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Bioaccumulation and effects of organochlorine and pyrethroid pesticides residues on *Oreochromis niloticus* and *Mugil cephalus* from some fish farms at El-Fayoum Governorate, Egypt.

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ABSTRACT

The residues of several organochlorine and pyrethroid pesticides were measured in the gills and intestine of *Oreochromis niloticus* and *Mugil cephalus* that were collected from three fish farms at El-Fayoum Governorate, during spring 2006. The impact of the accumulated pesticides on the total proteins, total lipids and the histological structure of the gills and intestine was studied. The results showed the existence of a wide variety of organochlorine pesticides residues (α-HCH, γ-HCH, Aldrin, Heptachlor, Dicofol, Chlordane, Dieldrin, Endrin and *o,p*-DDT) and pyrethroid pesticides residues (Thiram, Pencycuron, Atrazine, Butachlor, Diniconazole, Cypermethrin and Fenvalerate) in the gills and intestine of both fishes. The total protein and lipid contents in the gills and intestine of the studied fishes from the three fish farms showed a highly significant decline. Moreover, several histopathological alterations as severe degenerative, necrotic and proliferative changes were observed in the gill filaments and secondary lamellae. Furthermore, edema, curling and atrophy in secondary lamellae, as well as dilation and congestion in the blood vessels of gill filaments and haemorrhage between gill filaments were noticed. On the other hand, the intestine showed severe degenerative and necrotic changes in different intestinal layers with aggregation of necrotized cells in the intestinal lumen, atrophy in the muscularis, haemorrhage in the submucosa, edema between the intestinal layers, increased goblet cell population and dilation in the blood vessels of the submucosa.

Key words: Pesticides, bioaccumulation, proteins, lipids, histopathology, *Oreochromis niloticus*, *Mugil cephalus*, fish farms, El-Fayoum Governorate.

INTRODUCTION

Pesticides contamination of aquatic environment is one of the main causes of imbalance in the biota of the aquatic system. Several fish farms that are found around Lake Qarun in El-Fayoum Governorate are mainly dependant on agricultural drainage water, which is loaded with pesticides residues that exert a hazardous effect on fish.

Among all forms of chemical pesticides, organochlorines (such as DDT and others) are considered to be the most hazardous with respect to environmental pollution, since they are very persistent, non biodegradable and add to the residue build up in the food chain. They are lipophilic in nature, and the lipid content in an organism influences chemical bioconcentration (Schuurmann and Markert, 1998). In fish, bioconcentration from water *via* the gills, skin and ingestion of contaminated food are the possible routes for organochlorine pesticides to accumulate in different tissues. Svobodova *et al.* (1993) stated that organochlorine pesticides act as nerve poisons and are highly toxic to fish.

The natural and synthetic pyrethroids have emerged as a major class of highly active pesticides that have proved to be good substitutes for organochlorines, organophosphates and carbamates pesticides due to their lower persistence and comparatively lower mammalian toxicity (Parvez and Raisuddin, 2006a). The pyrethroids are highly active and used extensively in agriculture, for controlling pests and are consequently transported into aquatic environments. The high toxicity of pyrethroids in fish is mainly attributed to their high rate of gill absorption and deficiency in the fish enzyme system to hydrolyze them (Viran *et al.*, 2003). The primary target of the pyrethroid group is the nervous system as judged by their quick action and neuromuscular disorders (Reddy and Yellamma, 1991).

The accumulation of organochlorine and pyrethroid pesticides in the gills and intestine of fish has been studied by several investigators (Ahmed *et al.*, 2001; Jabber *et al.*, 2001; Sapozhnikova *et al.*, 2004; Abdallah and El-Greisy, 2006; Yang *et al* ., 2007).

Exposure to pesticides resulted in biochemical, physiological and histological alterations in the fish and many studies were previously carried out on the effect of pesticides on the levels of the tissue protein and lipid. Reduction in protein levels was observed in the gills of *Tilapia mossambica* exposed to cypermethrin (Reddy and Yellamma, 1991), *Channa punctatus* exposed to monocrotophos (Agrahari *et al.*, 2006) and *Cyprinus carpio* exposed to diazinon (Oruc and Usta, 2007) and in the alimentary tract of *Oreochromis niloticus* exposed to diazinon (Durmaz *et al.*, 2006). However, increases in the protein content were recorded in the gills of *C. carpio* exposed to cypermethrin (Philip and Rajasree, 1996) and *C. punctatus* exposed to paraquat (Parvez and Raisuddin, 2006b). Likewise, increases in the lipid content were observed in the gills of *T. mossambica* exposed to cypermethrin (Reddy *et al.*, 1991) and *C. punctatus* exposed to monocrotophos (Agrahari *et al.*, 2006).

Histopathological alterations have been also reported in the gills and intestine of many fish as a result of exposure to different pesticides (El-Elaimy *et al.*, 1990; Sakr, 1993; Braunbeck and Appelbaum, 1999; Das and Mukherjee, 2000a; Erkmen *et al.*, 2000; Cengiz *et al.*, 2001; Jiraungkoorskul *et al.*, 2003; Dezfuli *et al.*, 2006; Guimaraes *et al.*, 2006; Mohamed, 2006; Wijeyaratne and

Pathiratne, 2006; Yildirim *et al.*, 2006; Campagna *et al.*, 2007; Velmurugan *et al.*, 2007).

The present work aimed to evaluate the residue levels of some pesticides in the gills and intestine of *O. niloticus* and *M. cephalus* collected from three fish farms in El-Fayoum Governorate. Besides, the study investigates the impact of such pesticides on the total protein and lipid contents, as well as the histological structure of the gills and intestine of the two fish.

MATERIAL AND METHODS

1- Field of study:

Three fish farms were selected from several fish farms around Lake Qarun in El-Fayoum Governorate. The source of water for these farms is Dayer El-Berka agricultural drain. The first fish farm has an area of about 4200 m^2, with 750kg of mullet (*Mugil cephalus)* and 1000kg of tilapias fish production. The second fish farm has an area of about 4200 m^2, but with undefined fish production, whereas the third farm has an area of about 8400m^2, with about 850kg of *Mugil cephalus* and 2000kg of tilapias fish production.

2- Collection of fish samples:

Samples of adult *O. niloticus* and *M. cephalus* were collected from the three fish farms during spring 2006. The fish measured about 20.4 to 29.7 and 30.2 to 37.3cm in total length and 200.0 to 500.5 and 280.5 to 670.5g in weight for *O. niloticus* and *M. cephalus*, respectively. After dissection, the gills and intestine of each fish were carefully removed and prepared for pesticides residues analysis and biochemical and histological studies. Another fish sample was collected from Abbassa fish farm to be used as a control group.

3- Pesticides residue analysis:

Multiresidue analysis of fish gills and intestine samples were carried out in Pesticides Research and Analysis Laboratory in Environmental Poison Research Unit, Faculty of Agriculture, Ain Shams University. Extraction of pesticide residues in fish tissues (8g each sample) was carried out using acetonitrile-petroleum ether partitioning. Clean up was done on florisil column with three mixtures (6, 15 and 50% diethylether in petroleum ether) for elution, as described by Anonymous (1990). Gas chromatography apparatus [GC (Shimadzu, 12-A)] provided with FID (Flame Ionization Detector) and ECD (Electron Contraction Detector) was used for separation and identification of the pesticides residues in fish tissues (mg/kg wet wt.).

4- Biochemical analysis:

4.1- Total protein content in tissues:

Sample of 0.1g of gills or intestine was homogenized in a glass homogenizer for 3 minutes in 5ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was used for determination of total protein content (Doughaday *et al.*, 1952).

4.2- Total lipid content in tissues:

Sample of 0.1g of gills or intestine was homogenized in a glass homogenizer for 3 minutes in 5ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was discarded and the pellet obtained was washed with ice cold 10% trichloroacetic acid (TCA). The mixture was centrifuged for 10 minutes at 600 r.p.m and the supernatant discarded. This step was repeated twice with ice cold 5% TCA. The obtained dry pellet was extracted 3 times with a mixture of chloroform: ethanol: ether (1:2:2 [v/v/v]) (Little Field *et al.*, 1955). The combined extract was used for determination of total lipid content (Knight *et al.*, 1972).

5-Histological investigations:

Pieces of gills and intestine were fixed in Bouin's fluid for 24 hours, then dehydrated in ascending series of ethyl alcohol, cleared in xylene and then embedded in paraffin wax. Sections of 4-6μm thickness were cut, mounted on glass slides and stained with Harris' haematoxylin and eosin.

