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PROTECTIVE EFFECTS OF HSCAS AND MONTMORILLONITE AGAINST PCBS-INDUCED HISTOPATHOLOGICAL AND CYTOGENITICAL CHANGES IN NILE TILAPIA FISH (OREOCHROMIS NILOTICUS)

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SUMMARY

The present study was conducted to evaluate the protective effects of Hydrated Sodium Calcium Aluminosilicate (HSCAS) and Egyptian Montmorillonite (EM) against PCBs-induced pathological and cytogenitical changes in talipia fish. Sixty male Nile tilapia fishes were divided into six treatment groups and treated orally with HSCAS or EM with or without PCBs (1.6 ug/kg b.w) for six weeks (three times/week). Treatment with PCBs alone resulted in adverse pathological lesions in kidney, liver and spleen. PCBs increased total structural and numerical chromosomal aberrations in the kidneys, increased micronucli erythrocytes and the concentration of DNA in liver tissues. Addition of HSCAS or EM resulted in a significant improvement in the histological lesions but with an evidence of renal calculi formation. Both sorbents materials succeeded in the decrease of the genotoxicity of somatic cells (79% and 67.9% for HSCAS and EM respectively), and inhibited micronulei erthrocytes (83.1% and 77.2% for HSCAS and EM respectively), whereas, the inhibition of DNA concentration in liver tissue reached 58.2% and 56% for HSCAAS and EM respectively. It could be concluded that both HSCAS and EM had protective effects against PCBs toxicity. The proposed mechanism for such protection may be that these sorbent materials tightly bind and immobilize PCBs in the gastrointestinal tract resulting in the reduction of the toxin bioaviliability.

Key words: PCBs, HSCAS, montmorillonite, Sorbent materials, Clay, Pathology, cytogenetice, chromosomal aberrations, fish.

INTRODUCTION

Polychlorinated biphenyls (PCBs), members of the halogenated aromatic group, are persistent environmental contaminants, which are distributed throughout the ecosystem. The presence of PCBs in the environment has been extensively studied during the last 30 years, both in terms of total PCBs and since the last decade, as individual congeners (Kuriyama et al., 2003). Residues are found at every level of the food chain and as human beings are placed at the top, it is not surprising that significant levels of these compounds have been found in human adipose tissue and breast milk (Safe, 1994; Giesy and Kannan, 1998).

PCBs were once manufactured in large quantities in the USA, Germany, France, UK, Japan, Spain, and Italy. They are still being manufactured in North Korea and Russia (Berger et al., 2001), whereas, it is considered as a byproduct for the production of certain chemical industries in Egypt (Barakat, 2004). Human and animals are exposed to PCBs via oral ingestion of contaminated food products (Safe, 1994). Exposure to these compounds results in various harmful effects including reproductive toxicity, immune suppression, birth defects, cancer, and developmental and behavioral changes (Safe, 1994; Kimbrough, 1995). The degree of chlorinated and the molecular structure of PCBs determine their degree of toxicity and mode of action (Safe, 1984, 1990). PCBs elucidate adverse effect on aquatic organisms including fish and marine mammals. Such organisms considered as biomarkers for water contamination by different pollutants including PCBs. The PCBs could be incriminated as a cause of abnormal cellular changes in the renal corpuscle of bream (Abramis brama) and asp (Aspius aspius). The changes included dilation of glomerular capillaries, mesangial oedema and adhesion between Bowman's capsule layer (Kari et al., 2000). On the other hand, PCBs could induce liver and skin tumors in bullhead fish (Ameriurus nebulosus), and the compounds could be detected in the muscles of such fish (Pinkney et al., 2001). Hepatoma and liver hyperplasia in fishes exposed to PCBs were common (Vincent et al., 1998; Grinwis et al., 2001; Brusle, 1991). In teleston fish, as in all other vertebrates, detoxification of endogenous waste products and xenobiotics is carried out primary in the liver. The hepatocytes are also involved in intermediate metabolism of protein, carbohydrate, and lipids (Roberts, 1989). These metabolic activities are regulated by a variety of hormones including catecholamines and corticosteriods (Boon van der et al., 1991), which also participate in the primary stress response in fish (Donaldson, 1981). Away of the neoplastic effect of PCBs, they cause immunosupression in different fish species as they suppress B-cell mediated immunity in chinook salmon (onchorhynchus tshawytscha) (Arkoosh et al., 1994) or impaired natural killer (NK) and specific T cells in the harbour seals (phoca vitulina) while the highest dose

level caused a significant reduction in circulating lymphocytes (Chu et al., 1990).

Several reports indicated that a degradation of PCBs and other organic compounds in a water containing system may be protected by a non-ionic surfactant (Hilarides et al., 1994; Gray and Hilarides, 1995). This method, suggested for remediation of contaminated soil, appears to be not only technically feasible but it may also be economically competitive (Mucka et al., 2000). Sorption of different chemicals (i.e pyrethroids) to soils and sediments is well documented (Hill and Inaba, 1991; Jin and Webster, 1998). Ousou and Hansen (2002) reported that sorption to mineral surfaces may be important for retention and degradation of hydrophobic pesticides in subsoils and aquifers poor in organic matter.

