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Evaluation of Cytotoxicity and Genotoxicity of Treated Wastewater on Animal Model

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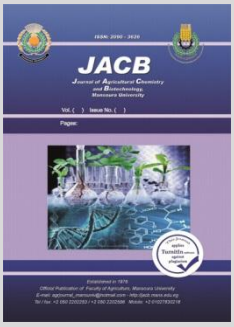

ABSTRACT

The water is important for all the living organisms. According to statistics, there is a problem in providing water for agriculture. Therefore, sewage treatment was resorted to. Wastewater contamination presents significant environmental and health challenges, but its comprehensive genetic and cellular impacts remain unclear. This study explored the genotoxic effects of different wastewater types on male albino mice, examining treated domestic (DWW), irrigation (IWW), and raw (RWW) wastewater sources. Twenty-five male mice were divided into five groups and exposed to various wastewater treatments for fifty days. Chemical analyses revealed that RWW and IWW contained significantly higher levels of pollutants and heavy metals comparing to FAW standards, whereas DWW was within acceptable limits. Histological examination of liver tissues indicated severe hydropic degeneration in RWW-treated mice. Chromosomal aberration analysis of bone marrow cells demonstrated a marked increase in structural and numerical aberrations in wastewater-treated groups, particularly in mice exposed to RWW and IWW. The mitotic index declined significantly with increasing wastewater contamination levels. Our study also evaluated the expression of apoptosis-regulating genes (P53, Bcl2, and Bax) in liver mice. The results showed significant damage associated with long-term use of wastewater. Compared to control, increased water pollution upregulated the expression of P53 and Bax while downregulating Bcl-2 expression. Notably, the MMC group (positive control) exhibited the most pronounced genotoxic and cytotoxic effects. These findings underscore the potential risks of using inadequately treated wastewater and highlight the importance of stringent wastewater management to mitigate environmental and health risks.

Keywords: Wastewater, Chromosomal aberration, Bone marrow, Apoptosis genes, and Histopathological examination.

INTRODUCTION

The need for freshwater, one of the most vital resources for life, continues to grow because of the world's expanding population. Due to the deterioration in freshwater ecosystems in some countries, it is necessary to reorganize the equitable distribution of water resources in terms of costs and benefits in order to maintain food and water security. Since agriculture is the largest consumer of fresh water, one way to solve the issue of water conservation is to use wastewater in agriculture (Dhiman *et al*., 2021). In ancient times, the purpose of irrigation with sewage water was one of the means to provide fertilizer for crops (Jaramillo and Restrepo, 2017).

The World Health Organization (WHO, 2006) clarified the concept of wastewater, which is nothing more than liquid waste resulting from the drainage units of homes, factories, and others. It also clarified the contents of this water of human and environmental waste (Karri *et al.,* 2021). About twenty million hectares of sewage water worldwide is reused directly in irrigating agricultural lands, whether treated or untreated (Mishra *et al.,* 2023). The greatest amounts of wastewater used for irrigation are in Mexico (4,493,000 m3/d), Egypt (1,918, 000 m3/d), and China (1,239,000 m3/d), according to World Bank research (De Anda and Shear 2021). Wastewater contains a high percentage of heavy metals, which are toxic to plants and cause disturbances in metabolic processes, as well as reduce plant growth and productivity (Goyal *et al.,* 2020).

Previous studies showed that poorly treated wastewater led to the deposition of heavy metals and other toxic elements in soil and plants, in addition to high pathogens that threaten human and animal health (Alengebawy *et al.,* 2021).

Histopathology, according to (Anklam *et al.,* 2022), is a useful tool for identifying and pinpointing the harmful effects of chemicals; nevertheless, these effects are often universal and may be applied to any drug or class of substances. Recent decades have witnessed an increase in cancer cases, which may be related to some of the genotoxic compounds found in wastewater. Research on the genotoxicity of wastewater is crucial since epidemiological studies have connected the use of drinking water contaminated by pollutants to a rise in cancer incidence (Du Plessis *et al.,* 2023). Only a few studies have examined the genotoxicity of hospital wastewater (Frédéric and Yves, 2014), and microbiological assays have been the most widely used method. After performing physicochemical and heavy metal analyses, animal bioassays were used to assess the genotoxicity of hospital effluent from southwest Nigeria. These included the micronucleus (MN), chromosome aberration (CA), mouse sperm morphology, and mean sperm count assays. These tests are helpful because they are particularly sensitive to mammalian germ-cell mutagens and so identify germ-cell mutagens, providing a direct and efficient method of discovering chemical agents that cause genetic damage in humans (Alabi 2011 and Oláh *et al.,* 2022).

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Increased levels of pollution can cause oxidative protein/DNA damage and the dysregulation of genes involved in the antioxidant system and intracellular redox homeostasis, which can lead to cellular death (Fulda *et al.,* 2010, Checa and Aran, 2020). When P53 detects DNA damage, the cell cycle is stopped at the G1/S checkpoint, and apoptosis is started (Maya-Mendoza *et al.,* 2014 and Watts 2022).

