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EVALUATION OF UNTREATED SUGAR CANE INDUSTRY EFFLUENT ON BIOCHEMICAL PARAMETERS AND HISTOPATHOLOGY IN MALE ALBINO RATS.

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ABSTRACT

The aim of present investigation was to evaluate the effects of untreated sugar cane industry wastewater (IWW) from Abu-Qurqas sugar factory on some biochemical parameters and histopathology in male Albino rats. Some physicochemical parameters of IWW and tap water were also determined. The physicochemical test results revealed that the pH of IWW sample is strongly acidic; the color is black (above normal, with smell of molasses) when compared with WHO standards while the pH and color of the tap water falls within the normal rang. The estimated heavy metals were recorded in the order Fe > Mn > Cu > Pb > Hg > Mo > Co > Cd. Fe, Pb, Hg and Mo concentrations in IWW sample were higher than WHO standards while Mn, Cd, and Cu were lower. It was observed that pH, Fe, Mn, Co, and Mo in tap water (TW) fall within the normal range when compared with WHO standards while Pb was higher while Hg, Cd and Cu were not detectable. The results of some hematological values showed statistical significant decrease ($p < 0.05$) in RBCs of rats given with IWW: at 25% v/v, 50% v/v and 100% v/v concentrations across all exposure periods relative to control groups while insignificant decrease in WBCs, PCV and MCV . The current data showed a non-significant decrease in all organs relative weight of rats orally exposed to 25% v/v, 50% v/v, and 100% concentrations of IWW compared with

the control groups while the highest reduction was recorded in the group exposed to IWW 100% for 3 months. A significant increase in the activities of serum ALT and AST of tested rat groups as the concentration of IWW increased was observed. The higher activity of serum ALT and AST was noticed in rats administrated to IWW 100% v/v concentration for 3 months. Oral administration of 25%, 50% and 100% ν IWW caused a very highly significant decrease ($p < 0.001$) in triglyceride (TG) of rat groups that in all exposure periods when compared with control groups while VHDL-c exhibited the same trend. A very highly significant increase ($p < 0.001$) in VLDL-c was recorded whereas an opposite trend decrease was noted in the value of total cholesterol (TC) in rat of tested groups compared to control groups. In contrast, the levels of both HDL-c and LDL-c in all tested groups were very highly significant ($p < 0.001$) decline during the exposure rats to different IWW concentrations for different periods compared with the control groups. Highly significant increase ($p < 0.001$) was observed in the serum total protein in rats treated with IWW 100% ν but no alterations in values were noticed due to administration of IWW 25 and 50% ν concentrations when compared with control groups. For albumin levels, in rats treated with IWW 50% and IWW 100% v/v showed very highly significant increase across all exposure periods in comparison to rats of control group whereas globulin levels were not affected. Thus A:G ratio consequently increase. Similarly, levels of serum urea were not significantly altered. All treatment groups showed a highly significant ($p < 0.001$) increase in MDA levels with a concomitant highly significant reduction in catalase (CAT) activity especially for 3 months.

The findings indicated that cane sugar effluent causes marked alterations in blood profile, lipid profile suggesting that heavy metal-containing water causes significant oxidative stress as well as mild liver and kidney damage which was proven histopathologically. The present investigation may be a valuable step in the toxicity assessment of cane sugar effluent in albino rats.

Keywords: cane sugar effluent, physicochemical parameters, blood profile, lipid profile, histopathology

INTRODUCTION

Industrial effluents are one of the principle sources of heavy metals responsible for pollution. Subsequently, contamination of soil, ground water, surface water and sediments with heavy metals is one of the major environmental problems. According to Jan, *et al.*, (2002), effluent generated from sugar industries contains contaminants, mainly heavy metals. They noted that the continuous discharge of effluent and consequent increase in concentration of heavy metals in different compartments of the environment can lead to bioaccumulation of metals in fauna and flora. Heavy metals are not biodegradable so they accumulate in primary organs in the body and over time begin to faster, leading to various symptoms of diseases. Thus, untreated or incompletely treated sugar effluent can be harmful to both aquatic and terrestrial life by adversely affecting on the natural ecosystem and long term health effects. Therefore, living organisms and other components of the environment in the immediate vicinity of any sugar factory are at risk of pollution unless treated.