Extensive studies have been carried out on the adsorption of chemical compounds on clay minerals. (Phillips et al., 2002; Mayura et al., 1998; Abdel-Wahhab et al., 1998, 1999, 2002). HSCAS and EM were found to have a high affinity for PCBs in vitro (Abdel-Wahhab and El-Kady, 2004, unpublished data) forming a stable adducts. Hence, this in vivo study aimed to investigate the adverse effects of PCBs on the histological, histochemical and cytological figure of talipia fish as a sensitive experimental model and the possible protective effect of HSCAS and EM against these hazards.

MATERIALS AND METHODS

Chemicals: DCMA polychlorinated biphenyl (PCBs) was purchased form Supel Co. Supelco Park, Belle fonte, PA USA. The chemical composition of PCBs mixture is depicted in table (1). Hydrated Sodium Calcium Aluminosilicate (HSCAS) was purchased from Engelhard Corporation (Cleveland, OH) whereas Egyptian Monmorillanite (EM) was provided by the Department of Ceramic, N. R.C., Cairo, Egypt.

Experimental Fishes: Four months old Nile tilapia (Oreachromis nilaticus) fish weighing 100 ± 5 g were purchased from EL-Wafaa Fish Farm (Giza, Egypt) and transported in a large plastic water containers supplied with battery aerators as a source of oxygen. Fishes were maintained on standard fish diet (zoocontrol fish food) free from any chemical contaminants, at the Animal House Lab., Faculty of Veterinary Medicine, Cairo University (Giza, Egypt). After an acclimation period of one week, fishes were divided into 6 experimental groups (10 fishes/ group) and each group was individually placed into an aquarium (1mx 0.5m x 0.3m) supplied with aerated, dechlorinated tap water. The water was circulated 15 times a day, and the average water temperature was 20 ± 3.7°C and the pH was in the range of 7.17 - 8.19.

Experimental design: Fishes within each treatment group were treated orally for 6 weeks (3 doses/ week) as follows: group 1 untreated control; group 2, treated with PCBs (0.6 ug/kg body weight dissolved in corn oil); group 3, treated with HSCAS alone; group 4, treated with EM alone, group 5, treated PCBs plus HSCAS, and group (6) treated with PCBs plus EM. Fish treated with sorbents alone or in combination with PCBs were given an amount of the sorbent equivalent to 0.5% of the estimated maximum daily intake of feed dissolved in corn oil. Mortality rate was recorded daily. Blood Films were prepared from the gills of each fish within different treatment groups at the end of the experimental period for the cytogenetic studies. After blood samples were collected, fishes were injected interperitonealy (i.p) with 0.03% colchicines. Three hours after the injection, samples were collected for cytogenetical examinations. All fishes were subjected to post mortem examination, and liver, spleen, kidney and intestine from all fishes within different groups were removed and fixed in 10%neutral buffered formalin, dehydrated in alcohol, cleared in xylol and embedded in paraffin. 4u thick sections were prepared and stained for histopathological and histochemical examinations (Carleton, 1976).

Chromosomal preparation: Samples of anterior kidney (head kidney) were collected and chromosomal preparations were carried out as described by Al-Sabti et al. (1983).

Micronucleus test: Blood smears were prepared on a glass slide. The slides were air-dried for 12 h and then fixed in methanol for 10 min, followed by 5% giemsa staining. 1000 erythrocytes for each fish were examined. To detect micronuclei in erythrocytes, the slides were analyzed using a 100X oil-immersion lens (De Flora et al., 1993).

DNA extraction and determination: The extraction and determination of DNA was carried out following the technique of Schneider (1957).

Statistical analysis: All data were subjected for statistical analysis using F test as described by Snedecor and Cochran (1961)

Table (1) Composition of PCBs mixture

PCBs components	Concentration µg/ml
2 chlorobiphenyl	100
3,3`-Dichlorobiphenyl	100
2,4,5- Trichlorobiphenyl	10
2,2`,4,4`-Tetrabiphenyl	10
2,3`,4,5`,6- Pentbiphenyl	10
2,2`,3,3`,6,6`- Hexabiphenyl	10
2,2`,3,4,5,5`,6- Heptabiphenyl	5
2,2`,3,3`,4,4`,5,5`- Octabiphenyl	5
2,2`,3,3`,4,4`,5,5`,6- Nonabiphenyl	5
2,2`,3,3`,4,4`,5,5`,6,6`- Decabiphenyl	5

RESULTS

1- Histopathological results:-

The effects of different treatments showed no fish death in any of the treatment groups except in the groups treated with PCBs alone and PCBs plus HSCAS. Six out of ten fishes (60%) of the group treated with PCBs alone died after 10 days from the beginning of the experiment (after 4 doses), whereas only one fish (10%) died after 12 days (5 doses) in the group treated with PCBs plus HSCAS. The post-mortom examination of the dead fishes in both groups showed accumulation of serous fluid in the abdominal cavity (ascites). The liver was friable and enlarged and the spleen was atrophied. The kidneys were dark and enlarged. At the end of the experimental period, post-mortom examination of fishes in the group treated with PCBs plus HSCAS showed lesions included exophthalmia (Fig. 1a) ascites and grayish white foci on the liver, whereas, the spleen and kidneys showed lesions typical to those found in dead fishes.