Wastewater extracts include a variety of xenobiotics that can trigger intrinsic and extrinsic apoptotic pathways. For example, benzene exposure was found to initiate an apoptotic pathway in rat bone marrow cells that is mediated by Bcl-2 (Shrivastava *et al.,* 2017).

Thereby, the current investigation intended to determine the genotoxic characteristics of raw wastewater and reused wastewater for domestic and irrigation purposes on mice examine the liver using histological examination, determine the frequency of chromosomal abnormalities in mice, and identify the apoptosis-related genes (P53, Bax, and BCL-2).

MATERIALS AND METHODS

Wastewater collection:

Wastewater samples from Al Gabal Al Asfar, Al Khankah, Al Qalyuobia Governorate, Egypt, were collected by Agricultural Research Center Production Sector (ARCPS) in three groups. The geographical coordinates for the collection site are 30°11'58"N, 31°22'35"E (obtained via Google Earth). Samples were analyzed at Department of Lands, Faculty of Agriculture, Ain Shams University. The first group was treated wastewater for domestic use (DWW) from the taps of houses in the area. The second group was treated wastewater for irrigation (IWW) from the main pumps to irrigate lands. The third group was raw (untreated) wastewater (RWW). About 18 liters of water were collected in each group in sterile plastic bottles that were sealed tightly. 500 ml was taken from each group for the analysis of Heavy elements (Co, B, Cr, Ni, Mn, Zn, Mn) Anions (HCO3, CO3, Cl, SO4) and Cations (Mg, Ca, K, Na) in the laboratories of the Department of Lands, Fac. of Agriculture, Ain Shams University.

Animal Experiment:

Twenty-five male albino mice, weighing 18 ± 2 g, were obtained from the National Research Center's animal house in Dokki, Cairo, Egypt. They were housed for two weeks to allow them to acclimate before the study began. Mice were kept at 22 ± 1°C with a 12-hour light/dark cycle, and they had unrestricted access to food and water before treatment.

Design and Treatments

The mice were split up into five groups, with five mice in each group. The first group was negative control, in which mice drank normal tap water (negative control). The second group drank treated domestic wastewater (DWW), and the third group drank treated wastewater for irrigation (IWW). Moreover, the fourth group drank Raw wastewater (RWW) without any purification treatment. Finally, the fifth group drank mitomycin (MMC) with a concentration of 0.6 mg/kg as a positive control.

Mitomycin (MMC) is used as a positive control as it is a well-known genotoxic agent that induces chromosomal aberrations and cytotoxicity. It has a predictable and reproducible effect on cellular systems, making it an ideal benchmark for comparing the genotoxic and cytotoxic effects of other substances (Li *et al.,* 2009).

The experiment was carried out for fifty days orally through glasses designed so that mice could drink.

Preparation of somatic cell chromosomes (bone marrow)

Upon completing the treatment period in the experiment, Mice were injected intraperitoneally with 0.4 % colchicine for 2 hr. before sacrifice. Mice were sacrificed, and then chromosomal aberration analysis was performed on somatic bone marrow cells. Chromosomes of somatic cells were prepared according to Shebl *et al.,* 2019. The muscles that adhered to the femora were removed and the tissue was cleansed. By flushing in saline (NaCl 0.9%), bone marrow cells were extracted from both femora. For ten minutes, the cells were spun in a centrifuge at 1000 rpm. To allow for osmotic swelling of cell suspensions, the pellet was redispersed, then it was incubated for 30 minutes at 40°C in a hypotonic solution containing 0.56 gm/100 ml of KCl. The cell suspensions underwent a 10-minute centrifugation at 1000 rpm, and the supernatant was thrown away. The cells were fixed in fixative 3 methanol: 1 glacial acetic acid for 10 minutes at room temp. fixative was added dropwise gently to the cells. At ten-minute intervals, centrifugation and fixation were performed three times. The cells were re-immersed in a tiny amount of fixative; a few drops from cell suspension were dropped onto chilled slides that were dipped in ice-cold ethanol 70%. The slides were flame-dried. Slides were dried completely before being stained for 30 minutes with 10% phosphate-buffered Giemsa and then allowed to dry once more. To score the various structural aberrations, including deletions, breaks, fragmentation, centromeric attenuations, and numerical changes, including polyploidy, and monosomy, at a 100x magnification, at least 50 metaphase spreads for each animal were examined under a microscope.

Mitotic index (MI)

The mitotic activity of bone marrow (BM) cells was measured using the mitotic index (MI), which is the number of dividing cells per 1000 cells. The mitotic index was also determined using the slides that were generated for the evaluation of chromosomal abnormalities. Slides that had been prepared were inspected to determine the filament index and total number of cells, no less than 2000 cells were counted in each mouse.