6- Statistical analysis:

The data were expressed as means (M) ± standard deviation (SD) and analyzed using *t*-test (Snedecor, 1962). The values were considered highly significant at $P \leq 0.01$.

RESULTS

I. Pesticides residues in fish tissues:

The residues of pesticides (mg/kg wet wt.) in the gills and intestine of *O. niloticus* and *M. cephalus* from the three fish farms are shown in Table (1). In *O. niloticus* of the first fish farm, only endrin (0.021mg/kg wet wt.) was detected in the gills, however, γ-HCH, Heptachlor, Dicofol, Pencycuron and Atrazine were detected in the intestine (0.012, 0.070, 0.078, 0.017 and 0.015 mg/kg wet wt., respectively). Residues of γ-HCH, *o,p'*-DDT, Atrazine and Butachlor were found in the gills of *M. cephalus*, reaching 0.058, 1.714, 0.043 and 2.049mg/kg wet wt., respectively. On the other hand, γ-HCH (0.034mg/kg wet wt.), Dieldrin (0.016mg/kg wet wt.), Atrazine (0.238mg/kg wet wt.) and Butachlor (2.665mg/kg wet wt.) were detected in the intestine of *M. cephalus*.

In the second fish farm, detecTable levels of γ-HCH, Chlordane and Butachlor were identified in the gills of *O. niloticus* and their concentrations were 0.062, 0.021 and 3.628mg/kg wet wt., respectively. Meanwhile, residues of γ-HCH, Endrin and Thiram were detected in the intestine of *O. niloticus*, reaching to 0.341, 0.013 and 0.448mg/kg wet wt., respectively.

The pesticides detected in the gills of *M. cephalus* were Aldrin, Dieldrin and Diniconazole and their concentrations were 0.014, 0.017 and 2.023 mg/kg wet wt., respectively. However, five pesticides were found in the intestine of *M. cephalus*, namely, γ-HCH (0.07mg/kg wet wt.), Dicofol (0.162mg/kg wet wt.), Chlordane (0.050mg/kg wet wt.), Butachlor (0.084mg/kg wet wt.) and Diniconazole (0.043mg/kg wet wt.).

Table (1): Pesticide residues (mg/kg wet weight) in the gills and intestine of *O. niloticus* and *M. cephalus* from the three fish farms (El-Fayoum Governorate).

Pesticides	Fish farms											
	I				II				III			
	O. niloticus		*M. cephalus*		*O. niloticus*		*M. cephalus*		*O. niloticus*		*M. cephalus*	
	Gills	Intestine	Gills	Intestine	Gills	Intestine	Gills	Intestine	Gills	Intestine	Gills	Intestine
α-HCH	ND	ND	ND	0.034	ND	ND	ND	ND	ND	ND	ND	ND
γ-HCH (Lindane)	ND	0.012	0.058	ND	0.062	0.341	ND	0.07	0.032	ND	ND	ND
Aldrin	ND	ND	ND	ND	ND	ND	0.014	ND	ND	ND	ND	0.019
Heptachlor	ND	0.070	ND	ND	ND	ND	ND	ND	0.078	ND	ND	ND
Dicofol	ND	0.078	ND	ND	ND	ND	ND	0.162	0.071	ND	ND	ND
Chlordane	ND	ND	ND	ND	0.021	ND	ND	0.050	0.016	0.091	ND	ND
Dieldrin	ND	ND	ND	0.016	ND	ND	0.017	ND	ND	0.095	ND	ND
Endrin	0.021	ND	ND	ND	ND	0.013	ND	ND	0.139	ND	ND	ND
O,p'-DDT	ND	ND	1.714	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thiram	ND	ND	ND	ND	ND	0.448	ND	ND	ND	ND	ND	ND
Pencycuron	ND	0.017	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Atrazine	ND	0.015	0.043	0.218	ND	ND	ND	ND	ND	ND	ND	0.203
Butachlor	ND	ND	2.049	2.665	3.628	ND	ND	0.084	ND	ND	ND	ND
Diniconazole	ND	ND	ND	ND	ND	ND	2.023	0.043	0.046	ND	ND	ND
Cypermethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.018	ND	ND
Fenvalerate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.076	ND
Deltamethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND= Not detected or the residues are existed in amounts below the limit of detection (0.01ppm).

In the third fish farm, the gills of *O. niloticus* revealed the occurrence of γ-HCH, Heptachlor, Dicofol, Chlordane, Endrin and Diniconazole by the values of 0.032, 0.078, 0.071, 0.016, 0.139 and 0.046mg/kg wet wt., respectively. Residues of Chlordane, Dieldrin and Cypermethrin were found in the intestine of *O. niloticus* and their concentrations were 0.091, 0.095 and 6.018mg/kg wet wt., respectively. Only Fenvalerate was detected in the gills of *M. cephalus* (0.076mg/kg wet wt.). However, detecTable levels of Aldrin and Atrazine were found in the intestine of *M. cephalus*, reaching 0.019 and 0.203mg/kg wet wt., respectively.

II-Biochemical parameters:

The biochemical data of *O. niloticus* and *M. cephalus* are presented in Table (2). The total protein and lipid contents in the gills and intestine of control fish were 5.33±0.23, 5.97±0.52, 2.02±0.25 and 3.77±0.36g/100g wet wt., respectively for *O. niloticus* and 4.83±0.23, 6.67±0.44, 2.50±0.09 and 4.03±0.27g/100g wet wt., respectively for *M. cephalus*. The total protein contents in the gills and intestine of *O. niloticus* and *M. cephalus* from the three fish farms were highly significantly ($P \leq 0.01$) lower than the control values. Similarly, the gills and intestine lipid content showed highly significant decrease in both fish from the three fish farms, except, the gills of *O. niloticus* from the first fish farm that showed insignificant decrease.

Table (2): Total protein and lipid contents (g/100g wet weight) in the tissues of *O. niloticus* and *M. cephalus* from the three fish farms (El-Fayoum Governorate).

Fish	Site	Gills protein content (g/100g wet weight)		Intestine protein content (g/100g wet weight)		Gills lipid content (g/100g wet weight)		Intestine lipid content (g/100g wet weight)	
		M±SD	t-value	M±SD	t-value	M±SD	t-value	M±SD	t-value
O. niloticus	Control	5.33±0.23	—	5.97±0.52	—	2.02±0.25	—	3.77±0.36	—
	Fish farm I	0.85±0.37 (-84.05)	25.45**	2.11±0.53 (-64.66)	12.73**	1.87±0.03 (-7.43)	1.49	1.64±0.70 (-56.50)	6.74**
	Fish farm II	1.85±0.06 (-65.29)	36.68**	1.89±0.44 (-68.34)	14.80**	1.00±0.11 (-50.50)	9.24**	0.63±0.21 (-83.29)	19.00**
	Fish farm III	1.16±0.38 (-78.24)	24.08**	2.25±0.14 (-62.31)	16.75**	0.88±0.15 (-56.44)	9.74**	0.40±0.03 (-89.39)	23.75**
M. cephalus	Control	4.83±0.23	—	6.57±0.44	—	2.50±0.09	—	4.03±0.27	—
	Fish farm I	1.65±0.15 (-65.84)	28.79**	1.31±0.72 (-80.36)	15.67**	0.52±0.08 (-79.20)	40.42**	0.74±0.03 (-81.64)	29.87**
	Fish farm II	0.74±0.01 (-84.68)	44.30**	2.17±0.70 (-67.47)	13.45**	0.56±0.07 (-77.50)	42.64**	0.88±0.02 (-78.16)	28.65**
	Fish farm III	1.19±0.24 (-75.36)	27.06**	2.01±0.57 (-69.87)	16.18**	0.58±0.05 (-76.80)	45.51**	1.78±0.17 (-55.83)	17.31**

M±SD = Mean ± Standard Deviation. Number of fish used (n) = 6
Figures between brackets are % change from control value. t-value = between control and fish farm value.
** Highly significant differences at $P \leq 0.01$

III- Histopathological alterations:

1- Gills:

The gills of the control fish showed normal histological structure (Fig. 1A), however, several histopathological alterations were detected in the gills of *O. niloticus* and *M. cephalus* from the three fish farms. These alterations included severe degenerative and necrotic changes in gill filaments and secondary lamellae (Figs. 1B,C&L, 2A, 3B,H&I, 4B,C&H, 5A) with necrotized cells aggregated between the gill filaments (Figs. 1D, 2B&C). Proliferative changes in the epithelium of gill filaments and secondary lamellae, resulting sometimes in obliteration of the space between the secondary lamellae, (Figs. 1E,F&G, 3D&K, 4E) and edema in gill filaments and secondary lamellae with separation of their epithelium from the lamellar supporting cells (Figs. 1H&I, 2G, 3C&J, 4D) were also noticed. Moreover, intravascular haemolysis (Fig. 1J), dilation (Figs. 1K, 2D&E) and congestion (Figs. 2D&E, 3F, 4G) were observed in the blood vessels of gill filaments. Also, atrophy (Figs. 1J, 3G) and curling (Figs. 3E&L, 5B) of secondary lamellae were noticed. In addition, in *M. cephalus* from the first and second fish farms, dilation in the blood vessels at the base of secondary lamellae (Fig. 2F) and haemorrhage between gill filaments (Figs. 2H, 3A, 4A) were observed. Besides, in *O. niloticus* from the third fish farm, haemorrhage was observed at the tips of secondary lamellae (Fig. 4F).