The histopatholigical examination showed no obvious lesions in the control group. The kidneys of the fishes in the group treated with PCBs alone showed degeneration in the renal tubules with a large number of hyaline bodies in the renal tubular epithelium (Fig. 1b). In the lumen of some renal tubules, bluish crystals were demonstrated including renal calculi. Moreover, in some fishes, nephrocalcinosis were noticed with a marked granular degeneration of the renal tubular epithe-

lium. The calcification appeared in the lumen of some renal tubules (Fig.1c) or in the interstitial tissue and surrounded with melanophores (Fig. 1d). A marked necrosis of renal tissue was noticed in only one fish in this group. This necrotic tissue was invaded by melanophores with areas of calcification surrounded by thin layer of fibrous connective tissue (Fig. 1e).

The histopathological examination of the hepatic tissues in fishes received PCBs alone showed focal areas of dysplasia accompanied with vesicular hepatocytic nuclei. In these areas, the hepatic tissue showed necrosis of some hepatocytes and melanophores aggregation (Fig.1f), and the hepatopencrease showed marked vaculation (Fig.1g). In one case of this group, hepatocellular carcinoma was prominent with marked necrosis and acinar arrangement of the hepatocytes (Fig.1h).

The spleen of the group received PCBs alone showed common lymphocytic depletion where the trabeculae appeared prominently and the melanomacrophage centers were devoid of melanophores and lymphocytes (Fig. 2a). Blood vessels were thickened and stained positive with Masson's Trichrom Stain (Fig. 2b), which indicates the increase in collagenous fibers with an increase in the connective tissue around the congested vessels. Moreover, golden brown pigments in the splenic tissue were also noticed and were confirmed by Prussian Blue Stain as hemosiderin pigments inside the melanophores as well as in the surrounding tissue (Fig. 2c).

Fishes treated with PCBs plus EM showed mild degenerative changes of both hepatic (Fig. 2d) and renal tissue, whereas, the spleen showed normal picture (Fig. 2e). On the other hand, fishes treated with HSCAS alone or plus PCBs showed obvious pathological lesions in the kidneys included the presence of renal calculi in both interstitial tissue and lumen of renal tubules. The calculi sometimes appeared as single small granule (Fig.2f) or multiple, large surrounded by fibrous connective tissues (Fig.2g). These calculi were stained positive with Alizaren red (Fig. 2h).

2- Cytogenetical results:-

The results of the present study indicated that oral administration of PCBs induced structural, numerical chromosomal abnormalities (Fig. 3), micronuclei and changes in the quantity of DNA. The effects of sorbents (HSCAS, EM) and PCBs on the micronucleated erythrocytes in gill of fishes are depicted in (Fig. 3 A& B) and table (2). Treatment with PCBs alone resulted in a significant increase ($P< 0.01$) in micronucleated erythrocytes. Whereas, fishes treated with HSCAS or EM alone were comparable to the control group combined treatment with HSCAS or EM and PCBs resulted in a significant ($P< 0.05$) inhibition

in micronucleated erythrocytes reached 82.1% and 77.2% for HSCAS and EM respectively.

The chromosomal examination of the kidney cells showed that structural aberrations included chromatid and chromosomal gaps (Fig. 3 E), chromatid (Fig. 3 D) and chromosomal breaks, deletions (Fig. 3 F), fragments and centromeric attenuations (Fig.3 H). The numerical aberrations represented by polyploidy (Fig. 3 G).

The abberations are summarized in table (3). The present study indicated that treatment with PCBs resulted in a significant (P<0.01) increase in chromosomal aberrations, whereas, fishes treated with sorbents alone were comparable to the control fishes (table 3) . Co treatment of fishes with PCBs and HSCAS or EM resulted in a significant decrease in total chromosomal aberrations in kidney cells (79% and 67.4% respectively) compared with those treated with PCBs alone group (Fig. 4). It is of interest to mention that both sorbents were effective in reducing the elevation of total number of chromosomal aberrations resulted from PCB although these two sorbents failed to normalize this parameter. The current study also indicated that all types of chromosomal aberrations were significantly increased (P< 0.01) in the group treated with PCB compared with control group. Fishes treated with PCBs plus HSCAS or EM showed that the main frequent type of structural aberrations was chromosomal gaps which was significantly increased (P< 0.05 and P< 0.001 respectively), whereas, no significant changes were found in the other types of aberrations.