Evaluation of the gene expression levels involved in apoptosis:

A total RNA extraction Kit (Thermo Scientific, Fermentas, #K0731) was applied to isolate total RNA from mice liver; the kit manufacturer's protocol was followed. Complementary DNA (cDNA) was carried out according to the manufacturer's protocol kits (Thermo Scientific, Fermentas, #EP0451). Gene expression was determined using cDNA as a template for apoptosis-related genes by Step One Plus real-time PCR system (Applied Biosystem, USA). The reference gene was the housekeeping gene β-actin utilized to estimate the alteration in target gene expression. Table 1 demonstrates the primer sequences designed for each gene under investigation (p53, Bcl-2, and Bax). qRT-PCR mix and condition were carried out according to Alsenosy *et al.*, 2024 and Sharawi, 2020. The $2^{-\Delta\Delta Ct}$ approach (Livak and Schmittgen, 2001) was utilized to ascertain the ratio of gene expression and estimate the cycle threshold (Ct) values for both the housekeeping gene and the target genes.

Table 1.The sequences of the forward and reverse primers used in quantitative RT-PCR.

Primer	Sequence	References
p53	F-5-CTCACTCCAGCTACCTGAAGA-3' R-5-AGAGGCAGTCAGTCAGTCTGAGTCA-3'	Alsenosy *et al.*, 2024 and Sharawi, 2020
Bcl-2	F-5'-TTCGCAGAGATGTCCAGTCA-3' R-5'-TTCAGAGACAGCCAGGAGAA-3'	Alsenosy *et al.*, 2024 and Sharawi, 2020
Bax	F-5'-GGCTGGACACTGGACTTCCT-3' R-5'-GGTGAGGACTCCAGCCACAA-3'.	Sharawi, 2020
B actin	F-5'-ACTATTGGCAACGAGCGGTT3' R-5-CAGGATTCCATACCCAAGAAGGA-3'	Alsenosy *et al.*, 2024 and Sharawi, 2020

Statistical analysis

All the data were expressed as means ± S.E., and SAS was used to perform a one-way analysis of variance (ANOVA) to evaluate the statistical significance. The mean of the treatments was compared using the least significant differences (LSD) method with a 5% probability.

RESULTS AND DISCUSSION

Results

1. Analysis of wastewater

The normal water (control) and the different forms of wastewater samples collected from Al-Jabal Al-Asfar area were analyzed to check their pH acidity, Electric conductivity (EC), Soluble Cations (Mg, Ca, K, Na), Soluble Anions (HCO3, CO3, SO4, Cl) and some mineral elements of heavy elements (Co, B, Cr, Ni, Mn, Zn, Mn) compared to the standard parameters of FAO and the control (Figure 1, and Table 2),

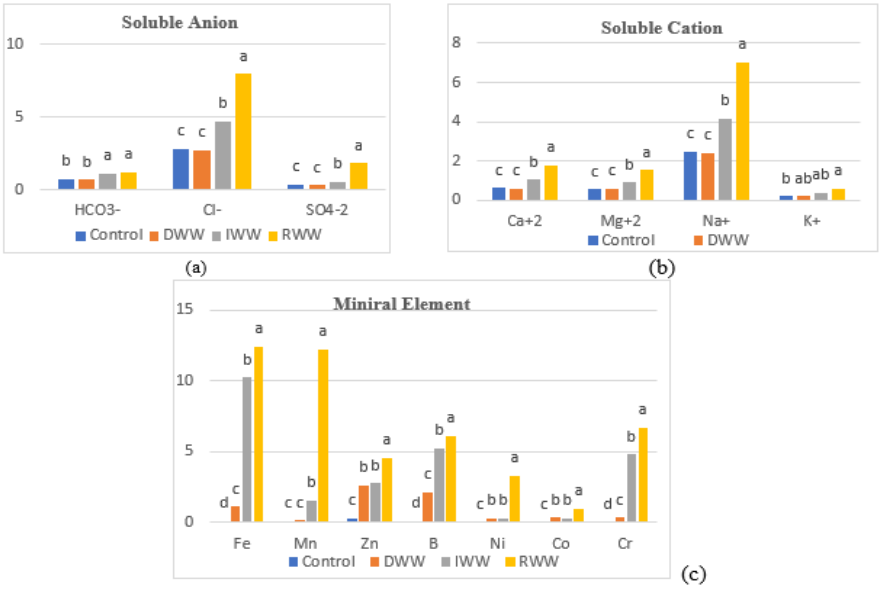

Figure 1. The values of parameters of different treatments of wastewater levels and the negative control; where (a) for Soluble anions, (b) for Soluble cations, and (c) for Mineral elements.

Different superscript letters showed significant differences at P< 0.05.

Table 2. Cations, Anions, and some mineral elements for wastewater levels and the control

