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(RESEARCH ARTICLE)

Hematological, biochemical, and hormonal effects of organochlorine pesticide residues found in canal and tap waters on albino white rats

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Abstract

Analysis of water from the canal that feeds the Damanhour water station and tap water in Damanhour, Beheira Governorate, Egypt, revealed the presence of gamma-HCH, beta-HCH, heptachlor epoxide, p, p D.D.E., dieldrin, endrin aldehyde, endosulfan sulfate, and endrin ketone. Therefore, this study aimed to investigate the adverse effects of administration of the OCl pesticide residues found in canal water (0.0448 µg/ kg b. wt.) and tap water (0.022 µg/ kg b. wt.) to white Albino rats. Results revealed that treatment of male and female rats with a single oral dose of OCl pesticide residues found in both canal and tap water caused slight alteration in Hemoglobin (Hb), red blood cells (RBC) in male rats, and packed cell volume (PCV) in male and female treated rats relative to control. There was a significant increase in Hb, a decrease in RBC counts in treated female rats, and a significant decrease in WBC count in both male and female rats. There was a slight alteration in GOT, GPT, and G.GT activity in both male and female rats and a significant increase in alkaline phosphatase (ALP) activity in male rats. Compared to controls, treated rats had substantial increases in uric acid and albumin concentrations and a slight alteration in total protein concentration in the serum. Administration of OCl residues to rats caused no significant effect on male or female progesterone. It caused a significant increase in testosterone concentration in treated female rats and a decrease in the serum of male rats. Compared to controls, treated male rats had slightly lower follicle stimulation hormone (FSH) levels, and treated female rats had higher levels. Also, no significant difference was observed in T4 levels between the control and treated females in treated rats. However, the T4 level increased significantly in the males administered the high O.C.s found in canal water. T3 levels decreased in the serum of male and female rats, but T3 was significantly reduced in male rats given an oral dose of OCl residues in canal water. Unlike their effects on T3 levels, the impact of OCl residues on T4 levels in this study was inconsistent in treated rats.

Conclusions: Administration of a single oral dose of OCl pesticide residues found in both canal and tap water to rats caused significant and non-significant alteration in some hematological, biochemical, and hormonal biomarkers in the serum of treated rats compared to control.

Keywords: OCl pesticide residues; GGT; T3; T4; FSH; GOT; GPT; ALP; Hb; RBC; WBC; PVC

1. Introduction

The extensive use of pesticides in agriculture, usually involving a wide broadcast application and spread out in large crop field areas, is recognized as a significant source of diffuse contamination, typically by leaching to the underlying aquifers or runoff to the surface waters [1]. Moreover, later ones are of particular concern since they are considered a supply resource for human drinking in Egypt. Therefore, monitoring pesticides in water samples has been an issue of great relevance in the last decades. Furthermore, the E.E.C. Drinking Water Directive [2] establishes a concentration threshold of 0.1 µg/l for each pesticide or 0.5 µg/l for the total amount of them, including their primary metabolites.

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Therefore, since these values are quite strict, it was considered of interest to carry out extensive pesticide monitoring in drinking waters.

Egypt has a high concentration of pesticides in the Nile and Rosetta branches. The reported concentrations for lindane and D.D.T. are five to ten times above European standards [3]. As agricultural activities are the principal source of pesticides in Beheira Governorate, concentrations in agricultural drains (El-Khairy drain) and the drinking water supply are expected to be even higher. Analysis of water samples from the El Mahmoudia canal (which is the canal that feeds the Damanhour water station) and tap water of Damanhour, Beheira Governorate, revealed the presence of gamma-HCH, beta-HCH, heptachlor epoxide, p, p D.D.E., dieldrin, endrin aldehyde, endosulfan sulfate, and endrin ketone. The concentrations of these OCl compounds were more pronounced in El-Mahmodia drinking water than in tap water [4]. Therefore, this study aimed to investigate the potential adverse effects of these OCl pesticide residues in drinking waters (canal and tap waters) on white rats,

2. Material and methods

2.1. Animals

Male and female albino rats (*Rattus norvegicus*) with an average weight of 110–140 g were supplied from a breeding culture in the Animal Health Research Center (Cairo). Rats were housed in an 8-cage system and maintained for 20 days, acclimatized prior to treatments, allowed free access to water, and fed on an adequate, stable diet.