2- Intestine:

The intestine of the control fish showed normal histological features (Fig. 5C), while several histopathological alterations were noticed in the intestine of *O. niloticus* and *M. cephalus* from the three fish farms. These

alterations included vacuolar degeneration in the serosa (Figs. 5D&E, 8C&D), severe atrophy in the muscularis (Figs. 5D&G, 8E&F), severe degenerative and necrotic changes in different intestinal layers (serosa, muscularis, submucosa and mucosa) (Figs. 5H, 6A,B,C,D,G&H, 7A,B,G&H, 8A,C,D&G, 9C,D&E) with aggregations of inflammatory cells (Fig. 5F), as well as necrotized cells aggregated in the intestinal lumen (Figs. 5H, 7B, 8A&F). Moreover, edema was seen between different intestinal layers (Figs. 6F, 7D, 8B, 9A,F&G) and also in muscularis (Fig. 7C) and submucosa (Figs. 8E&H). In addition, in *M. cephalus* from the first fish farm, haemorrhage was observed in the submucosa (Fig. 6E). Besides, in *O. niloticus* from the second fish farm, separation of the serosa (Fig. 6G) and increased goblet cell population (Figs. 7E&F) were noticed. Also, in *O. niloticus* and *M. cephalus* from the third fish farm, dilation was observed in the blood vessels of submucosa (Figs. 9B&F).

DISCUSSION

Pesticides have been widely used in Egypt to control various pests and disease vectors. Indiscriminate use of pesticides creates serious problems to aquatic environment since these pesticides usually find their way to the aquatic ecosystem through various routes.

Mohamed and Gad (2007) detected residues of several organochlorines (γ-HCH, Chlordane, Endrin and *o,p'*-DDT) and pyrethroids (Thiram, Atrazine, Butachlor, Fenvalerate and Deltamethrin) in the water of the three studied fish farms (Table, 3). Similarly, Abdel-Tawab (1999) and Sweilum (2004) detected several pesticides (carbaryl, dursban, fenitrothion, fenpropathrin, endrin, heptachlor and reldan) in the water of some fish farms at El-Fayoum Governorate.

Table (3): Pesticide residues (mg/L) in the three fish farms (El-Fayoum Governorate) (according to Mohamed and Gad (2007)).

Pesticides	Fish farms		
	I	II	III
α -HCH	ND	ND	ND
γ -HCH (Lindane)	ND	ND	0.017
Aldrin	ND	ND	ND
Heptachlor	ND	ND	ND
Dicofol	ND	ND	ND
Chlordane	ND	ND	0.080
Dieldrin	ND	ND	ND
Endrin	ND	ND	0.016
o,p'- DDT	0.092	0.042	0.021
Thiram	ND	0.021	ND
Pencycuron	ND	ND	ND
Atrazine	ND	0.015	ND
Butachlor	ND	ND	0.076
Diniconazole	ND	ND	ND
Cypermethrin	ND	ND	ND
Fenvalerate	0.228	ND	ND
Deltamethrin	0.543	ND	1.377

ND= Not detected or the residues are existed in amounts below the limit of detection (0.01ppm).

Fish gills represent a multifunctional organ, which carries out ion transport activities, gas exchange, acid-base regulation and waste excretion *via* the branchial epithelium (Wendelaar Bonga, 1997). It was found that the gills accumulate more pesticides due to their great ability of absorption (Yang *et al.*, 2007). Also, the intestine is one of the most important sites where toxicants are absorbed. Many environmental toxicants enter the food chain and are absorbed together with food from the intestine (Rozman and Klaassen, 2001). The present study showed that the gills and intestine of *O. niloticus* and *M. cephalus* accumulated a wide variety of organochlorine pesticides (α-HCH, γ-HCH, Aldrin, Heptachlor, Dicofol, Chlordane, Dieldrin, Endrin and *o,p*-DDT), as well as pyrethroid pesticides (Thiram, Pencycuron, Atrazin, Butachlor, Diniconazole, Cypermethrin and Fenvalerate). As indicated in Table (1), the most frequent pesticide noticed in the studied fish tissues was γ-HCH, while the least frequent ones were α-HCH, *o,p'*-DDT, Thiram, Pencycuron, Cypermethrin and Fenvalerate. The presence of pesticides residues in the fish tissues of the present study supports the findings reported by several investigators. Ahmed *et al.* (2001) detected residues of endosulfan, heptachlor and dicofol in the gills of *M. cephalus* from Lake Temsah, Suez Canal, Egypt. Jabber *et al.* (2001) found that the levels of organochlorine pesticides residues in *Lates calcarifer* from an estuary in Bangladesh followed the order: gut > muscle > liver. Yang *et al.* (2007) reported that the gills of fish from high mountain lakes and Lhasa River in Tibetan Plateau accumulate more pesticides than the other tissues.

In the present study, the control values of total protein and lipid contents of the gills and intestine of *O. niloticus* and *M. cephalus* were within the same range for perch and roach (Tkatcheva *et al.*, 2004) and *O. niloticus* (Durmaz *et al.*, 2006). The results indicated that the detected pesticides residues in the gills and intestine of *O. niloticus* and *M. cephalus* from the three fish farms induced highly significant reduction in the gills and intestine protein and lipid contents of both fish, however, the gills lipid contents of *O. niloticus* from the first fish farm showed insignificant decrease. The observed reduction in the gills and intestine protein and lipid contents may be due to general stress response. Under stressful conditions, fish secrete high amounts of catecholamines and corticosteroids which produces an enhanced metabolic rate, which in turn deplete metabolic reserves (as proteins and lipids) to provide the energy demand (Durmaz *et al.*, 2006). Moreover, the decrease in tissue protein concentration is an indicator for reduced protein synthesis, low assimilation of food and low amino acid uptake for protein synthesis (Das and Mukherjee, 2000b). Similarly, Abd El-Salam and Lotfy (1993) reported that the depletion of protein content suggests an increased proteolysis or retarded protein synthesis with eventual use of amino acids for energy production. Goal and Agrawal (1981) stated that the depletion of cellular proteins might be caused by one or more of the following factors: inhibition of amino acid incorporation, breakdown of protein into amino acids and diffusion out of the cells. The present results agree with the findings of Reddy and

Yellamma (1991); Agrahari *et al.* (2006); Durmaz *et al.* (2006) and Oruc and Usta (2007).

In the present study, the gills of *O. niloticus* and *M. cephalus* from the three fish farms showed marked histopathological alterations. These alterations included severe degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae, edema in secondary lamellae, intravascular haemolysis and dilation in the blood vessels of gill filaments, atrophy and curling of secondary lamellae. and haemorrhage between gill filaments. Some of the structural changes may serve as a defense mechanism in protecting the fish from the pesticides-contaminated water by increasing the diffusion distance. The observed thickening (proliferation) of the gill epithelium is thought to be an adaptive response directed to increase the blood-water distance and to suppress exchange with water (Tkatcheva *et al.*, 2004). According to Malatt (1985), lifting of epithelia could be served as a mechanism of defense, because the separation of the epithelium of secondary lamellae from the lamellar supporting cells increases the distance across where water-borne pesticides must diffuse to reach the blood stream. Increases in the diffusion distance can adversely affect the gas exchange and might impair the respiratory function of the gills. Ionic regulation might also be seriously affected, since fish gills are involved in ion exchange for osmoregulatory purposes (Dutta *et al.*, 1993).

According to Karlsson *et al.* (1985), the increase of cellular layers of lamellar epithelium may be due to an increase in the mitotic divisions of the lamellar epithelium.