	Parameter	Unit	Control	DWW	IWW	RWW	FWO standard range
	EC	dS/m	0.38	0.38	0.65	1.09	non ;<0.7 slight to moderate (0.7 - 3.0) (severe > 3.0)
	TDS	ppm(mg/l)	246	241	415	699	640-960
	pH	_	7.30	7.40	6.60	7.50	> 7
Soluble anions	CO_3^{-2}	meq/l	n.d	n.d	n.d	n.d	
	HCO_3^-		0.71	0.69	1.12	1.19	Non: <1.5 slight to moderate (1.5-7.5) (severe > 7.5)
	Cl^-		2.81	2.75(97.62mg/l)	4.74(168.27mg/l)	7.98(283.29mg/l)	above 100 mg/L most plants
	SO_4^{-2}		0.33	0.32	0.55	1.81	0-20
Soluble cations	Ca^{+2}	meq/l	0.62	0.59 (11.82mg/l)	1.05(21.04mg/l)	1.77(35.47mg/l)	Below 40 mg/L (plant deficiency)
	Mg^{+2}		0.54	0.57(6.93mg/l)	0.91(11.06mg/l)	1.54(18.72mg/l)	Below 25 mg/L (plant deficiency)
	Na^+		2.47	2.42(55.66 mg/l)	4.16(95.68 mg/l)	7.01(161.23 mg/l)	Above 50 mg/L
	K^+		0.21	0.19(7.41mg/l)	0.36(14.04mg/l)	0.60(23.4mg/l)	No high level of concern for plant growth.
Some mineral elements	Fe	ppm(mg/l)	0.12	1.12	10.25	12.41	Above 5.0 Is toxicity
	Mn		0.09	0.14	1.57	12.25	0.20
	Zn		0.30	2.62	2.76	4.58	2.0
	B		0.12	2.14	5.26	6.14	0.5 - 2.0
	Ni		0.05	0.31	0.30	3.28	0.20
	Co		0.03	0.34	0.32	0.98	0.05
	Cr		0.01	0.35	4.82	6.71	0.10

The data obtained from the analysis showed that heavy metal elements, cations, and anions significantly increased by increasing the water pollution level in RWW, and IRR in comparison with the control. There were no significant differences between DDW and control in the cations and anions concentrations, whereas the heavy metal

element (Fe, Co, B, Cr, Ni, Zn) concentrations showed significant differences between them. Moreover, there were significant differences between RWW and IRR in all the cations, anions, and heavy metal concentrations except (HCO3, and K).

2. Histological examination of mice liver

The liver is the main detoxification organ in the body; therefore, it is the most vulnerable organ. Histological change depended on the duration of exposure and the dose used for harmful substances. The effect of sewage water (RWW, IWW, and DWW) on the liver tissues of mice was studied. The sewage water treatments were compared with tap water Uncontaminated (negative control) and positive control group treated by Mitomycin c (MMC). Figure (2-A) shows the normal liver cells for the control mice. The effect of MMC on liver tissues is present in Figure (2-b), in which the blue arrowhead indicates focal hepatic degeneration while the red arrowhead indicates mild periportal mononuclear cell infiltration.

Hepato-Histological examination of RWW: The section in Figure (2-C) showed the presence of severe hydropic degeneration (vacuolation of hepatocytes), It also shows irregular hepatic cords in this section.

Hepato-Histological examination of IWW: A section of liver from mice treated for IWW showed mild hydropic degeneration (vacuolation of hepatocytes) (Figure 2-D).

Hepato-Histological examination of DWW: The liver tissues in this treatment showed a few vacuolations of hepatocytes (Figure 2-E).

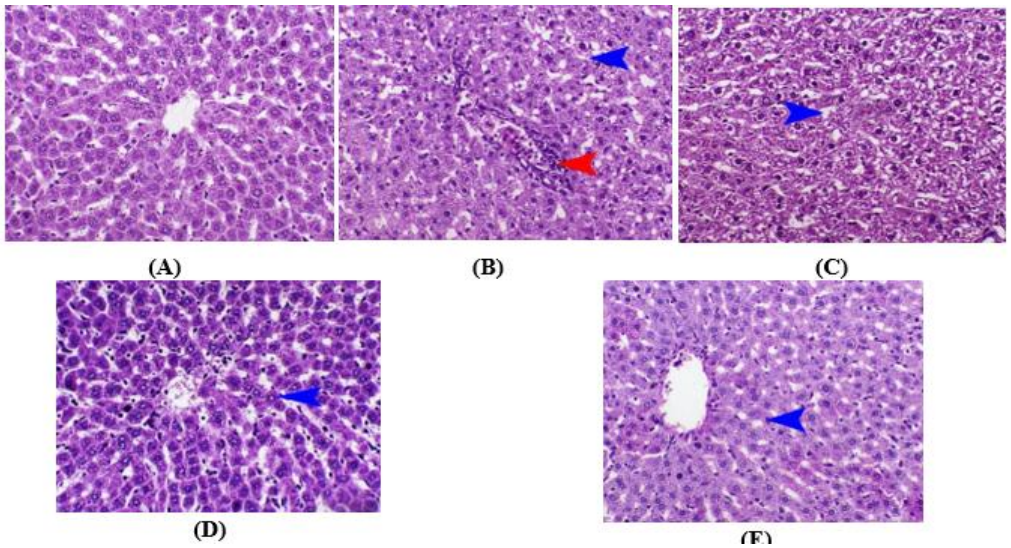

Figure 2. A section in liver mice shows: (A) normal hepatocytes (H&E, X200) in normal mice. (B) showing focal hepatic degeneration (blue arrowhead) and mild periportal mononuclear cell infiltration (red arrowhead), H&E, X200 in mice treated with mitomycin c (MMC). (C) severe hydropic degeneration (vacuolation of hepatocytes) and irregular hepatic cords, H&E, X200 in mice treated with RWW. (D) mild hydropic degeneration (vacuolation of hepatocytes), H&E, X200 in mice treated with IWW. (E) a few hydropic degeneration (vacuolation of hepatocytes), H&E, X200 in mice treated with DWW.