2.2. Experiments

Six groups of rats, each of 4 rats, were used. The rats of the first three groups, males, and the second three groups, females, received a single oral dose equal to the OCl pesticide residues in 1.5 ml from the extracts of canal water (0.0448 µg/Kg b.wt.) and tap water (0.022 µg/Kg b.wt.). Rats in control were divided into two groups and given orally the same volume of distilled water as used in single-dose treatments. In all experiments, four rats were used for each dose and animals were killed by decapitation after 24h from the insecticidal treatment. The collected blood samples were transferred to serum separation tubes and heparinized tubes.

- **The determination of hematocrit value** (packed cell volume) was done according to the method of [5, 6] using a micro hematocrit centrifuge model SH120...
- **Total hemoglobin** was determined according to the Wintrobe method [7]. To create cyanmethemoglobin, potassium ferricyanide first oxidizes hemoglobin into methemhoglobin.
- Hemoglobin Cyanide + Ferricyanide ---------- > Cyanmethemoglobin
- **Counting of red blood cells (R**.B.C.s): A hemocytometer, microscope Novex, and red cell count pipette were used to achieve the goal of this experiment according to the methods of [8] and [9].
- **White blood cell counts (WBC**s) were measured by using a hemocytometer, microscope, and WBC counts pipet according to the methods of [8] and [9].
- **Using diagnostic kits from Boehringer Mannheim Gmbh, uric acid concentration** was measured by the technique described in [10] and [11].
- **Total protein was determin**ed according to the method described by Henry [12] using Boehringer Mannheim Gmbh's diagnostic kits.
- Utilizing diagnostic kits from Boehringer Mannheim Gmbh
- **Creatinine** was measured according to the protocol [13].
- Serum albumin was measured using kits from (Sentinel Ch., Via Principe Eugenio 5.20155 Milan, Italy) according to [14]'s instructions.
- **Glutamic-oxaloacetic transaminase** (serum G.O.T.) activity was determined according to the method described by [15] using Boehringer Mannheim GMBH Diagnostic's Kits.
- **The serum G.P.T**. activity was determined according to the method described by [15] using Boehringer Mannheim Gmbh Diagnostics Kits. The method depends on a photometric estimation of pyruvate by monitoring the concentration of pyruvate hydrazone formed with 2, and 4-dinitrophenyl hydrazine.
- **Measurement of alkaline phosphatase** (ALP/AP) activity was measured according to the method reported by [16] using Diamond Diagnostics Kits.
- **The determination of gamma-glutamyl transferase** (G.G.T.) was carried out according to the method reported by [17], [18], and [19] using linear chemicals (S. L. Kits).

2.3. Determination of Hormones

- **Follicle-stimulating hormone (F.S.H.) determination** was carried out according to the method reported by [20] using the International Immuno Diagnostics Kits.
- **Testosterone hormone determination** was carried out according to the method reported by [21] using International Immuno Diagnostics Kits.
- **The progesterone hormone** was determined according to the method reported by [22].
- **The triiodothyronine (T3) hormone** was determined according to the method reported by [23] using the International Immuno Diagnostics Kits.
- **Thyroxine (T4) hormone** determination according to the method reported by [24] using International Immune Diagnostics Kits was used in this study.

2.4. Statistical analysis

Statistical analysis using the Statistical Analysis System (S.A.S.) 2013) software as a factorial C.R.D. (utterly randomized design) was used in this study.

3. Results

Rats given single oral doses of OCl pesticide residues (gamma- and beta-HCH, heptachlor epoxide, p, p-DDE, dieldrin, endrin aldehyde, endosulfan sulfate, and endrin ketone), which were found in tap water (0.022µg /kg body wt.) and in canal water (0.0448 µg/ kg body wt.) did not show signs of acute toxicity or mortality in treated rats. However, in treated rats' hematological, biochemical, and hormonal alterations were observed.

3.1. Hematological effects

In the blood of OCl-treated rats, results show no significant alteration in hemoglobin (Hb) content, red blood cells (RBC) count in male rats, or packed cell volume (PCV) in treated male and female rats relative to control. In the treated female rats, results show a significant Hb increase and decrease in RBC and WBC counts (Table 1).