The lamellar and inter-lamellar epithelial necrosis observed in the present study suggests the direct toxic effect of the pesticides on the gill tissue. Elnemaki and Abuzinadah (2003) have indicated these changes in response to the insecticide contra/insect 500/50 E.C. The dilation of the lamellar blood vessels and the presence of edematous fluid in the secondary lamellae may be due to increased permeability induced by the exposure to the pesticides. This edematous fluid separated the respiratory epithelium from the underlying tissue and led to its desquamation, as well as necrosis (Balah *et al.*, 1993; Erkmen *et al.*, 2000). The histopathological changes observed in the gills of the studied fish are in agreement with those observed in other fish species under the influence of different pesticides (Das and Mukherjee, 2000a; Erkmen *et al.*, 2000; Jiraungkoorskul *et al.*, 2003; Dezfuli *et al.*, 2006; Guimaraes *et al.*, 2006 and Wijeyaratne and Pathiratne, 2006). Also, Yildirim *et al.* (2006) reported that exposure of *O. niloticus* to 5µg/l deltamethrin resulted in lamellar fusion in the gills. Exposure of *Danio rerio* to aldrin and heptachlor was found to induce cell proliferation between secondary lamellae, lifting of respiratory epithelial cells, fusion of several secondary lamellae and dilation of blood vessels in the gills (Campagna *et al.*, 2007). Sublethal concentrations (1.5-3.0ppb) of fenvalerate resulted in epithelial hyperplasia, epithelial necrosis, desquamation, lamellar fusion, epithelial lifting, edema, swelling at the tips of secondary lamellae and

curling of secondary lamellae in the gills of *Cirrhinus mrigala* (Velmurugan *et al.*, 2007).

In the present study, several histopathological alterations were observed in the intestine of the studied fish. These alterations included severe degenerative and necrotic changes in the different intestinal layers with necrotized cells aggregated in the intestinal lumen, atrophy in the muscularis, edema between the intestinal layers, haemorrhage in the submucosa, increase in goblet cell population and dilation in the blood vessels of the submucosa. According to Desai *et al.* (1984) and Mohamed (2004), the degenerative and necrotic changes observed in the different intestinal layers of the studied fish may be due to a direct effect of the detected pesticides on the cells, to an accumulation of acetylcholine in the tissues or to a reduction in oxygen supply. The increase in the goblet cell population observed in the intestine of the studied fish may act to immobilize pesticides by binding it to mucus (Naidu *et al.*, 1983). The present results are in agreement with those observed by many investigators about the effects of different pesticides on fish intestine (El-Elaimy *et al.*, 1990; Sakr, 1993; Braunbeck and Appelbaum, 1999; Mohamed, 2006 and Dezfuli *et al.*, 2006). Moreover, Cengiz *et al.* (2001) observed edema, degeneration, accumulation of lymphocytes and disintegration of villi in the intestine of *G. affinis* subjected to thiodan. Velmurugan *et al.* (2007) observed atrophy of epithelial cells, necrosis of epithelial cells, desquamation of mucosal epithelium and infiltration of lymphocytes into the lamina propria in the intestine of *C. mrigala* exposed to fenvalerate.

In conclusion, the present results indicated that the detected organochlorine and pyrethroid pesticides residues in the gills and intestine of *O. niloticus* and *M. cephalus* from the three fish farms (at El-Fayoum Governorate) induced changes in the total protein and total lipid contents of the gills and intestine of the studied fish. Besides, these pesticides residues caused several histopathological changes in the gills and intestine of the fish. Therefore, the agriculture drainage water (the source of water for the fish farms) should be treated before being used in aquaculture.

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EXPLANATION OF FIGURES

Fig. (1): Sections of gills showing control (A) (X100), severe degenerative and necrotic changes in gill filaments and secondary lamellae (B&C), necrotized cells aggregated between the gill filaments (D), proliferation in the epithelium of gill filaments and secondary lamellae (E, F&G), edema in secondary lamellae with separation of their epithelium from the lamellar supporting cells (H&I), intravascular haemolysis in blood vessel of gill filament and atrophy of secondary lamellae (J), dilation in blood vessel of gill filament (K) *(O. niloticus*, 1st fish farm) (X400) and severe degenerative and necrotic changes in gill filaments and secondary lamellae (L) (*M. cephalus*, 1st fish farm) (X400).

Fig. (2): Sections of gills showing severe degenerative and necrotic changes in gill filaments and secondary lamellae (A), necrotized cells aggregated between the gill filaments (B&C), dilation and congestion in the blood vessels of gill filaments (D&E), dilation in the blood vessels at the base of secondary lamellae (F),edema in gill filaments and secondary lamellae (G) and haemorrhage between the gill filaments (H) (*M. cephalus*, 1st fish farm) (X400).

Fig. (3): Sections of gills showing haemorrhage between the gill filaments (A) *(M. cephalus*, 1st fish farm)(X400), severe degenerative and necrotic changes in gill filaments and secondary lamellae (B), edema in secondary lamellae (C), proliferation in the epithelium of gill filaments and secondary lamellae (D), curling of secondary lamellae (E), dilation and congestion in blood vessel of gill filament (F), atrophy in secondary lamellae (G)(*O. niloticus*, 2nd fish farm) (X400), severe degenerative and necrotic changes in gill filaments and secondary lamellae (H&I), edema in secondary lamellae (J), proliferation in the epithelium of gill filaments and secondary lamellae (K) and curling of secondary lamellae (L) (*M. cephalus*, 2nd fish farm) (X 400).

Fig. (4): Sections of gills showing haemorrhage between gill filaments (A)*(M. cephalus*,2nd fish farm) (X400), severe degenerative and necrotic changes in gill filaments and secondary lamellae (B&C), edema in secondary lamellae (D), proliferation in the epithelium of gill filaments and secondary lamellae (E), haemorrhage at the tips of secondary lamellae (F), dilation and congestion in blood vessel of gill filament (G)(*O. niloticus*, 3rd fish farm) (X400) and severe degenerative and necrotic changes in gill filaments and secondary lamellae (H)(*M. cephalus*, 3rd fish farm) (X400).

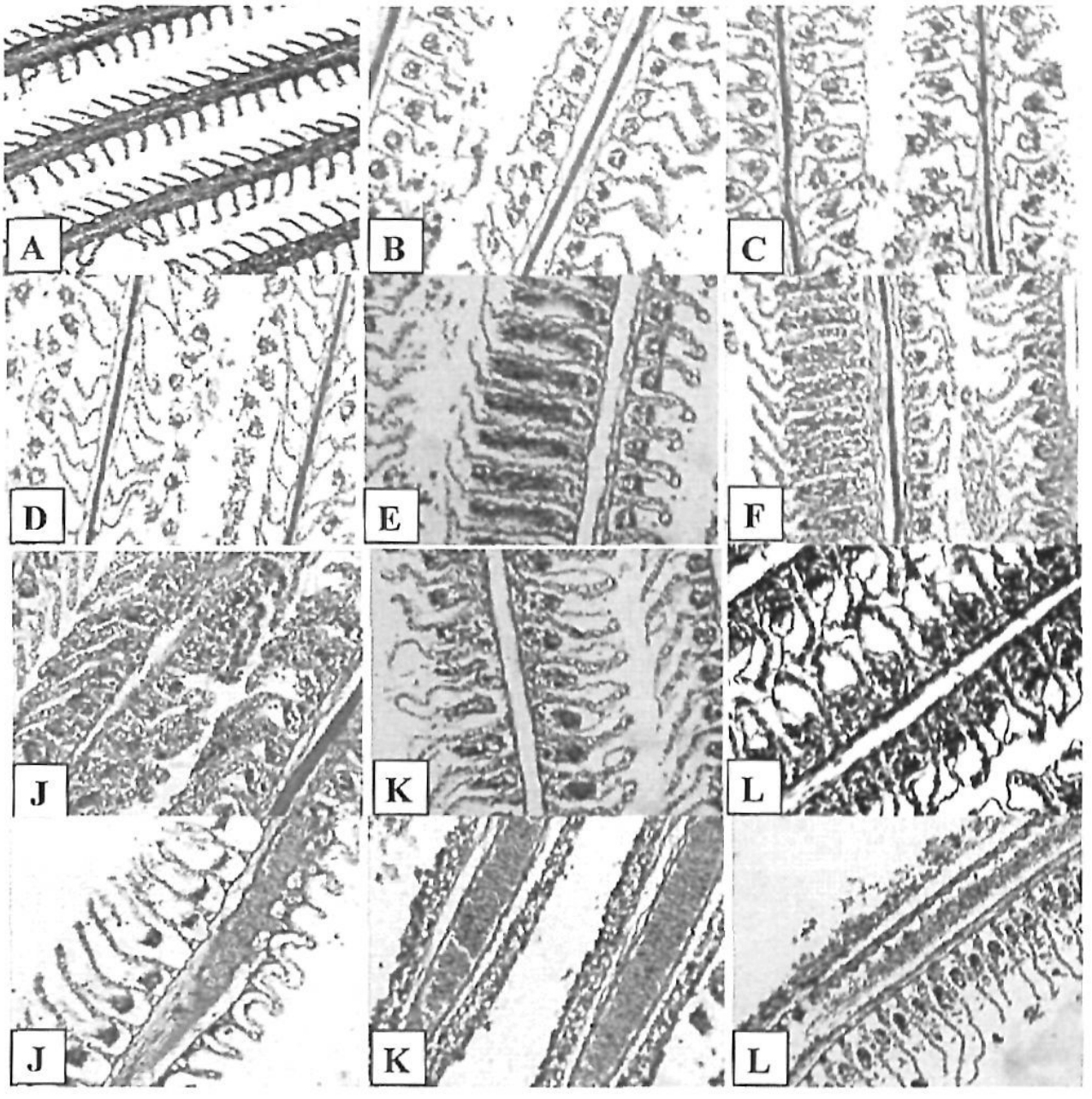
Fig. (5): Sections of gills and intestine showing severe degenerative and necrotic changes in gill filaments and secondary lamellae (A), curling of secondary lamellae (B) (*M. cephalus*, 3rd fish farm) (X400), control intestine (C)(X100), vacuolar degeneration in the serosa and atrophy in the muscularis (D), vacuolar degeneration in the serosa (E), aggregations of inflammatory cells in submucosa and mucosa (F), atrophy in the muscularis (G) and severe degenerative and necrotic changes in submucosa and mucosa with necrotized cells aggregated in the intestinal lumen (H)(*O. niloticus*, 1st fish farm) (X 400).