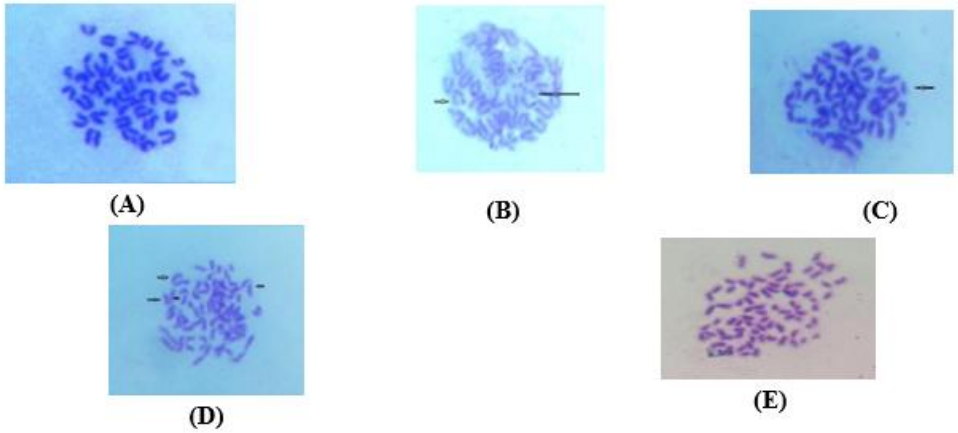

Figure 3. Photomicrograph as examples of metaphase spread from the mice bone marrow at a 100X magnification: (A) untreated mice showing normal spread. (B) treated mice showing break. (C) treated mice showing fragments. (D) treated mice showing deletion. (E) treated mice showing Centromeric attenuation.

3. Effect on mitotic activity (MI):

The slides prepared for the evaluation of chromosomal abnormalities utilized the rate of cell division to calculate the mitotic index (MI). After exposure to different forms of wastewater treatments, the mitotic activity of bone marrow cells in male mice was presented in Table (3). The percentage of mitotic activity in the positive control (MMC), the raw wastewater (RWW), and the irrigation wastewater (IWW) decreased significantly to 54%, 66%, and 78.6% in treated animals, respectively, compared to the negative

control, which was 93.6%. The MI of the RWW treatment approached the MI of the MMC (positive control). Meanwhile, the treated domestic wastewater (DWW) decreased slightly to 83% with no significant difference.

4. Chromosomal aberrations (CA) analysis in the bone marrow of mice

Fifty metaphase spreads in each treatment, and positive and negative controls were carried out. The metaphase examination of bone marrow cells exhibited a variety of chromosomal abnormalities (Table 3), including structural and numerical aberrations. Figure (3) presents the different types of chromosomal aberrations. Chromatid deletion is noted to be more frequent than other aberrations. The percentage of structural aberrations in bone marrow cells (break deletion, centromeric attenuation, break, and Fragments) was illustrated in (Figure 4). The percentage of the observed numerical aberrations that involve changes in chromosome number were hypoploidy and hyperploidy as shown in (Figure 5).

Table 3. Means and standard error of chromosomal aberrations in bone marrow cells of male mice with different treatments of wastewater levels and the controls (positive and negative)

Treatment	Cell with structural aberration				Total structural aberrations	Cell with numerical aberration		Total numerical aberrations	Mitotic Index (MI)
	Deletion	Centromeric attenuation	Break	Fragments		Hyperploidy	Hypoploidy		
Control	1.4 ± 0.51^{d}	0 ± 0^{b}	0 ± 0^{c}	0 ± 0^{c}	1.4 ± 0.51^{c}	1.0 ± 0.45^{c}	0.60 ± 0.6^{e}	1.6 ± 0.75^{d}	93.6^{a}
DWW	1.8 ± 0.37^{cd}	0 ± 0^{b}	0 ± 0^{c}	0.2 ± 0.20^{bc}	2.0 ± 0.32^{c}	$2.0 \pm 0.95c$	1.2 ± 0.49^{d}	3.2 ± 1.24^{c}	83^{b}
IWW	3.0 ± 0.71^{bc}	0 ± 0^{b}	0.2 ± 0.20^{bc}	0.2 ± 0.20^{bc}	3.4 ± 0.68^{bc}	4.6 ± 1.40^{b}	3.2 ± 1.07^{c}	7.8 ± 2.11^{b}	78.6^{c}
RWW	3.8 ± 0.58^{b}	0 ± 0^{b}	$*0.4 \pm 0.25^{b}$	0.4 ± 0.40^{b}	4.6 ± 0.68^{b}	4.6 ± 1.40^{b}	3.8 ± 1.07^{b}	8.4 ± 2.16^{b}	66^{d}
MMC	13.0 ± 1.55^{a}	2.4 ± 0.75^{a}	$*1.2 \pm 0.58^{a}$	1.4 ± 0.75^{a}	18.0 ± 1.95^{a}	8.0 ± 2.00^{a}	9.8 ± 2.46^{a}	17.8 ± 1.46^{a}	54^{e}

values are expressed as mean ± SE different, letters in the same column are significantly different (P ≤ 0.05

