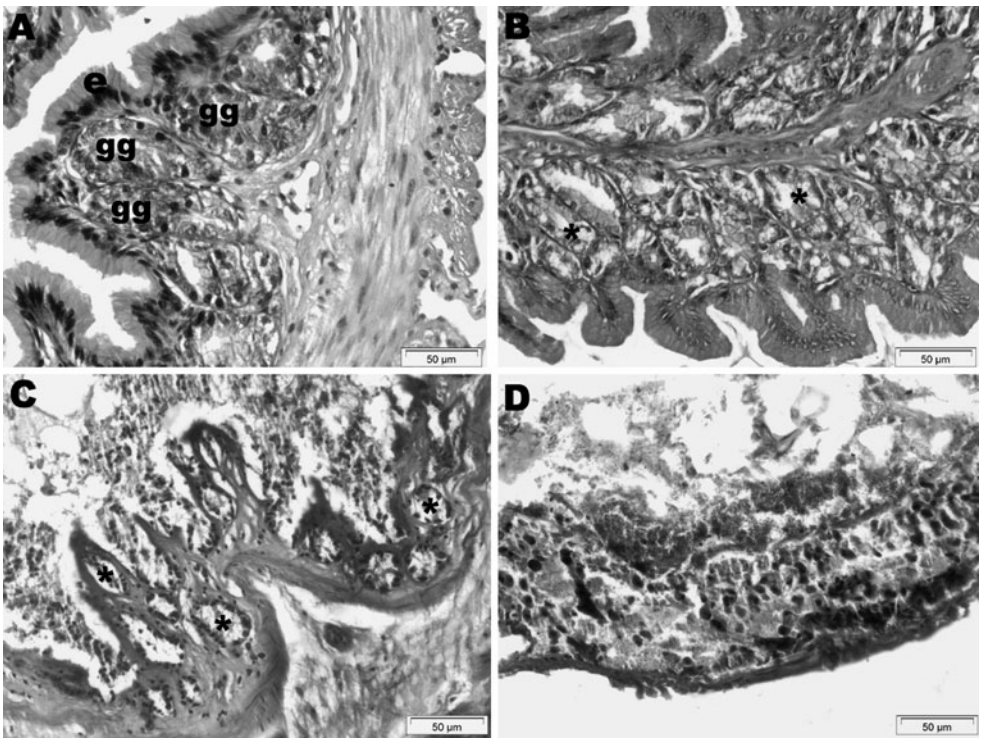

Fig. 2 **a** General view of stomach in *O. elegans*. Note that normal appearance of gastric glands (*gg*) and epithelial layer (*e*), **b** vacuolization in epithelial cells of gastric glands (*asterisk*) of stomach in low dose group, **c** a high degree of degeneration in both epithelial (*e*) and gastric gland (*asterisk*) cells in medium dose, **d** disintegration in epithelial layer and gastric glands in high dose group

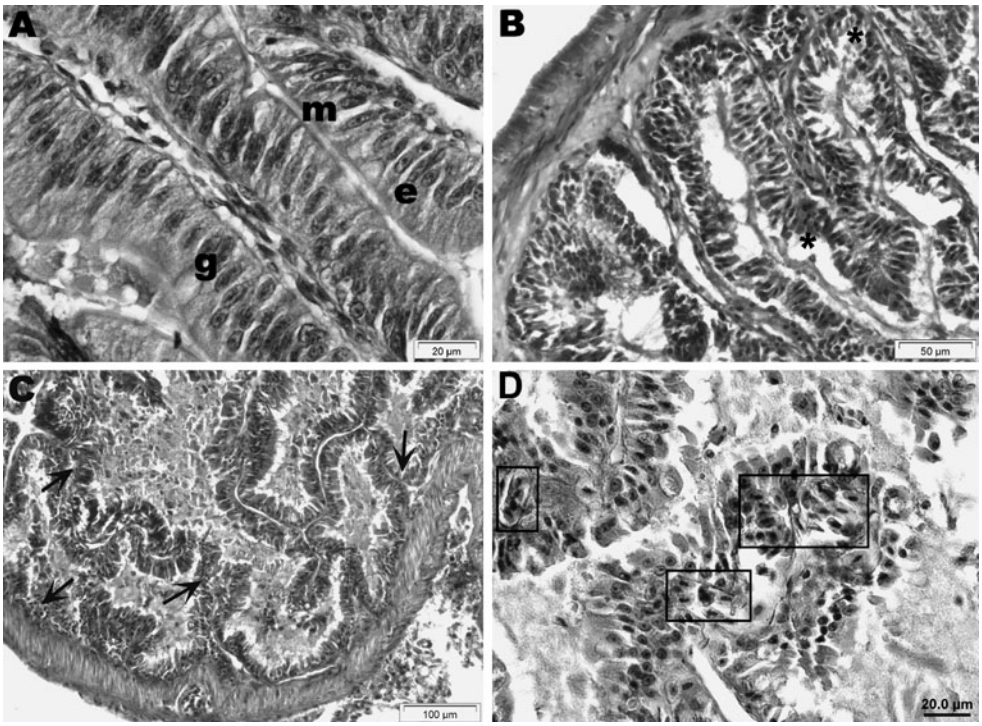

Fig. 3 **a** Epithelial cells (*e*), goblet cells (*g*) and microvilli (*m*) in small intestine of control group, **b** disruption (*asterisk*) in epithelial cells of small intestine in medium dose, **c** villi degeneration (*arrow*), **d** hemorrhage (*rectangulated*) in small intestine of high dose group animals