Table (2): Effect of HSBS and EM pretreatment on PCBs induced micronuclei in erythrocyte of Nile Tilapia fish

	Mn	Bi	Total	Inhibations %
	M ± SE	M ± SE		
Control	1.6 ± 0.51	0.0 ± 0.0	8	
PCBs	31.6 ± 2.66***	3.4 ± 0.21*	158	
PSBs + HSCAS	8.8 ± 1.46	0.0 ± 0.0	44	82.10%
PSBs + EM	10.6 ± 2.48*	0.6 ± 0.40	53	77.20%
HSCAS	3.8 ± 0.58	0.0 ± 0.0	19	
EM	4.4 ± 0.98	0.2 ± 0.20	22	

* P < 0.05
** P < 0.01
*** P < 0.001
mn: Micronuclei

Table (3): Effect of HSBS and EM pretreatment on PCBs induced chromosomal aberrations in in kidney of Nile Tilapia fish

	Gap	Chromosomal gap	break	Chromosomal break	delation	fragment	Centromeric attenuations.	Total ex. g.	Inhibations %	Polyploidy
	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE		M ± SE
Control	0.8 ± 0.37	0.2 ± 0.20	0.4 ± 0.24	0.0 ± 0.0	0.2 ± 0.20	0.4 ± 0.24	0.6 ± 0.40	1.8 ± 0.37		0.8 ± 0.37
PCBs	7.0 ± 0.71**	5.6 ± 0.51***	7.2 ± 0.37***	5.4 ± 0.51***	6.6 ± 0.51***	8.4 ± 0.51***	8.4 ± 0.51***	41.6 ± 1.03***		16.6 ± 1.7***
PSBs + HSCAS	4.2 ± 0.37	4.6 ± 1.03*	2.0 ± 0.63	1.6 ± 0.51	1.2 ± 0.58	1.2 ± 0.49	0.6 ± 0.60	10.8 ± 2.35*	79%	4.0 ± 0.71
PSBs + EM	5.0 ± 0.84**	6.2 ± 0.97	3.4 ± 0.93	2.8 ± 0.86	1.8 ± 0.58	1.2 ± 0.37	1.0 ± 0.32	16.0 ±1.70**	67.90%	5.6 ± 0.51
HSCAS	2.2 ± 0.66	0.4 ± 0.24	1.0 ± 0.32	0.4 ± 0.24	0.4 ± 0.24	0.6 ± 0.40	0.4 ± 0.24	2.2 ± 0.58		2.2 ± 0.66
EM	3.2 ± 0.80	1.0 ± 0.32	1.2 ± 0.37	0.6 ± 0.24	0.6 ± 0.40	1.0 ± 0.32	0.4 ± 0.40	4.6 ± 1.03		3.6 ± 1.03*

Table (4). Means ± SE for DNA concentration in liver of fish treated with PCBs with or without sorbent materials

Group	DNA mg/g tissue	Inhibition %
Control	1.15 ± 0.08^{a}	
PCBs	5.86 ± 0.46^{b}	
HSCAS	1.67 ± 0.12^{a}	
EM	2.20 ± 0.20^{c}	
PCBs + HSCAS	3.42 ± 0.30^{c}	58.2
PCBs + EM	3.81 ± 0.15^{d}	56

Within each column, means superscript with the different letters are significantly difference ($P < 0.05$)

DNA concentrations in liver cells were significantly increased ($P < 0.01$) in fished treated with PCBs alone. Fishes treated with EM alone showed a significant increase ($P < 0.05$) in DNA concentration in liver tissues whereas, those treated with HSCAS were comparable to the control group (table 4). Co treatment with PCBs plus sorbents resulted in a significant inhibition of the increased level of DNA concentrations in liver cells. This inhibition reached 58.2% and 56% for HSCAS and EM respectively (table 4).

DISCUSSION

Numerous reports have demonstrated the deleterious effects of PCBs on fish (Lipsky et al., 1978; Klannig et al., 1979; Donaldson, 1981 Aandersson et al., 1988;Quabius et al., 1998;). The most recent approach in the area of detoxification of different environmental contaminants is the use of chemisorbent materials.

Recent studies have demonstrated that HSCAS and EM prevented aflatoxicosis in rats (Mayura et al., 1998; Abdel-Wahhab et al., 1998; 1999; 2002). The basic mechanism appears to involve sequstion of AFB1 in the gastrointestinal tract and chemisorption (i.e. tight binding) to HSCAS or EM, which results in a reduction in toxin bioavailability (Phillips et al., 2002). Further studies have suggested that aflatoxin may bind within the inter layer, on the basal surface and at the edge of HSCAS (Phillips et al., 2002) and/or EM (Abdel-Wahhab et al., 2002).