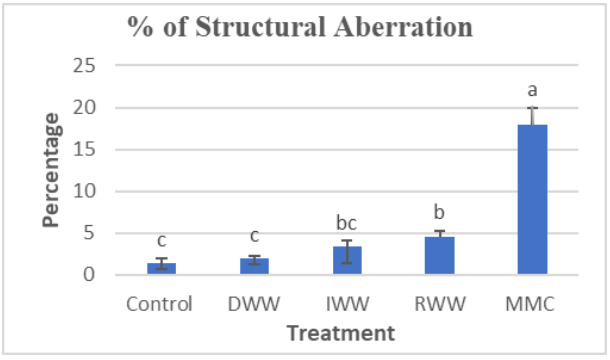

Figure 4. Percentage of total structural chromosomal abnormalities in bone marrow cells of male mice induced by different forms of wastewater treatments (DWW, IWW, and RWW) and the control (positive and negative).

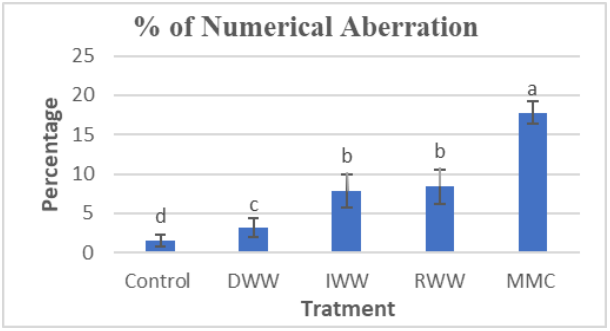

Figure 5. Percentage of total numerical chromosomal abnormalities in bone marrow cells of male mice induced by various forms of wastewater treatments (DWW, IWW, and RWW) and the control (positive and negative).

The results showed the genotoxic effect of MMC on the bone marrow cells of mice. MMC exposure demonstrated a highly significant raise in chromosomal aberrations in comparison to the negative control group. The mean value of total structural aberrations increased to (18.0 ± 1.95) ($p<0.05$). Also, cells with hyperploidy and hypoploidy increased significantly (8.0 ± 2.00) and (9.8 ± 2.46), respectively, compared to the negative control.

Effect of (DWW) treatment: Through the mean values of total structural aberrations (2.0 ± 0.32), it was found that there is no significant difference after DWW treatment. Meanwhile, the mean values of the total numerical aberrations (3.2 ± 1.24) showed a significant increase.

Effect of (IWW) treatment: A significant raise in the mean values of structural aberrations (3.4 ± 0.68) in chromosomal aberration was observed after treatment with IWW, where the most common deletion cells (3.0 ± 0.71). Moreover, there is a significant increase in the mean values of total numerical aberrations generated by IWW (7.8 ± 2.11), where the most common hyperploidy cells (2.0 ± 0.95).

Effect of (RWW) treatment: After RWW treatment, there was a considerable rise in the cell number with total structural aberrations (4.6 ± 0.68) ($P < 0.05$) relative to the negative control. Deletions were the most common aberration observed (3.8 ± 0.58). As well it significantly increased the number of cells with total numerical aberrations (8.4 ± 2.16) ($P < 0.05$) compared to the negative control. This included a significant increase in the mean of hyperploid cells (4.6 ± 1.40).

5. Evaluation of the expression levels of genes responsible for apoptotic regulation:

Gene Expression Analysis of Apoptotic Regulators Quantitative real-time PCR analysis revealed distinct patterns in the expression of apoptosis-related genes (P53, Bcl-2, and Bax) across different wastewater treatments. The study found an inverse relationship between P53/Bax and Bcl-2 expression levels in mouse liver tissue. The tumor suppressor gene P53 exhibited a significant, dose-dependent increase in expression across different wastewater treatments. Compared to the control group, P53 expression was elevated by 1.6-fold in the DWW group, 3.27-fold in the IWW group, and 4.5-fold in the RWW group (Figures 6-A). Similarly, the pro-apoptotic gene Bax followed a comparable trend, with expression levels increasing by 1.83-fold, 2.22-fold, and 4.29-fold in the DWW, IWW, and RWW groups, respectively (Figures 6-B).

In contrast, the anti-apoptotic gene Bcl-2 showed significant downregulation. Expression levels decreased to

0.44-fold, 0.3-fold, and 0.28-fold in the DWW, IWW, and RWW groups, respectively (Figures 6-C).

The mitomycin (MMC) group, used as a positive control, demonstrated the most significant gene expression alterations. P53 expression exhibited a remarkable 10.34-fold increase, while Bax gene expression reached 5.98-fold (Figures 6-A & B), both suggesting considerable cellular stress and possible genetic damage. Whereas, it demonstrated the most significant reduction of Bcl-2, with expression down to 0.12-fold (Figures 6-C).

There may be some chemicals or pathogens in wastewater that have a harmful effect on tumor suppressors. Abnormal activation of Bcl2 may lead to increased activity of abnormal cells and the formation of tumors (Czabotar and Garcia-Saez, 2023 and Green, 2022).