Sugar industry is seasonal in nature and operates only for 120-200 days in the season and it is one of the most important agro based industry segment in Egypt. Sugar industry is one of the main sources of pollutants to surface water bodies and the adverse impacts of sugar mills effluents are well known. Due to the nature of its operations which is chemical and water intensive-eventually resulting in

high wastewater generation. Sugar industries generate about 1000 liters of waste-water for every tone of sugar cane crushed. In developing countries, untreated or partially-treated industrial wastewater is directly discharged on the nearby wetland and/or water bodies (Sorsa, *et al.*, 2015).

Several chemicals are used in sugar industries mainly for coagulation of impurities of the end products. Ca(OH)_2 is used to clarify and to increase pH of juices. A small quantity of H_3PO_4 is added prior to liming for improve clarification. CO_2 gas is bubbled through the defecated juice to lower pH, which result in the improvement of precipitation of impurities. Polyelectrolytes, which are polymer based chemicals, are also used for the coagulation of impurities during defecation and carbonation process. SO_2 is bubbled through the defecated raw sugar to remove colour. Diluted solutions of NaOH or Na_2CO_3 are used for the periodic descaling of heaters followed by neutralizing it with dilute HCl . Lead sub acetate is used for the analysis of sugar content. All these chemicals, one-way or another, are contributing towards increasing the organic strength, dissolved solids and suspended matter (Jadhav, *et al.*, 2013).

Use of industrial effluent and sewage sludge on agricultural land has become a common practice in India and developing countries as a result of

which these toxic metals can be transferred and concentrated into plant tissues from the soil. These metals have damaging effects on plants themselves and may become a health hazard to man and animals (Saranraj and Stella, 2014).

Sugar factory effluent produces obnoxious odor and unpleasant color when released into the environment without proper treatment. Farmers have been using these effluents for irrigation, found that the growth, yield and soil health were reduced (Ozoh and Olasimeji, 1984). Contaminants such as chloride, sulphate, phosphate, magnesium and nitrate are discharged with the effluent of various industries which create a nuisance due to physical appearance, odor and create a nuisance due to physical appearance, odor and taste. Such harmful water is injurious to plants, animals and human beings. The effects of various industrial effluents on seed germination, growth and yield of crop plants have captivated the attention of many workers.

Egypt is among the more productive states of sugar after India and Brazil. Sugar industry plays an important role in the rural economy of Egypt by uplifting the livelihood and creation of employment. Sugar production in Egypt depends on the sugar cane and sugar beet. Abu Qurqas sugar factory is one of the oldest sugar factories in Egypt, located in southern province of Minia, with three working shifts a day, its production depends on

sugar cane and sugar beet, and it works about 120-200 days a year, season of sugar cane and sugar harvest. Due to no enough reports on effect of sugar cane industry wastewater on rats necessitated this study. Therefore, the aim of present investigation was to evaluate the effects of cane sugar industry wastewater from Abu-Qurqas sugar factory (IWW) on some serum biochemical parameters and histopathology in male Albino rats. Some physic-chemical parameters and heavy metal constituents of IWW were also analysed.

MATERIAL AND METHODS

1- Wastewater Collection:

The industrial wastewater (IWW) samples have been collected from the outlet Abu-Qurqas sugar factory before entering into Effluent Treatment Plant. The samples had a smell of molasses and it was dark in colour. Temperature and pH of samples were measured, filled in plastic jerry cans of 20 Litres capacity, transferred to the laboratory and preserved in bottles 1L, 2L, 4L, and then kept frozen at -20°C until needed for analysis. This (IWW) was applied as drinking water at three concentrations of 25% v/v , 50% v/v , and 100% using distilled water.

2- Animals:

Sixty healthy male Wistar Albino rats of 4-8 weeks old, weighing 120-150g were selected after physical and

behavioural veterinary examination in Animal experimental Lab., Agric. Chem. Dept., Fac., Agric., El-Minia Univ., El-Minia, Egypt. They were housed in standard cages and left to acclimatize for 7 days to standard laboratory conditions-light/dark (12hr light: 12hr darkness) at a temperature of 25⁰C, before commencement of the experiment and standard laboratory conditions throughout the experiment (Compbell, 1961). The animals were maintained on standard laboratory feed (commercial diets, 100% vegetarian feed, 21% protein from production of Cairo Feed Company) and portable water *ad libitum*.