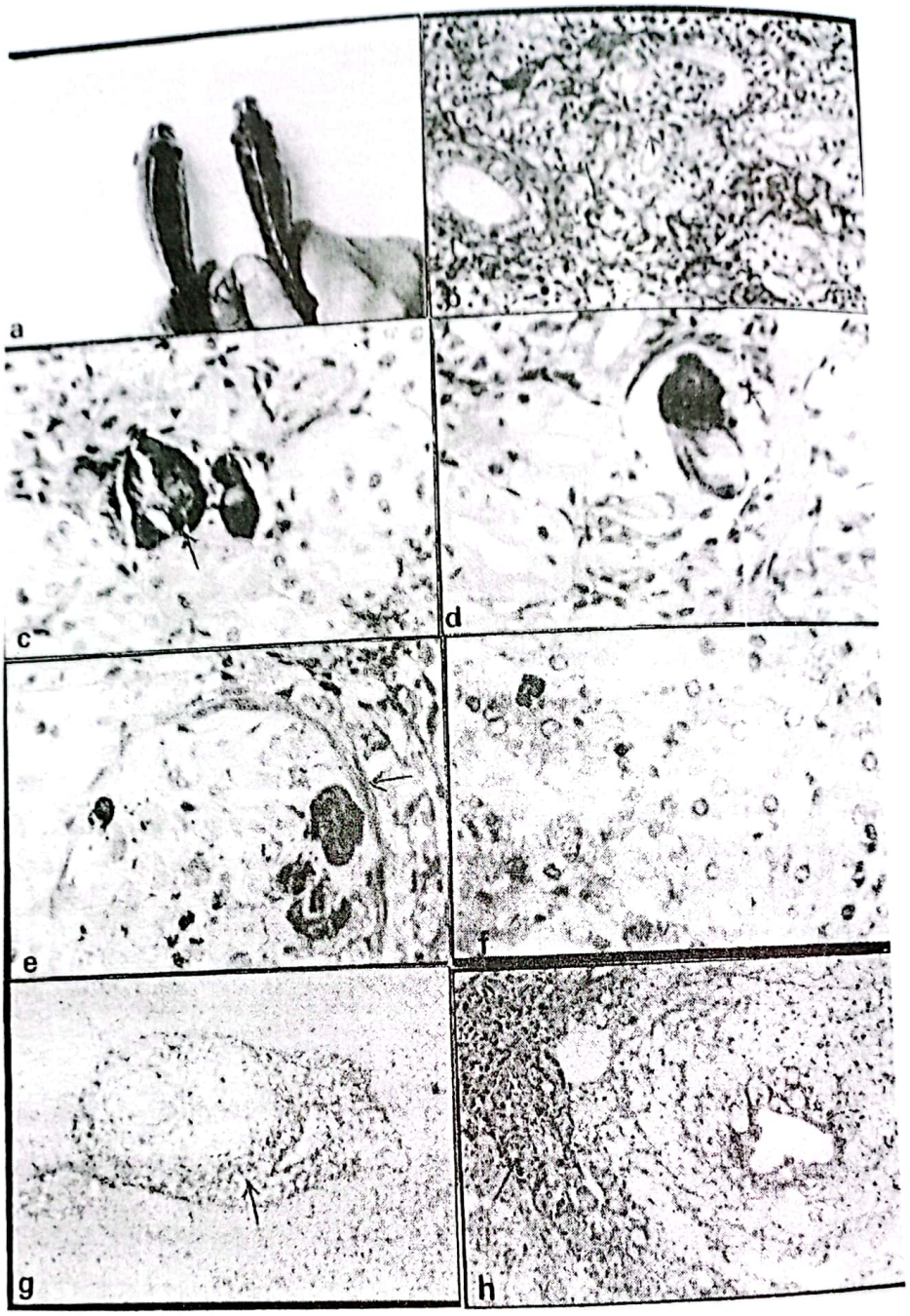

Fig 1: Oreochromis niloticus treated with PCB alone showing:
a. Exophthalmia (left) in comparison with control (right) **b.** Kidney showing hyaline bodies in the renal tubular epithelium (Small arrow) with bluish crystals in the lumen (large arrow).H&E stain x 400 **c.** Marked calcium deposition in the lumen of renal tubules. H&E stain x 1000.**d.** The calcified area surrounded with melanophores (arrow). H&E stain x 1000.**e.** Necrosis of the renal tissue, melanophores aggregation, calcium deposition and thin fibrous connective tissue capsule (arrow). H&E stain x 1000.**f.** Liver showing vesicular nuclei of the hepatocytes and scattered melanophores. H&E stain x 1000.**g.** The hepatic tissue showed dysplasia and vacuolation of hepatopancrease (arrow). H&E stain x 200.**h.** The hepatic tissue showed hepatocellular carcinoma .Notice, acinar arrangement of the hepatocytes (arrow); H&E stain x400).