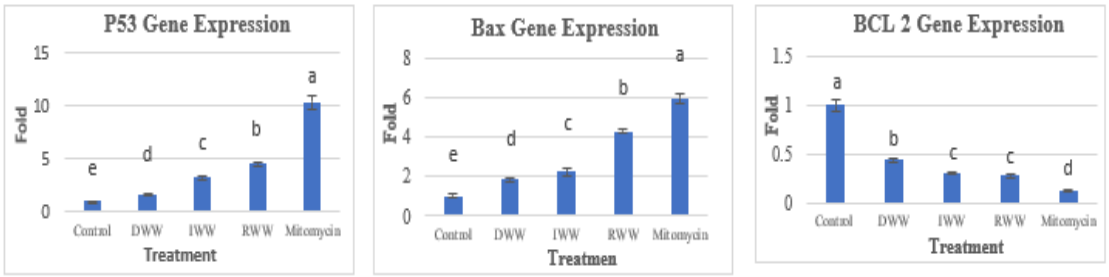

Figure 6. The effect of each raw wastewater (RWW), irrigation wastewater (IWW), and treated domestic wastewater (DWW) on apoptosis-related genes: relative expression of P53 (A), Bax (B), and Bcl2 (C) genes were evaluated using quantitative RT-PCR. P < 0.05, compared to the control group.

Discussion

The present study demonstrates the significant impact of wastewater quality on both cellular and genetic levels in living organisms. Our findings reveal a clear correlation between increasing water pollution levels and adverse biological effects, particularly in liver tissue and chromosomal integrity and gene expression.

The analysis of water quality indicated that the levels of heavy metals, particularly in RWW and IWW samples, exceeded FAO standards. This finding supports Abd-Elaty *et al.,* (2020), who emphasized on the critical need for proper wastewater treatment before agricultural reuse. The elevated concentration of heavy metal that we have observed present significant risks, as underscored by the research conducted by Karri *et al.,* (2021) concerning pollutants in municipal wastewater. These findings are particularly relevant given Singh (2021) who estimated that over twenty million hectares of agricultural land worldwide are irrigated with treated and untreated municipal wastewater.

Histological analysis of liver tissues demonstrated the severity of tissue damage correlating with the level of wastewater treatment. The severe hydropic degeneration observed in RWW-treated mice aligns with findings by Rabelo *et al.,* (2018) who reported similar hepatic deterioration in mice exposed to tannery effluent. This cellular damage pattern was also consistent with Riaz *et al.,* (2020), who observed vacuolation and inflammation in metal-exposed rat liver tissue. Further Additional evidence is provided by Renu *et al.,* (2021), who showed that low concentrations of wastewater can lead to notable hepatic alterations, such as glycogen vacuolation and leukocyte infiltration.

The genotoxic effects observed in our study warrant particular attention. The chromosomal aberration analysis revealed a compelling pattern: RWW induced significant structural and numerical chromosomal changes, while DWW showed minimal impact. This gradient of genetic damage correlates with Mathur *et al.,* 2022's findings on hospital wastewater's genotoxic effects. The observed decrease in mitotic index particularly aligns with their conclusions about cell cycle disruption. Additionally, our findings are consistent with those of Anouzla (2024), who conducted a study on hospital wastewater and demonstrated significant increases in chromosomal aberrations and aberrant cell morphology. The prevalence of deletions as the principal form of aberration in MMC and RWW-treated cells aligns with the observation of Yahaya *et al.,* (2021), regarding the critical implications of chromosomal deletions for cellular function.

Perhaps most significantly, our gene expression analysis revealed an intricate relationship between water pollution and apoptotic regulation. The inverse relationship between p53/Bax and Bcl-2 expression levels suggests complex cellular responses to wastewater exposure. This molecular mechanism aligns with the research conducted by Singh *et al.,* (2019) regarding the regulation of the apoptotic pathway. Moreover, it confirms the findings of Green (2022) as well as those of Czabotar and Garcia-Saez (2023) concerning mitochondrial permeability transition in cell death processes. Additionally, the observed changes in p53, Bax, and Bcl-2 expression align with the results of Alvarado-Ortiz *et al.,* (2021), who reported the p53's crucial role in maintaining proliferation-apoptosis balance. Neganova *et al.,* (2011) explained the relationship between these genes, which provides a mechanistic framework for understanding our observations.

The study's implications for agricultural practices are particularly relevant in water-scarce regions. While Farhadkhani *et al.,* (2018) demonstrated the potential for properly treated wastewater in Mediterranean agriculture, our findings suggest caution is warranted. The significant differences we observed between RWW and treated water samples underscore Seaf Elnasr *et al.,* (2017), who concluded the necessity of complete treatment before agricultural use.

The presence of heavy metals in wastewater raises particular concerns about carcinogenic potential, even trace amounts of elements like nickel, aluminum, and chromium can modify cancer-related genes through various mechanisms (Mishra *et al.,* 2010). The specific mechanisms of nickel-

induced carcinogenesis, described by Zhou *et al.,* (2009) and Romaniuk *et al.,* (2017) involving histone modification and DNA hypermethylation, provide a molecular basis for understanding potential long-term risks.