Fig. (6): Sections of intestine showing severe degenerative and necrotic changes in submucosa and mucosa (A)(*O. niloticus*, 1st fish farm)(X400), severe degenerative and necrotic changes in different intestinal layers (B,C&D), haemorrhage in the submucosa (E), edema between the submucosa and mucosa (F) (*M. cephalus*, 1st fish farm)(X400), separation of the serosa and severe degenerative and necrotic changes in the muscularis, submucosa and mucosa (G) and severe degenerative and necrotic changes in the muscularis, submucosa and mucosa (H)(*O. niloticus*, 2nd fish farm)(X400).

Fig. (7): Sections of intestine showing severe degenerative and necrotic changes in the muscularis, submucosa and mucosa (A&B), edema in the muscularis (C), edema between the muscularis and submucosa (D), increase in goblet cell population (E&F)(*O. niloticus*, 2nd fish farm)(X400) and degenerative changes in the serosa, submucosa and mucosa (G&H) (*M. cephalus*, 2nd fish farm) (X400).

Fig. (8): Sections of intestine showing degenerative and necrotic changes in the mucosa with necrotized cells aggregated in the intestinal lumen (A), edema between the submucosa and mucosa (B)(*M. cephalus*, 2nd fish farm)(X400), vacuolar degeneration in the serosa and severe degenerative and necrotic changes in the submucosa and mucosa (C&D), severe atrophy in the muscularis and edema in the submucosa (E), severe atrophy in the muscularis and necrotized cells aggregated in the intestinal lumen (F), severe degenerative and necrotic changes in the submucosa and mucosa (G) and edema in the submucosa (H)(*O. niloticus*, 3rd fish farm) (X400).

Fig. (9): Sections of intestine showing edema between the submucosa and mucosa (A), dilation in the blood vessel of the submucosa (B)(*O. niloticus*, 3rd fish farm) (X400), severe degenerative and necrotic changes in the different intestinal layers (C,D&E), dilation in blood vessel of the submucosa and edema between the submucosa and mucosa (F) and edema between the submucosa and mucosa (G) (*M. cephalus*, 3rd fish farm) (X400).



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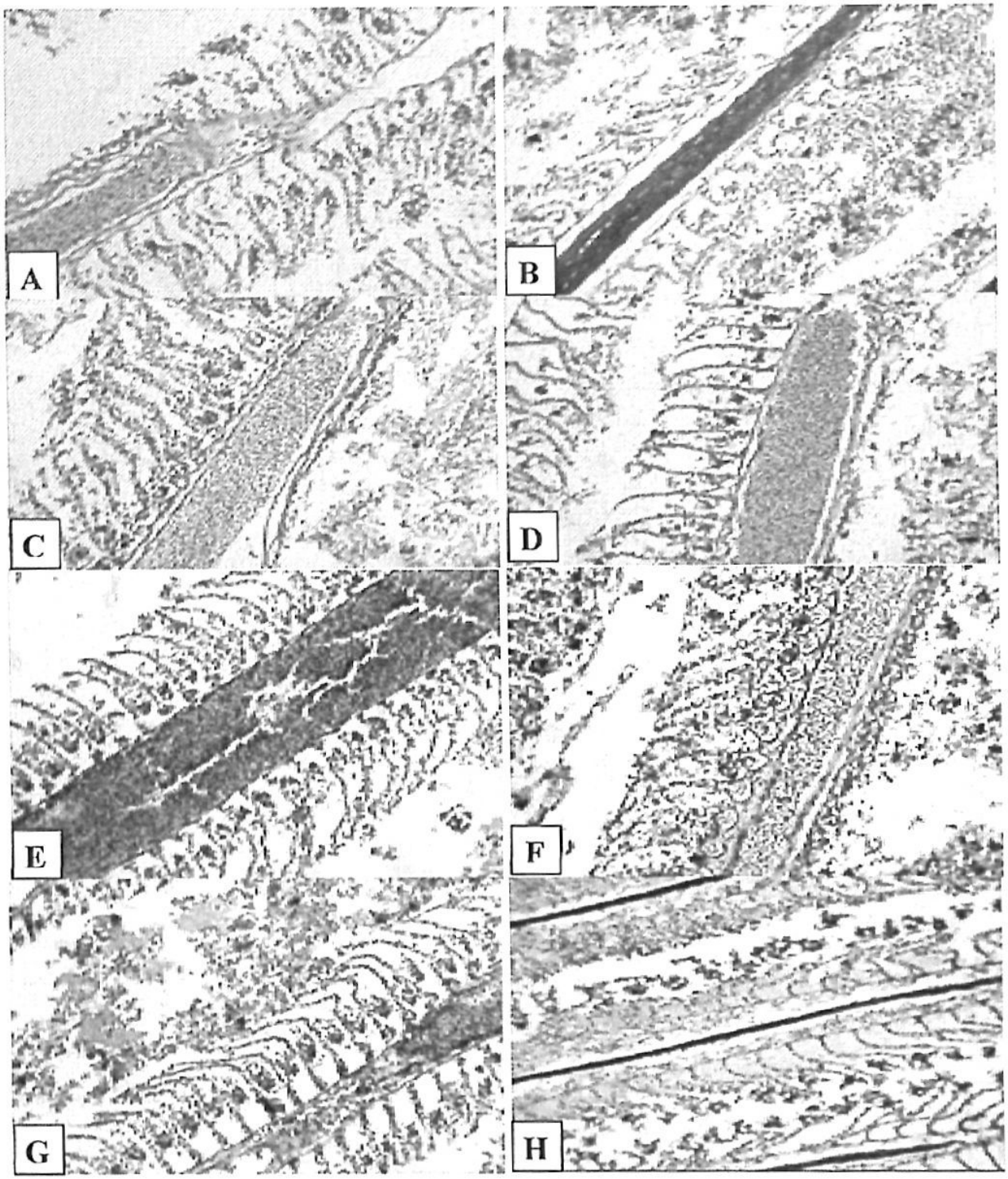