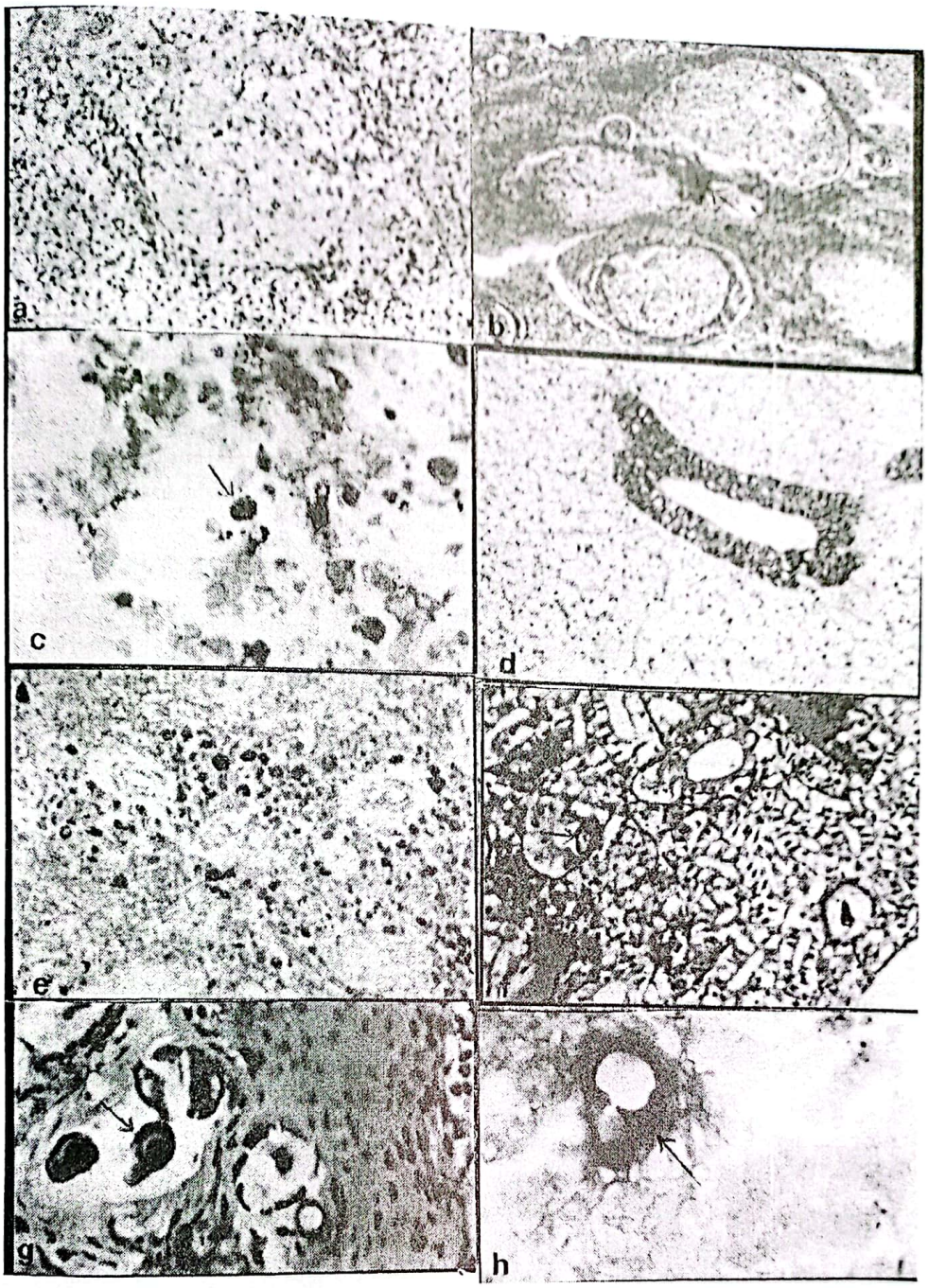

Fig.2: Oreochromis niloticus **a.** Treated with PCB alone showing lymphocytic depletion of spleen and devoid melanin pigments in the melanomacrophage centers(H&E stain x 400) **b.** Marked thickening of the blood vessels wall of the spleen (Massonís trichrom stain x 200) **c.** The hemosiderin pigments were observed inside and outside the melanophores (Prussian blue stain x 1000) **d.** Liver of Oreochromis niloticus treated with PCB and EM showing mild degenerative changes of the hepatocytes and normal hepatopancrease (H&E stain x 200) **e.** The same group showing normal spleen (H&E stain x 400) **f.** Oreochromis niloticus treated with PCB and HSCAS showing small renal calculi (arrow).; H&Estain x 400 **g.** Kidneys of the same group showing multiple renal calculi surrounded with fibrous connective tissue (H&E stain x 1000) **h.** The renal calculi stained positively by Alizarine red (x 1000).