Table 1 Effects of single oral doses of OCl residues in water on the concentrations of treated rats' blood components

Treatments	**Hb (g/dl)**		**PCV %**		**RBC (X 10^6/mm^3)**		**WBC (X 10^2/mm^3)**	
	Male Mean ± SE*	**Female Mean ± SE***	**Male Mean ± SE***	**Female Mean ± SE***	**Male Mean ± SE***	**Female Mean ± SE***	**Male Mean ± SE***	**Female Mean ± SE***
Control	9.175±0.623c	9.00±0.408c	29.00±4.546a	31.00±2.160a	1.95±0.100ab	2.225±0.171a	24.25±1.258a	24.00±1.414a
Tap water. (0.022 µg/kg b. wt)	9.50±0.408 bc	10.125±0.478 ab	34.25±6.13 a	34.00±3.55a	1.700±0.294 b	1.750±0.100 b	22.25±0.957ab	22.25±0.957ab
Canal water (0.0448 µg/kg b.wt.)	9.50±0.00 bc	10.375±0.946 a	32.500±3.78 a	31.75±3.77 a	1.875±0.206 b	1.775±0.250 b	21.00±0.816 b	22.00±1.414 b
L.S.D. 5%	0.83818		6.1899		0.27627		1.75077	

*The data shown with the same symbols in the vertical column did not differ at 0.05 statistical levels.

3.2. Blood-biochemical effects

Results in Table 2 revealed that the activities of serum GOT, GPT, and GGT are slightly changed in rats administered OCl pesticide residues in tap and canal water. In contrast, significantly higher activity of ALP was observed in the female rats administered OCl residues in canal water than in the control.

Table 2 Effects of single oral doses of OCl residues in water on the activity of GOT, GPT, ALP, and GGT in the serum of treated rats

Treatments	G.O.T. activity (U/l)		G.P.T. activity (U/l)		A.L.P. activity (U/l)		G.G.T. activity (U/l)	
	Male Mean ± SE*	Female Mean ± SE*	Male Mean ± SE*	Female Mean ± SE*	Male Mean ± SE*	Female Mean ± SE*	Male Mean ± SE*	Female Mean ± SE*
Control	44.54±9.33 ab	35.45±8.00 ab	33.80±8.90 a	36.16±3.6 a	205.47±97.36 abc	110.4±113.08 bc	3.52±0.69 a	1.66±0.787 a
Tap water. (0.022 µg/kg b. wt)	46.28±11.23 a	43.56±11.17 ab	34.68±4.181 a	36.64±5.45 a	263.42±1.41 a	80.28±84.48 c	2.592±0.263 a	3.88±1.75 a
Canal water (0.0448 µg/kg b.wt.)	39.39±10.255 ab	27.35±20.07 b	33.00±4.814 a	37.40±7.20 a	226.85±140.66 ab	268.94±63.05 a	2.915±1.05 a	4.027±3.63 a
L.S.D 5a%	1.8298		8.883		139.846		2.614	

. *The data shown with the same symbols in the vertical column did not differ at 0.05 statistical levels.

Alteration in creatinine and uric acid levels indicated injury in kidney function. Results in Table 3 revealed that creatinine, uric acid, albumin, and total protein are influenced to some extent by OCl pesticide residues administered to rats in drinking waters. Uric acid increased significantly in the serum of male rats given OCl found in tap water. However, creatinine slightly increased in rats, and total protein decreased significantly in female rats relative to the control. Also, a significant increase in albumin in the serum of treated male rats was observed.

Table 3 Effects of single oral doses of OCl residues in water on the concentrations of creatinine, uric acid, total protein, and albumin in the serum of treated rats

Treatments	Creatinine (g/dl)		Uric acid (mg/dl)		Total protein (g/dl)		Albumin (g/dl)	
	Male Mean ± SE*	Female Mean ± SE*	Male Mean ± SE*	Female Mean ± SE*	Male Mean ± SE*	Female Mean ± SE*	Male Mean ± SE*	Female Mean ± SE*
Control	18.90±2.33 c	27.77±2.89 ab	2.96±0.72 ac	2.967±0.32 Bc	9.522±1.05 bc	11.645±1.64 a	3.507±0.711 b	5.567±1.069 ab
Tap water.(0.022 µg/kg b. wt.)	26.04±7.94 a.b.c.	30.86±3.90 a	3.84±0.466 a	3.55±0.177 Ab	9.11±0.962 bc	8.997±1.739 bc	5.68±2.019 ab	7.557±2.004 a
Canal water (0.0448 µg/kg b.wt.)	20.09±6.34 ac	30.18±7.21 a	2.82±0.532 bc	2.705±0.56 C	10.442±1.36 ab	7.887±1.066 c	6.217±1.545 a	5.55±1.337 ab
L.S.D 5%	8.236		0.735		1.988		2.262	

. *Data shown with the same symbols in the vertical column did not differ at 0.05 statistical levels.

3.3. Hormonal effects

Results of the effect of OCl residues on circulating hormones in treated rats were recorded in Table 4 and revealed that this treatment had no significant impact on progesterone in both male and female rats. Conversely, testosterone is significantly increased in the serum of treated females and decreased in treated males relative to the control. In contrast, the follicle stimulation hormone (FSH) concentration in male rats is slightly altered, and it increased by 30.8% in treated females compared to the control.