(Velmurugan et al. 2007). In *O. elegans*, the high-dose group showed considerable hemorrhage in the small intestine. Ozelmas and Akay (1995) showed the hyper-secretion in mucous-secreting activity and perinuclear vacuolisation in the small intestine of the low dose group in the lizard, *Lacerta parva*, after exposure to different concentrations of malathion (1, 2, 3 mg/kg). Degeneration of villi, atrophy and ruptured villi were observed in the small intestine of the medium and high-dose groups like in *O. elegans*.

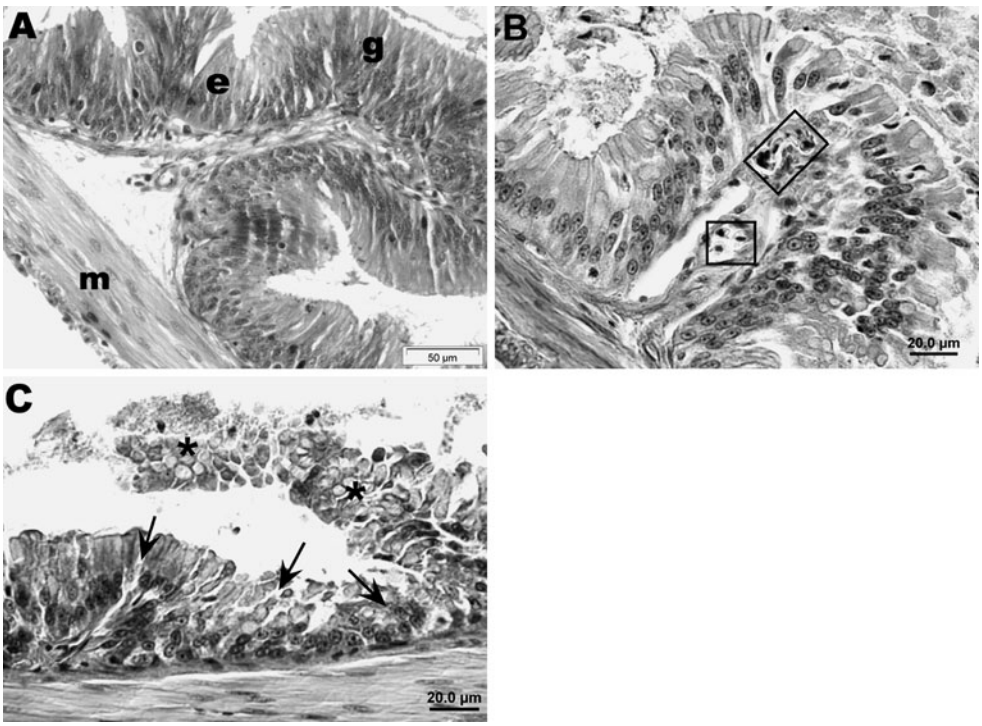

Fig. 4 **a** Epithelial cells (*e*), goblet cells (*g*) and muscle layer (*m*), **b** a weak hemorrhage (*rectangulated*) in large intestine of medium dose, **c** secretion granulles (*asterisk*) of scattered goblet cells and disintegration of epithelial cells (*arrow*)

The epithelial layer of the large intestine was composed of columnar epithelial cells arranged as a single layer forming small folds. There were many goblet cells among the epithelial cells (Fig. 4a). A weak hemorrhage was observed at the medium dose (Fig. 4b). Disintegration of epithelial cells and secretory granules of scattered goblet cells were determined in the high-dose group (Fig. 4c). Scattered mucous secretory granules were present in the lumen of the large intestine of *O. elegans* in the high-dose group. Sastry and Gupta (1979) determined the intestinal degeneration and an increase in the mucous secretion of goblet cells in the intestine of *H. fossilis*. In *C. punctatus,* the mucosal epithelium of the intestine was degenerated, mucous secreting goblet cells showed hyperactivity and the intestinal lumen was filled with mucous (Sastry and Malik 1979).

According to the results of this study, carbaryl caused adverse effects on the digestive system of the lizard, *O. elegans*. Due to the fact that, there is no sufficient study related to reptiles, especially lizards and snakes which are the least studied taxa within ecotoxicological studies, it should be noted that our findings could be of importance in terms of providing direction for other reptilian ecotoxicological studies that intend to focus on the area concerning the exposure of reptiles to contaminants.

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