3- Experimental design:

The animals were randomly evenly divided into twelve groups of five rats each as follows:

Group 1: Standard Feed + Potable water served as the control group for 30 days.

Group 2: Standard Feed + (25% IWW) for 30 days.

Group 3: Standard Feed + (50% IWW) for 30 days.

Group 4: Standard Feed + (100% IWW) for 30 days.

Group 5: Standard Feed + Potable water served as the control group for 60 days.

Group 6: Standard Feed + (25% IWW) for 60 days.

Group 7: Standard Feed + (50% IWW) for 60 days.

Group 8: Standard Feed + (100% IWW) for 60 days.

Group 9: Standard Feed + Potable water served as the control group for 90 days.

Group 10: Standard Feed + (25% IWW) for 90 days.

Group 11: Standard Feed + (50% IWW) for 30 days.

Group 12: Standard Feed + (100% IWW) for 90 days.

4-Estimation of heavy metals in wastewater samples:

Heavy metals (*Fe*, *Mn*, *Co*, *Pb*, *Hg*, *Cd*, *Cu*, and *Mo*) were estimated in water samples using an Atomic Absorption Spectrophotometer (Perkin Elmer, Analyst A 800) as per the standard protocols of APHA (2005) at the Centre for Agricultural Research, Regional Centre for Food and Feed, Cairo, Egypt.

5- Biological evaluation and Animals Sample Preparation:

Food consumption was monitored daily and the body weights were measured once a week. Daily body gain (DBG), Daily feed intake (DFI) and the feed efficiency ratio (FER) were calculated for the nutritional experiment part.

At the end of each experimental period (30, 60 and 90 days), the animals after fasting overnight were sacrificed following mild ether anaesthetics. The blood samples were collected by cardiac puncture into

clean centrifuge tubes and allowed to coagulate and centrifuged at 3000 rpm for 20 minutes to separate the blood serum samples. Separated serum was stored at -20⁰C for subsequent biochemical analysis. The kidney and liver tissues were excised and stored in freshly prepared formalin for histological processing and microscopy.

Liver of rats was dissected out the sliced and extracted with 0.9% phosphate buffer saline (pH 7.4) solution containing heparin. After homogenization and centrifugation the filtrate was stored in ice and used for malondialdehyde (MDA) and catalase (CAT) activity determinations.

6- Biochemical Analysis:

1.6- Antioxidant Parameters:

Lipid peroxidation (LPO) was measured as malondialdehyde (MDA) and its concentration was determined in terms of thiobarbituric acid reactive substances (TBARS) with colorimetric method as described by Ohkawa *et al.* (1979). Catalase activity was also assayed according to the method of Ohkawa *et al.* (1979).

Haematological determinations:

Erythrocytic Count (RBC), Total leucocyte Count (WBC), Haematocrite (Paked Cell Volume) PCV value and Wintrob Erythrocyte Indices (mean corpuscular volume, MCV) were determined and calculated by Dacie and Lewis (1991).

Serum proteins determination: Serum total protein was determined by the biuret reagent as described by Gornall *et al.*, (1949). Serum albumin (A) concentration was determined according to the method of Doumas *et al.* (1971) using reagent kits (Spin react Company, Spain). Globulin (G) was determined by subtraction of albumin from total protein (Javed and Usmani, 2015) and A: G ratio was calculated according to the results of albumin and globulin.

Liver Function Tests:

The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using commercial test kits (Randox Laboratories, UK) based on the colorimetric method of Reitman and Frankel, (1957).

Kidney Function Tests:

Serum urea and creatinine concentrations were colorimetrically measured according to Kroll *et al.* (1987).

Parameters of Lipid Profile

Enzymatic colorimetric method for determination of triglycerides (TG) was carried out according to method of Fassati and Prencipe (1982). Total cholesterol (CHL) was estimated by the method of Burstein *et al.* (1970) and high density lipoprotein cholesterol (HDL-Cholesterol) was determined according to method of Allain *et al.* (1974). Low density

lipoprotein-cholesterol (LDL-c), very low density lipoprotein- cholesterol (VLDL-c) and risk factor (RF) were calculated according to Friedewald *et al.* (1972) equation (mg/dl) by the following formula:

$$\text{VLDL (mg/dL)} = \text{Triglycerides (TG)}/5$$

$$\text{LDL-c (mg/dL)} = \text{Total Cholesterol} - [\text{HDL-c} + (\text{TG}) /5]$$

$$\text{RF} = \text{LDL-c} - / \text{HDL-c}$$

Histopathological Studies of Liver and Kidney

Histopathological studies were carried out on liver and kidney tissue samples from different groups of rats at the end of experimental period using the modified method described by Okoro, (2002).