Table 4 Effects of single oral doses of OCl pesticide residues in water on the concentrations of FSH, Testosterone, and Progesterone hormones in the serum of treated rats

Treatments	FSH (mIU/ml)		Testosterone (ng/ml)		Progesterone (ng/ml)	
	Male	Female	Male	Female	Male	Female
	Mean ± SE*	Mean ± SE*	Mean ± SE*	Mean ± SE*	Mean ± SE*	Mean ± SE*
Control	2.818 ±0.777 b	3.156±0.913 ab	11.879±0.83 a	10.721±0.155 c	30.88±0.018 b	31.21±0.088 a
Tap water. (0.022 µg/kg b.wt.)	2.627±0.520 b	4.131±0.805 a	11.75±0.246 ab	11.56±0.434 ab	30.94±0.042 b	31.22±0.101 a
Canal water (0.0448 µg/kg b.wt.)	3.156±1.201 ab	3.622±0.624 ab	11.095±0.32 bc	9.67±0.184 d	30.87±0.005 b	31.16±0.0852 a
L.S.D. 5%	1.241		0.6257		0.1184	

*Data shown with the same symbols in the vertical column did not differ at 0.05 statistical levels

Table 5 Effects of single oral doses of OCl pesticide residues in water on the concentrations of Triiodothyronine (T3) and Thyroxine (T4) hormones in the serum of treated rats

Treatments	Triiodothyronine (T3) (ng/dl)		Thyroxine (T4) (µg/dl)	
	Male	Female	Male	Female
	Mean ± SE*	Mean ± SE*	Mean ± SE*	Mean ± SE*
Control	435.79±31.35 a	405.26±44.3ab	4.017±0.315b	9.633±0.761a
Tap water. (0.022 µg/kg b.wt.)	328.68±97.68 c	355.65±28.1 bc	3.328±0.061 b	7.387±2.653 a
Canal water (0.0448 µg/kg b.wt.))	320.52±16.82 bc	346.5±38.9 bc	8.592±3.623 a	8.353±2.032 a
L.S.D. 5%	78.354		3.031	

*Data shown with the same symbols in the vertical column did not differ at 0.05 statistical levels.

4. Discussion

The extensive use of pesticides in agriculture is recognized as a significant source of water pollution, typically by leaching to the underlying aquifers or by runoff to the surface [1]. The latter is of particular concern since they are often used to supply water resources for human consumption. Analysis of water samples from El Mahmodia canal (a canal that feeds Damanhour water station) and tap water in Damanhour city revealed the presence of gamma- HCH, beta-HCH, heptachlor epoxide, p, p DDE, dieldrin, endrin aldehyde, endosulfan sulfate, and endrin ketone [4]. Rats were given a single oral dose of OCl pesticide residues in tap water (0.022 µg/ kg b. wt.) and canal water (0.0448 µg/ kg b. wt.) did not show any signs of acute toxicity or mortality in treated rats. However, many hematological, biochemical, and hormonal alterations were found in the blood of treated rats. Hematological results revealed a significant increase in Hb, and decreased RBC counts in treated female rats. This observation suggests that the OCl pesticide may exhibit sex selectivity in action. This may be because the sex-dependent hematological effect of OCl is not apparent. However, it

could be suggested that the female sex hormone potentiates the metabolites of the pesticides towards such an impact on the blood. Also, white blood cell (WBC) counts showed a significant decrease in treated male and female rats relative to the control. Some investigations also obtained similar results [25] and [26].

Serum enzymes are helpful in diagnosis as they pick up any disturbances to the system early enough to allow for projection and possible remedies. In light of this, the activities of selected enzymes (GOT, GPT, ALP, and GGT) in the serum of treated rats were determined. Both male and female treated rats showed no significant GOT or GPT activity changes.

Alkaline phosphatase (ALP) is a membrane-bound enzyme applied to diagnose liver damage [27]. Two abnormal levels of this enzyme are known, an abnormally high level, which usually suggests leakage from tissues, and a deficient level, which may imply enzyme inhibition. Our results revealed a non-significant change in alkaline phosphatase (ALP) activity in treated male rats and significantly higher activity of ALP in treated female rats (2.4 folds) relative to control. This result shows that the OCl pesticide residues may have been metabolized to harmful metabolites, which may have been responsible for the leakage of the ALP enzyme into the serum. This observation conformed to the results of many authors [28, 29, 30] who reported high serum ALP levels in rats treated with chemically polluted water.