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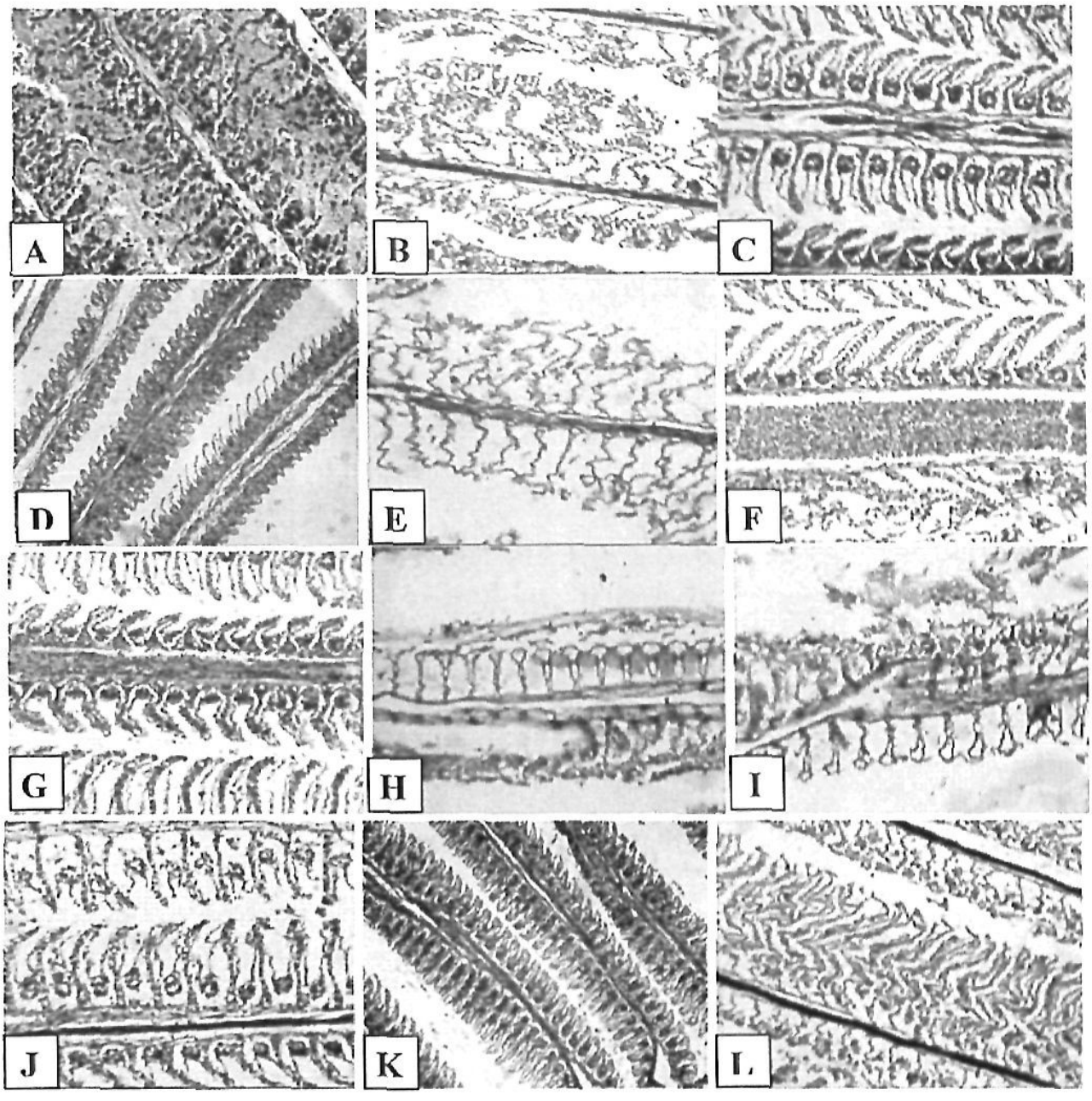

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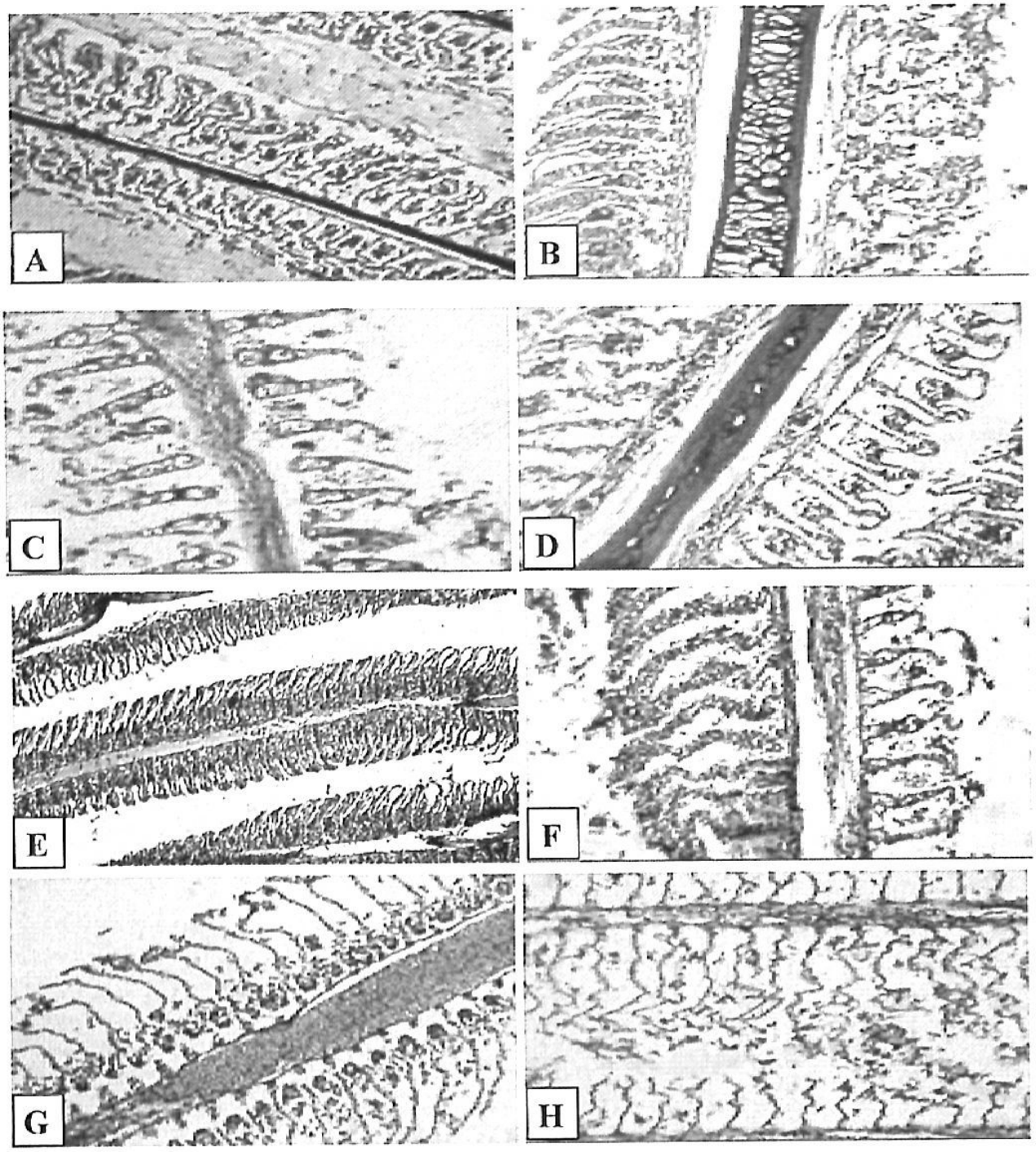

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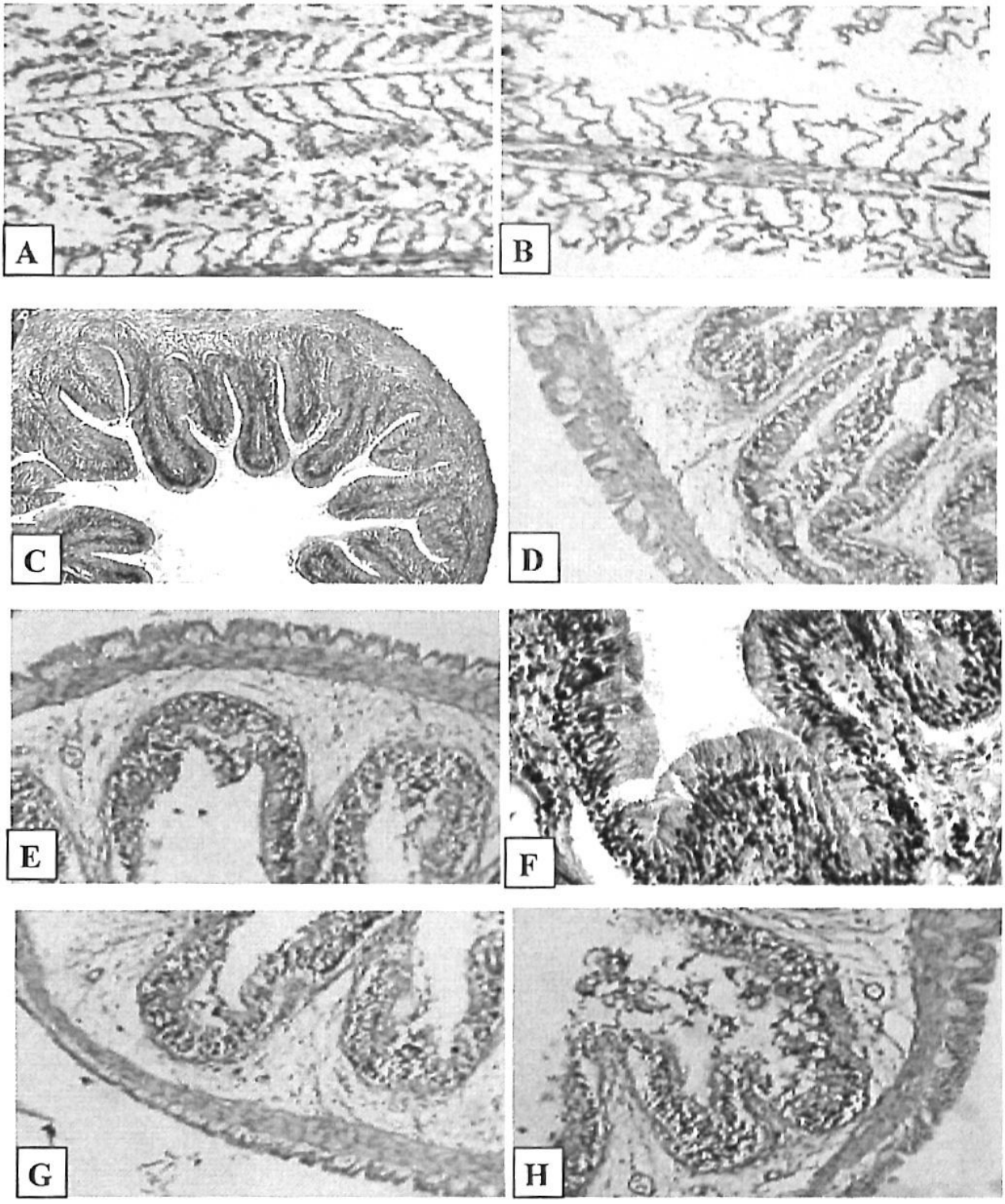