2.6- Statistical Analysis

Statistical Analyses were performed by using graph pad prism statistical software package (version 6). Results were presented as means with their standard Deviation (mean \pm SD). Statistical differences between the means of the various groups evaluated using one way analysis of variance (ANOVA) followed by Tuckey s test. Dates were considered statistically at $p < 0.05$.

RESULTS AND DISCUSSION

1- Some physicochemical parameters and heavy metals in water samples:

Table (1) shows the results of some physicochemical tests carried out

on samples from cane sugar factory of Abu-Qurqas wastewater (IWW), heavy metal concentration and tap water (TW) used as control in comparison with WHO permissive standards as recorded by Olajire and Imeokpara (2000); Alabi, *et al.* (2014). . Physicochemical analysis of water has long been employed to assess its quality. Temperature, pH, DO, TSS and TDS (not determined) are often used as measures of water quality because any change in them reflects the pollution status of natural waters. It was observed that the pH of IWW sample is strongly acidic; the color is black (above normal, with smell of molasses) when compared with WHO standards while the pH and color of the tap water falls within the normal range when compared with WHO standards. This is in correlation with a previous study by Alabi *et al.* (2014). The pH value obtained, 4.4, for the IWW is outside the WHO recommended for drinking water which made the IWW potentially hazardous especially if it used in irrigation or drinking without proper treatment (Ozoh and Oladimeji (1984). The low pH value of this IWW may be due to the acidic materials used during the manufacturing process, and the dark (black) may due to the molasses backward from the manufacturing process. The estimated heavy metals were recorded in the order $\text{Fe} > \text{Mn} > \text{Cu} > \text{Pb} > \text{Hg} > \text{Mo} > \text{Co} > \text{Cd}$. As shown in Tabl (1), Fe, Pb, Hg and Mo concentrations in IWW sample were higher than international maximum permissible levels. Mn, Cd, and Cu

were below the international recommendable minimum concentrations. As a result, heavy metal toxicity may result from consumption of the IWW under consideration. Moreover, the long term use of this sugar effluent IWW for irrigation which contaminates soil and crops to such an extent that it becomes toxic to plants and causes deterioration of soil (Fakayode, 2005).

2- Effect of IWW at different concentrations on some blood parameters in tested rats (Haematological Indices, HI):

Hematological indices are useful because they are used to ascertain in the future disease state of the body (Okeke *et al.*, 2006). They also explain the blood relating functions of substances that enter the body. Effects of oral administration of IWW at various concentrations on some hematological parameters in tested rat's blood for different periods are presented in Table 2. Oral administration of IWW (25%) to rats for one month resulted in statistically highly significant decrease ($p < 0.01$) in RBCs relative to control group and resulted in statistically significant decrease ($p < 0.05$) in RBCs of rats given with IWW across all exposure concentrations for the different periods compared to the control groups. Also, data in Table 2 showed insignificantly decreased in WBCs, PCV and MCVfL due to treatment of rats with different concentration of IWW samples during different periods. The noticeable decrease in RBCs concentration may

be attributed to retardation, disturbance in the erythropoiesis in bone marrow. Also, due to much faster rate of destruction of peripheral red blood cells in spleen (Lee *et al.* 1999). Our findings were confirmed with previous reports which showed that untreated silk dye effluent (50% and 75%) for 30, 45, and 60 days exposed mice had severe haematological disorder. These current results agree with Alabi, *et al.* (2014).

3- Effect of IWW at different concentrations on body weight of tested rats:

Monitoring of body weight during treatment provides an index of general health status of the animals and such information may be important for the interpretation of health. The effect of IWW on the body weight, feed intake and feed efficiency was given in Table (3). No significant changes were observed in pattern of body weight of rats orally exposed to all concentrations of IWW except IWW 50% and 100% dose groups for 3 months, respectively, showed a significant ($p < 0.05$) and highly significant ($p < 0.01$) decrease in daily body weight (DBW) relative to control groups. It was obviously noticed that the reduction in daily body weight (8.74% relative to control) of rats exposed to IWW 100% for 3 months was correlated with daily feed intake. The results are in agreement with Kanthariya and Tank (2015) who found a significant decrease in the body weight of rats as textile effluent concentration dose increase.