Our findings concerning the efficacy of water treatment confirm the importance of considering social, health, and economic conditions in wastewater management (Boelee *et al.,* 2019). The gradient of effects observed among the RWW, IWW, and DWW regimens is consistent with the findings of Qadri and Faiq (2020), who differentiated between the acute and chronic hazards associated with exposure to wastewater.

The study underscores the critical need for stringent wastewater management practices to reduce environmental and health risks. Although treated domestic wastewater (DWW) showed fewer adverse effects, the presence of residual genotoxic and cytotoxic agents calls for the development of more advanced treatment technologies, such as membrane filtration or advanced oxidation processes. Additionally, the adoption of monitoring frameworks to assess the biological impacts of wastewater on living organisms is essential for ensuring public and environmental safety.

CONCLUSION

In conclusion, the result indicated that wastewater exhibited an alteration in structural chromosomes in mice as indicated by its harmful action of increasing the levels of chromosomal aberrations; also, there was a negative effect on the mechanism of programmed cell death. Therefore, we appeal to the concerned authorities to increase control over wastewater treatment operations for the health and safety of the environment.

Declaration

Ain Shams University Committee on Experimental Animal Care and Studies Ethics, Agriculture Sector Committee, authorized all studies involving the use of animals (permission No. 15-2023-01).

This research study was conducted by ethical standards for the use of animals in research. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Agriculture (permission No. 15-2024-04).

All procedures involving animals were performed following the guidelines established by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The welfare of the animals was prioritized throughout the study, ensuring minimal distress and pain.

We affirm that all personnel involved in the study were trained in the proper handling and care of animals, and that all efforts were made to reduce the number of animals used in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement).

By adhering to these ethical standards, we aim to ensure the integrity of our research and the humane treatment of all animals involved.

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تقييم السمية الخلوية والسمية الوراثية لمياه الصرف المعالجة على النموذج الحيواني

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الملخص

الماء مهم لجميع الكائنات الحية. وفقًا للإحصائيات، هناك مشكلة في توفير المياه للزراعة، ولذلك تم اللجوء إلى معالجة مياه الصرف الصحي لحل هذه المشكلة، لما لها من فوائد عديدة عند استخدامها في الزراعة. ولكن تلوث مياه الصرف الصحي يمثل تحديات بيئية وصحية كبيرة، بينما تظل تأثيراتها الجينية والخلوية غير واضحة تمامًا. استهدفت هذه الدراسة استكشاف التأثيرات السمية الوراثية لأنواع مختلفة من مياه الصرف على 25 من ذكور الفئران البيضاء وتم تقسيمها الى خمس مجموعات حيث تعرض ثلاثة مجموعات منها الى معاملات مختلفة من مياه الصرف لمدة خمسين يومًا بالإضافة لمجموعة المقارنة ومجموعة المقارنة الإيجابية (MMC). وقد استخدم لذلك مياة الصرف المعالجة للاستخدام المنزلى (DWW)، والمعالجة للرى (IWW)، ومياه الصرف الصحى الخام (RWW).أظهرت التحليلات الكيميائية أن مياه الصرف الخام (RWW) ومياه الصرف المعالجة للري (IWW) تحتويان على مستويات مرتفعة بشكل كبير من الملوثات والمعادن الثقيلة مقارنة بمعايير منظمة الأغذية والزراعة (FAW)، بينما كانت مياه الصرف المعالجة للاستخدام المنزلي (DWW) ضمن الحدود المقبولة. أظهر الفحص النسيجي لأنسجة الكبد وجود تنكس مائي شديد في الفئران المعالجة بمياه الصرف الخام (RWW). كما أظهر تحليل الانحرافات الكروموسومية في نخاع العظم زيادة ملحوظة في الانحرافات الكروموسومية بعد المعالجة مقارنة بالمجموعة الضابطة، خاصة في الفئران المعرضة لمياه الصرف الخام (RWW) ومياه الصرف المعالجة للري (IWW). انخفض مؤشر الانقسام (MI) بشكل كبير مع زيادة مستويات تلوث مياه الصرف. كما قامت دراستنا بتقييم التعبير عن الجينات المنظمة للموت المبرمج للخلايا المبرمج P53 ، Bax، و Bcl2 في كبد الفئران. وأظهرت النتائج ضررًا كبيرًا مرتبطًا بالاستخدام الطويل الأمد لمياه الصرف الصحي .وبالمقارنة مع المجموعة الضابطة، أدى تلوث المياه المتزايد الى زيادة التعبير لجينات P53 و Bax بينما قل من التعبير الجيني ل Bcl-2. الجدير بالذكر أن مجموعة الميتوميسين (MMC)، التي استخدمت كعنصر تحكم إيجابي، أظهرت أشد التأثيرات السمية الوراثية والخلوية. تؤكد هذه النتائج على المخاطر المحتملة لاستخدام مياه الصرف غير المعالجة بشكل كافٍ وتبرز أهمية إدارة مياه الصرف الصحي بشكل صارم للحد من المخاطر البيئية والصحية.