In the present study, we evaluated the ability of HSCAS and EM to protect the tilapia fish from the effects of PCBs. The selected dose of PCBs was literature based (Quabius et al., 1998) whereas, the selected dose of HSCAS and EM were based on our previous work (Abdel-Wahhab et al, 2002). The histological examination of fishes treated with PCBs showed pathological lesions typical to those reported in the literature. Hepatic necrosis and hepatocellular carcinoma were observed in fishes exposed to PCBs. The kidneys showed depletion in the hemopoietic tissue as well as hyaline bodies in the renal tubules. Similar to our observations, Vincent et al. (1998) reported that hepatoma and liver hyperplasia were common in fishes exposed to PCBs. Moreover, Pinkney et al. (2001) detected tumours in liver and skin of bull head fish exposed to PCBs. Although the hepatic carcinoma is malignant tumour and usually metastasized into different organs in

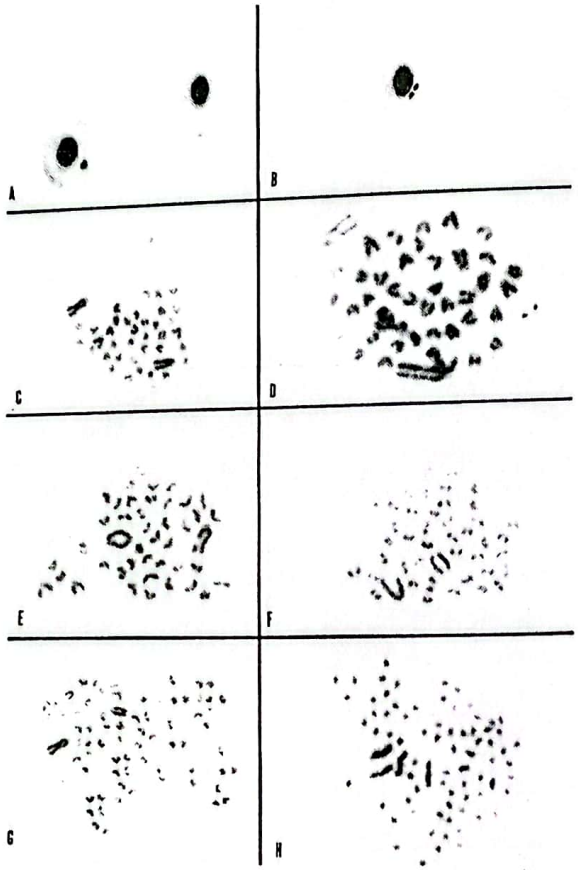

Fig.3:
A- Gill smear showing ertythrocytes with and without micronuclei.
B-Erythrocytes with bimicronuclei.
C- Normal metaphase spread of Oreochromis niloticus.
D-Treated Oreochromis niloticus showing break.
E- Chromosomal gaps.
F- Deletion
G- Polyploidy
H- Centromeric attenuation

mammals and higher vertebrates, we did not observe any metastasis in this study. The teleosts (bony fish) liver appears less vulnerable to metastases from neoplasms than other organs of higher animals and this may be attributable to the lack of kuppfer cells in hepatic tissues of the fish (Roberts, 1989). The hemopoietic depletion reported in this study may be due to the immunosuppressive effect of PCBs. There are a number of confounding factors in the studies of the toxic effects on fish liver. First, it has been shown that liver morphology (Ishii and Yamamoto, 1970; Yamamoto and Egami, 1974) and the responsiveness to organic toxicants (Braunbeck et al., 1984) may differ considerably between male and female fish. Second, in field and laboratory studies on the ef-