Table (1): Some physico-chemical parameters and heavy metal content of IWW samples and tap water (TW) using WHO values as standards.

Water sample and WHO standards	Some physico-chemical parameters				Metal concentration (ppm)							
	Ordour	Colour	pH	Temp	Fe	Mn	Co	Pb	Hg	Cd	Cu	Mo
IWW	Smell of molasses	Black with impurities	4.4	25	7.62	0.27	0.08	0.90	0.190	0.010	0.26	0.09
TW	Odourless	Colourless	7.4	20	0.29	0.18	0.10	0.47	0.000	0.002	0.02	0.89
WHO* MPC	--	--	8.0	--	1.30	0.50	ND	0.10	0.010	0.003	1.50	0.07
Values for fresh water	RMC	--	6.5	--	0.30	0.10	ND	0.05	0.001	0.005	1.00	0.01

*Source: Olajire and Imeokpara (2000); Alabi, *et al.* (2014).

Key: WHO = World Health Organization, MPC = Maximum permissible concentration, RMC = Recommendable minimum concentration, ND = not determined.

Table (2): Some hematological parameters of tested rats treated with IWW sample (25; 50 and 100% v/v).

Treatment		RBCs ×10 ⁶ /mm ³	W B Cs ×50/mm ³	P C V	M C V(fL) (10 ⁻¹⁵ litre)
Control	One month	7.01±0.12	3.43±0.05	49.43±0.42	70.57±1.70
	Two month	5.10±0.41	1.53±0.05	47.97±1.40	77.36±32.35
	Three month	5.50±0.33	4.20±0.01	49.50±1.71	91.10±5.67
I W W25%	One month	5.29**±0.64	3.41 ^{ns} ±0.06	52.87 ^{ns} ±1.80	100.7 ^{ns} ±9.111
	Two month	4.65 ^{ns} ±0.15	2.45 ^{ns} ±0.02	55.97 ^{ns} ±1.74	120.4 ^{ns} ±3.75
	Three month	4.64 ^{ns} ±0.14	3.90 ^{ns} ±0.04	53.50 ^{ns} ±0.7000	115.3 ^{ns} ±1.97
I W W50%	One month	5.49*±0.015	4.83 ^{ns} ±0.01	49.83 ^{ns} ±3.26	90.83 ^{ns} ±6.02
	Two month	4.74 ^{ns} ±0.54	2.28*±0.04	51.27 ^{ns} ±3.20	109.5 ^{ns} ±18.35
	Three month	7.42*±0.32	3.53 ^{ns} ±0.01	51.83 ^{ns} ±2.84	69.90 ^{ns} ±1.22
I W W100%	One month	5.62*±0.42	4.03 ^{ns} ±0.01	52.63 ^{ns} ±1.16	93.87 ^{ns} ±5.35
	Two month	5.13 ^{ns} ±0.74	2.90 ^{ns} ±0.01	52.90 ^{ns} ±4.19	105.3 ^{ns} ±16.34
	Three month	4.91±1.90	3.55 ^{ns} ±0.01	51.80 ^{ns} ±1.56	120.8 ^{ns} ±54.75

Values are means ± (S.E.), Number of rats per group = 5, ns = non- significant difference.

* Significant difference (p<0.05), ** highly significant difference (p<0.01).

Table (3): Effect of IWW on initial body weight (IBW), final body weight (FBW), daily body gain (DBG), daily feed intake (DFI) and feed efficiency ratio (%) in rats.