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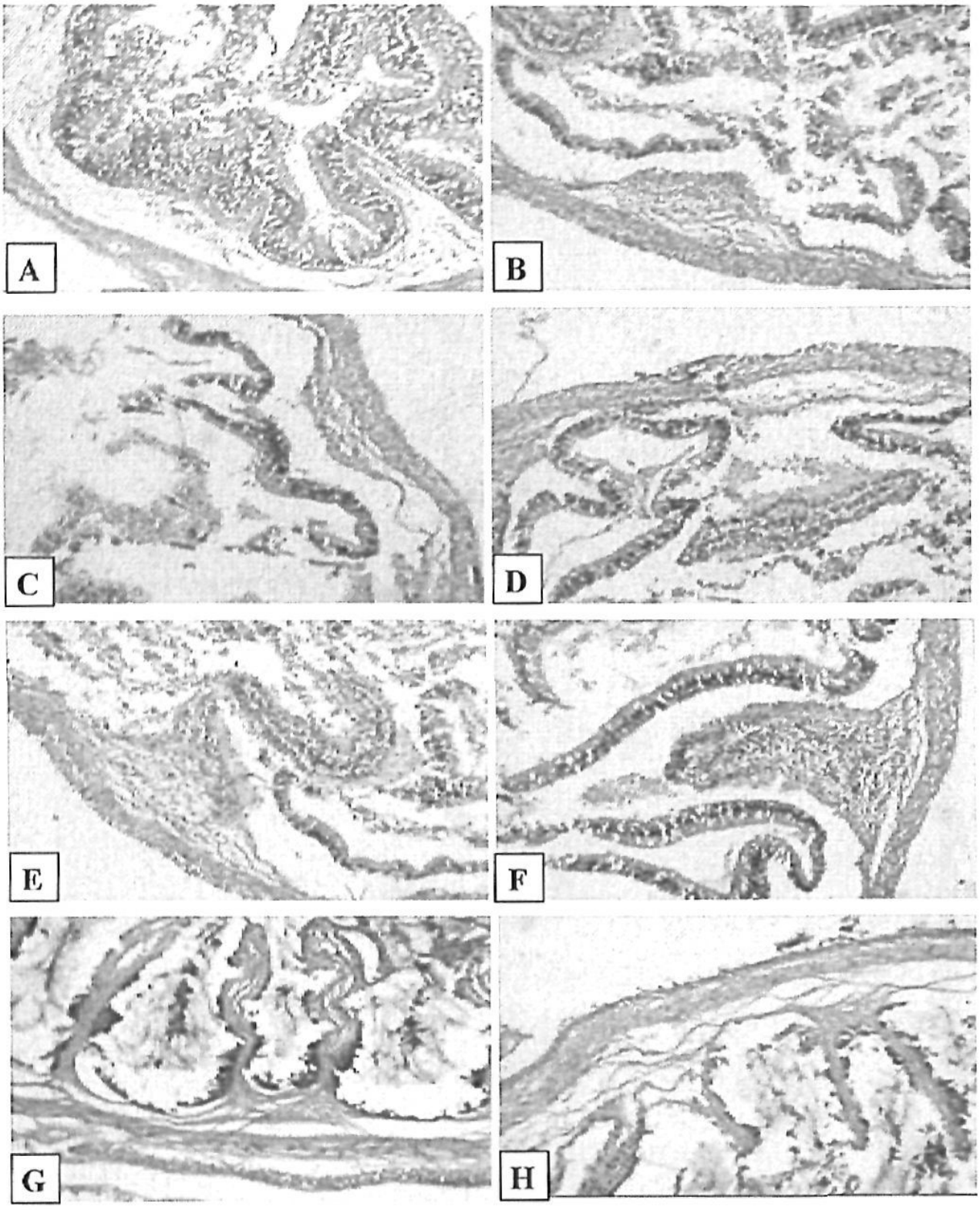

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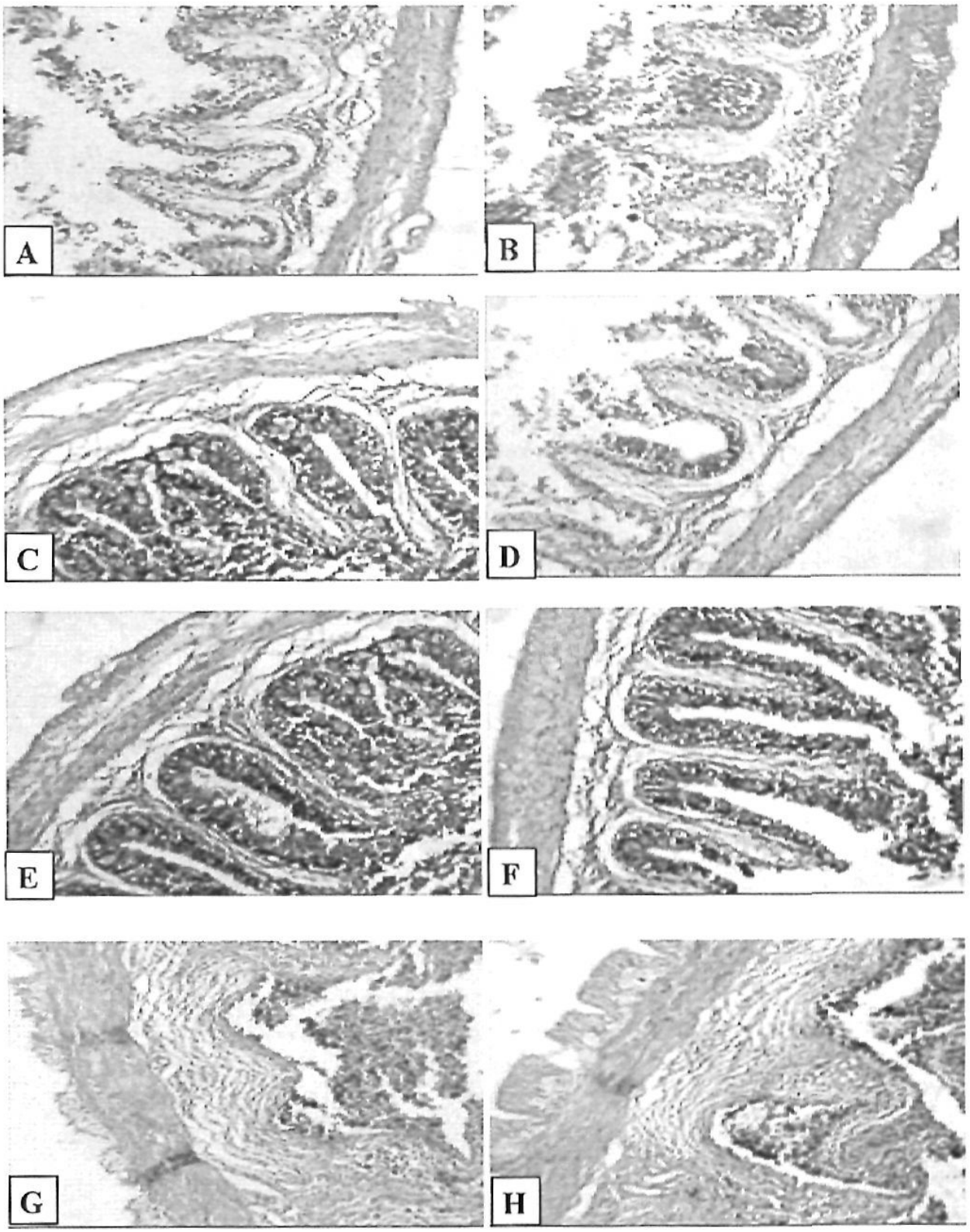