Gamma-glutamyl transferase (GGT) is a primarily membrane-bound enzyme in many organs: the kidney, pancreas, liver, spleen, and small intestine. GGT participates in the transfer of amino acids across the cellular membrane and in glutathione metabolism. Also, this enzyme could interact with the neurotransmitter (acetylcholine), which may be removed from the binding with AChE and may result in decreased activity of AChE. GGT is currently the most sensitive enzymatic indicator of hepatobiliary disease [31, 32]. Our results revealed no significant changes in G.G.T. activity in both OCl-treated male and female rats. Also, results revealed that creatinine, uric acid, albumin, and total protein are, to some extent, influenced by OCl pesticide residues administered to rats in drinking water. Alteration in the creatinine and uric acid levels indicated injury in kidney function. The average elevation of blood urea, nitrogen, and plasma creatinine can be used as an index of decreased glomerular filtration in the kidney [27].

Results of the effect of administration of OCl residues, found in both canal and tap water, on circulating hormones in treated rats revealed slight alteration in progesterone concentration. Conversely, this treatment caused a significant increase in testosterone in the serum of females and a decrease in the serum of male rats. Also, this treatment caused insignificant alteration in the concentration of follicle stimulation hormone (F.S.H.) in male rats and significant in females. In addition, a substantial increase in T4 levels in male rats and a decrease in T3 levels in female rats administered OCl residues of canal water. This finding may be because T3 is considered a biologically active thyroid hormone [33]. Unlike their effect on T3 levels, the impact of OCl residues on T4 levels in this study was inconsistent in both male and female rats. Overall, these results would indicate an impairment of the negative steroid feedback and an impairment of the inhibition B negative feedback on the hypothalamic-pituitary axis, as suggested by the increased F.S.H. release accompanying the impaired spermatogenesis [34]. Thyroid hormones affect virtually every tissue in the human body [35], and an imbalance in thyroid hormone levels can lead to a wide range of clinical conditions [36]. Several environmental agents, most notably organochlorine pesticides and polychlorinated biphenyls (P.C.B.s), have been found to influence thyroid hormone activity and metabolism [37] and [38]. The overall results of this study revealed that organochlorine pesticide residues in both canal drinking water and tap water did not cause acute toxic symptoms in rats. However, these doses cause alterations in hematological components, biochemical biomarkers of liver and kidney injury, and hormonal disruption in the treated rats. The OCl residues of canal water were more effective in this respect than those in tap water. It may be because of the higher concentration of these OCl pesticide residues in El-Mahmodia drinking water than in tap water [4].

List of Abbreviations

- Hb = Hemoglobin
- RBC = red blood cells
- PCV = packed cell volume
- ALP = alkaline phosphatase
- GGT = gamma-glutamyl transferase
- FSH = follicle stimulation hormone
- GOT = glutamic-oxaloacetic transaminase
- GGT = gamma-glutamyl transferase
- T3 = Triiodothyronine hormone
- T4 = Thyroxine hormone

5. Conclusion

The results of the present study have shown different effects of OCl pesticide residues found in both canal and tap water on the blood of male and female rats administered these residues. Thus, these organochlorine residues did not cause acute toxic symptoms or mortality in treated rats. However, they caused alterations in hematological parameters, biochemical biomarkers of liver and kidney injury, and hormonal disruption in the treated rats. The OCl residues in canal water were more effective in this respect than those in tap water. This may be due to the higher concentration of these OCl pesticide residues in El-Mahmodia water (canal water) than in tap water.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors confirm no known conflicts of interest are associated with this publication.

Statement of ethical approval

The experimental work on rats was performed with the approval of the Animal Care and Experimental Committee, Faculty of Agriculture, Damanhur University, Egypt, and according to the Guide for Care and Use of Laboratory Animals (N.R.C., Acad. Press; Washington, DC, U.S.A.,1996

Statement of informed consent

The authors consent to the publication of identifiable details in the journal, which can include images and other information within the text.

Availability of data and materials

This article includes all the data generated or analyzed during this study. Also, the related datasets are available from the corresponding author upon reasonable request.

Author's contributions

All authors contributed to the study's conception and design. They performed material preparation, data collection, and analysis. M.A. Abbassy conceived and designed the experiments, wrote the paper, and submitted it for publication on behalf of the co-authors.; E. Nour Eldin performed the experiments and analyzed the data; and M.A. Khalifa and Omar A. Omar contributed reagents/materials/analysis tools.

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