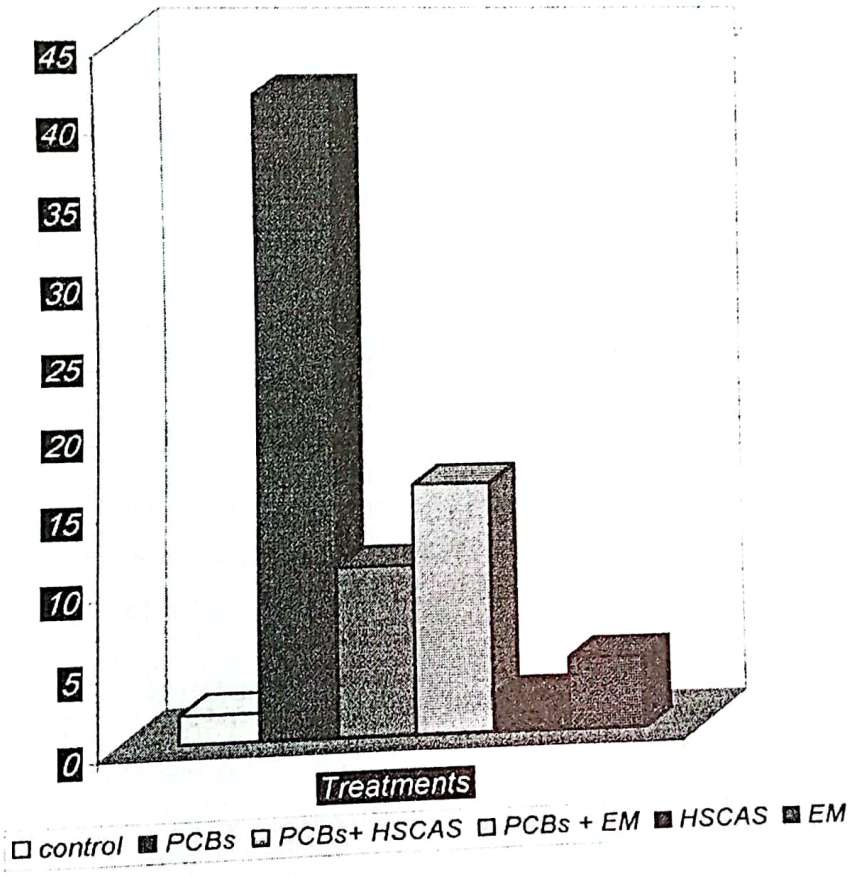

Fig.4:
Effect of PCBs alone and in combination with HSCAS and EM on total structural aberrations

fects of toxicants on liver structure and function, stressful conditions can not be avoided. Accordingly, under stressful conditions, the ability of the fish to cope with toxicants is generally impaired (Larsson et al., 1984 Gill and Epple, 1993;). The presence of hyaline droplets in the renal tubules reported in the current study is usually associated with altered glomerular filteration (Ferguson, 1989).

The use of sorbent materials (i.e. HSCAS or EM) succeeded to reduce the toxicity of PCBs as the necrotic changes and neoplastic lesions were not detected in fish received the combined treatment. The formation of calculi in the kidneys found in the current study in the groups of fishes treated with PCBs alone, sorbents alone or the combined treatment may be attributed to the enhancement of these substances for the nephrocalcinosis and calculi formation in renal tissue of fishes. Similar to these observations, McGavin et al. (2001) reported that PCBs induced necrosis in the renal tubules and the necrotic tissue is favorable media for calcification. On the other hand, Watkins and Souttiern, (1992) stated that a synthetic sodium aluminosilicate has been shown to influence calcium and phosphorus utilization in chicken and subsequently disturb Ca/P ratio. These findings may explain the role of clays in calculi formation. Generally, the pathogenesis of the calculi formation due to the ingestion of clay minerals in the present study required further investigation to detect the actual causes of nephrocalcinosis due to aluminosilicate administration.

The present study revealed that PCBs administration resulted in significant increase in total and numerical aberrations and all types of structural aberrations in kidney cells of fish. In this regards, WHO (1993) reported that PCBs induce a significant increase in chromosomal aberrations include chromatid breaks and rearrangement in mammalian cells. Furthermore, these authors indicated that PCBs bind to DNA in vivo and in vitro, and both single strand DNA breaks and the induction of DNA repair have been detected in mammalian cells in vitro. When taken together, the data indicate that PCBs have a little, if any, in vivo genotoxic potential. However, some of the congeners, especially those with a low degree of chlorination, may cause mutagenic and DNA damage. A proposed mechanism for those genotoxicity is the metabolism of these compounds to arene oxide intermediates, which are able to alkylate critical cellular macromolecules (Safe, 1984; Safe, 1990;). Another studies revealed that PCBs alone was lipophilic and therefore have the potency of accumulating in the fat stores of animals. It has a mutagen properties, which increase in DNA breakage and increase the micronuclei erythrocytes in vivo in fish (BeLopaeme et al., 1996). The use of adsorbent materials against populated compounds is a new field that must be completely. In the current study, HSCAS and EM were capable to diminish

the cytotoxic effects of PCBs in somatic cells of fish. In a previous report, Abdel-Wahhab et al. (1998) reported that HSCAS and bentonite had protective effects against aflatoxin-induced chromosomal aberrations in rats. Another report showed that HSCAS and EM succeeded in the prevention of aflatoxicosis in rats (Phillips et al., 1995; Mayura et al., 1998 and Abdel-Wahhab et al., 1999, 2002;). In those reports, the authors stated that these sorbents tightly bind and immobilize aflatoxin in the gastrointestinal tract of the animals. Moreover, EM may possess three types of active binding sites, (i) those located at basal planes within interlayer channels, (ii) those located on the surface and (iii) those located at the edges of clay particles (Abdel-Wahhab et al., 2002). Our results clearly indicated that PCBs increase DNA in the liver of fish, whereas, addition of HSCAS or EM had no significant changes. The presence of tumors in the PCBs-treated fish was explain the increased of DNA based on the increased cellular division.

In conclusion, our results have indicated the effectiveness of HSCAC and EM for PCBs intoxication in fish. These sorbent materials succeeded to diminish the neoplasms and other pathological lesions besides, adverse cytogenetically effects. Away of the nephrocalcinosis, this study demonstrates the potential for significant improvement associated with the inclusion of selective sorbent materials. Moreover, sorbent materials should be rigorously tested individually and thoroughly characterized in vivo, paying particular attention to their effectiveness and safety in sensitive animal models and their potential for deleterious interactions.

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