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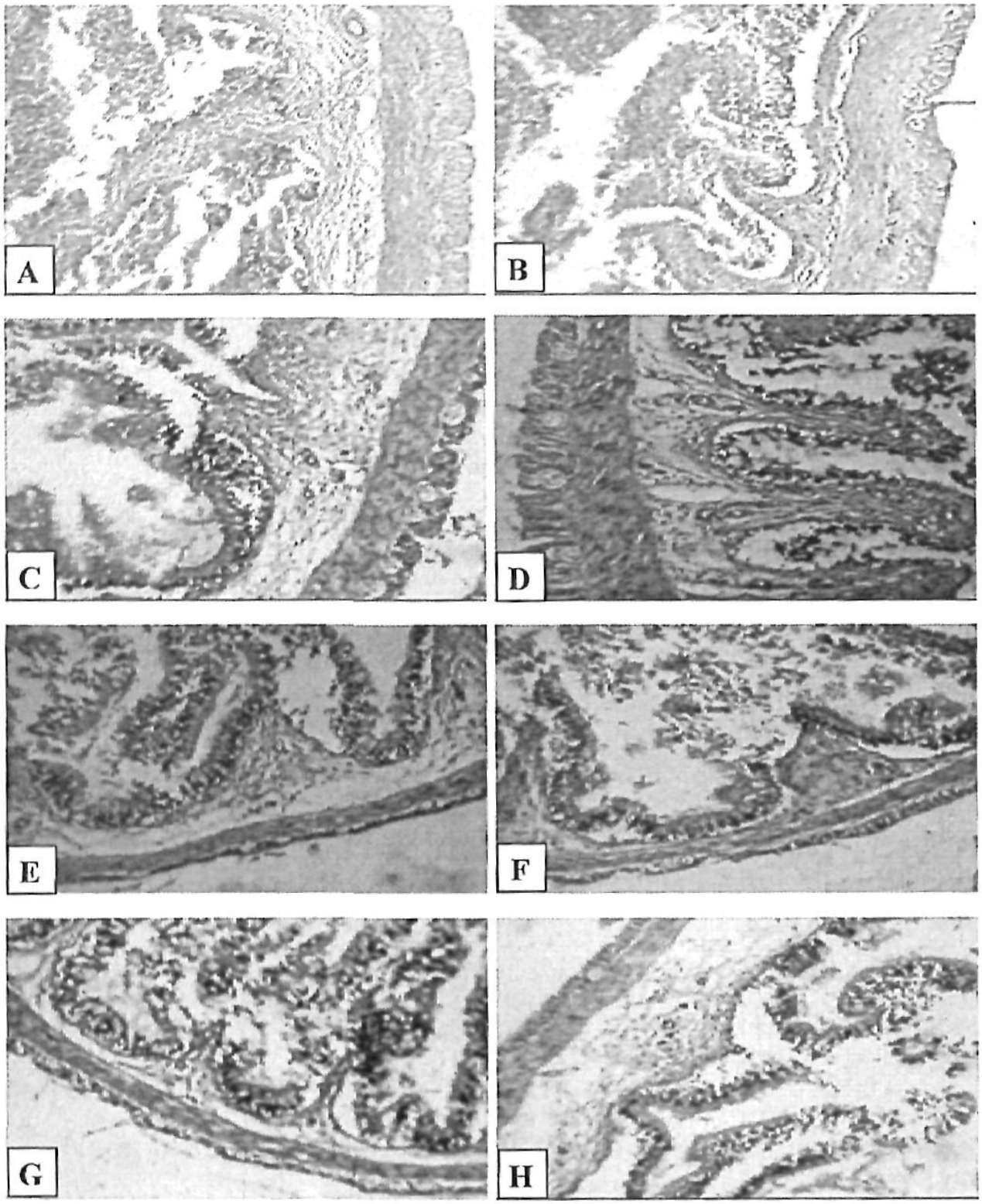

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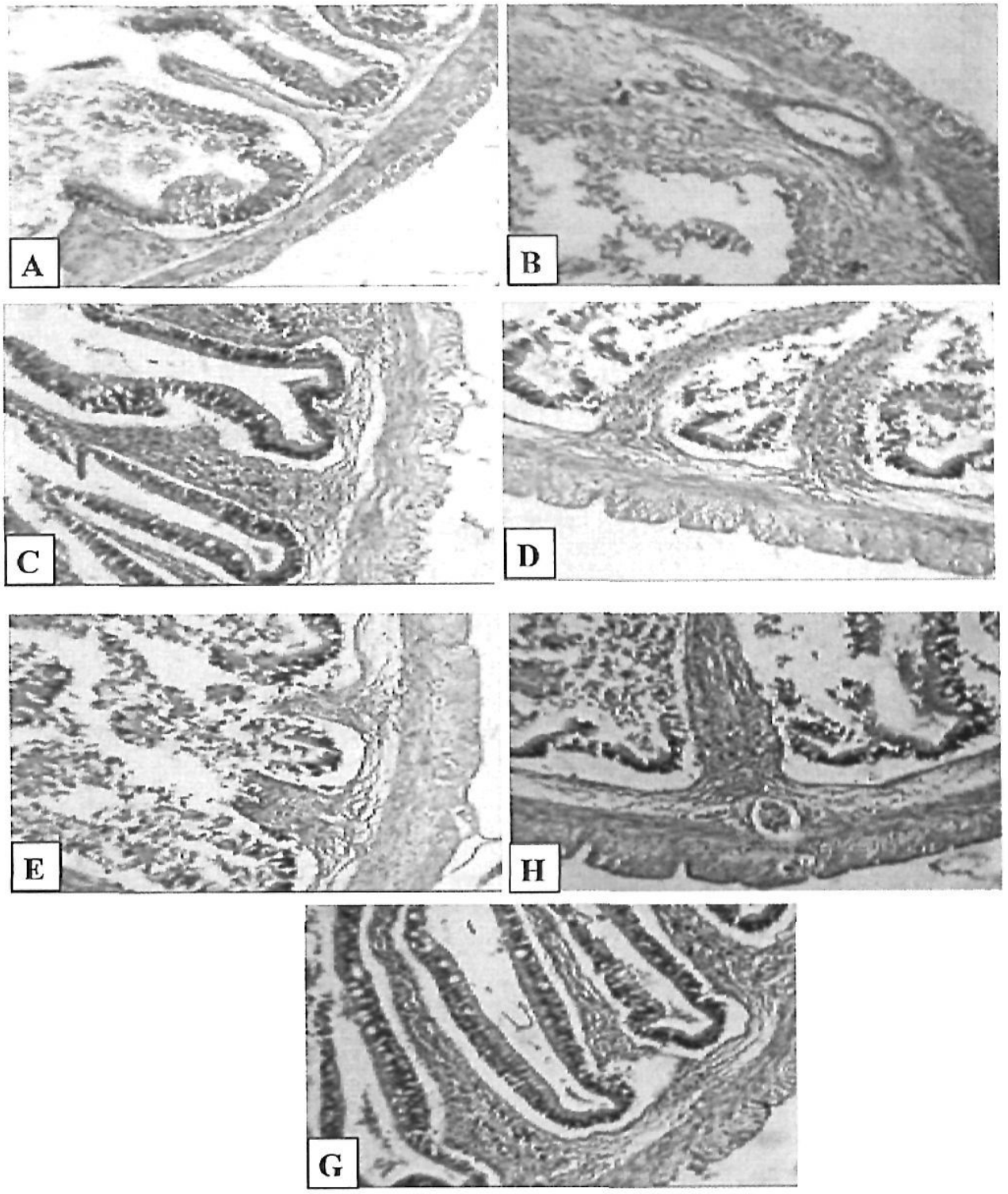

Fig. (9)