Treatment		IBW (g)	FBW (g)	DBW(g)	DFI (g)	FER (%)
Control	One month	20.60±1.49	34.88±3.51	0.47±0.08	18.83	2.52 ± 0.45
	Two month	19.64±1.11	41.16±3.37	0.36±0.05	19.90	1.81 ± 0.26
	Three month	17.52±0.59	48.04±5.69	0.34±0.06	19.43	1.75 ± 0.32
IWW 25%	One month	26.16*±1.49	37.12 ^{ns} ±2.09	0.44 ^{ns} ±0.13	19.68	2.24 ^{ns} ± 0.63
	Two month	25.32 ^{ns} ±3.81	40.68 ^{ns} ±4.40	0.26 ^{ns} ±0.04	18.44	1.39 ^{ns} ± 0.20
	Three month	22.64 ^{ns} ±3.61	50.84 ^{ns} ±6.49	0.31 ^{ns} ±0.04	19.42	1.62 ^{ns} ± 0.21
IWW 50%	One month	26.00*±2.29	43.08 ^{ns} ±1.66	0.57 ^{ns} ±0.07	19.85	2.86 ^{ns} ± 0.35
	Two month	25.40 ^{ns} ±3.73	40.04 ^{ns} ±5.15	0.25*±0.03	18.91	1.30 ^{ns} ± 0.17
	Three month	22.48 ^{ns} ±1.63	44.72 ^{ns} ±6.12	0.25 ^{ns} ±0.07	18.20	1.38 ^{ns} ± 0.35
IWW 100%	One month	26.20*±2.08	42.28 ^{ns} ±4.17	0.54 ^{ns} ±0.11	19.55	2.79 ^{ns} ± 0.59
	Two month	25.36 ^{ns} ±3.55	41.04 ^{ns} ±1.09	0.26 ^{ns} ±0.06	19.08	1.37 ^{ns} ± 0.32
	Three month	22.68 ^{ns} ±1.96	43.84 ^{ns} ±5.25	0.22**±0.03	18.32	1.20* ± 0.15

Values are means ± (S.E.), Number of rats per group = 5, ns = non- significant difference, * Significant difference (p<0.05), ** highly significant difference (p<0.01).

4- Effect of IWW at different concentrations on relative organs weight (%) of tested rats:

Data in Table (4) reveals effect of IWW at various concentrations on relative organ weights % e.g. heart,

liver, kidney, spleen, lung and brain. There was a non-significant decrease in all organs relative weight ratio of rats orally exposed to 25% v/v, 50% v/v, and 100% concentrations of IWW compared with the control, while a

significant and highly significant reduction in relative heart weight observed in the group exposed to 1WW 25% for 2 months and the group exposed to 1WW 50% for 2 months, respectively. This is in line with the work of Barnes and Denz, 1954, who established that changes in organ weight is one of the criteria of toxicity and diseased condition.

5- Effect of IWW at different concentrations on aminotransferase activities (ALT) and (AST) in tested rats:

Transaminases (ALT and AST) are well-known cytosolic enzymes of hepatocyte used as biomarkers to predict possible toxicity. Generally, damage to liver cells will result in elevations of these transaminases in the serum (Singh *et al.* 2001). Furthermore, measurement of enzymic activities of ALT is of clinical and toxicological importance as changes in its activities are indicative of liver damage by toxicants or a diseased condition (Singh *et al.* 2001). Table 5 shows the specific activities of ALT and AST in serum of rats exposed to different concentrations of IWW compared with the control. Relative to the control, there was a significant increase in ALT and AST activities as the concentration of the IWW and exposure time increased. It was noticed that the specific activities of both enzymes were highly significant increased ($p < 0.01$) in rat groups given IWW at 25 and 50% v/v concentrations for 3 and 1 months, respectively, but this increasing in the activity of both

enzymes was most very highly significant ($p < 0.001$) in rats placed on 50% for 2 and 3 months and 100% v/v throughout 3 months when compared with control. The observed significant increase in the activities of serum ALT and AST of rat groups as the concentration of IWW increased indicated hepatocellular damage. This is may due to alteration in the liver cellular system which results in enzyme leakage into the serum. Moreover, alkaline phosphatase, ALP (not determined) is a marker enzyme for the plasma membrane and endoplasmic reticulum, therefore, damage to membranes of tissues may affect ALP activity. The effluent may also contain substances which are capable of inhibiting or inactivating ALP in situ (Umezawa and Hooper 1982). Normally, enzyme will not always be found in the serum except there is damage to one or more organs or tissues of the body. Therefore, enzymes from diseased tissues or organs (e.g. cardiac, hepatic and neoplastic diseases) and from drug assault or other xenobiotics may become manifested in the serum resulting in increased activity since they must have leaked from the diseased tissue as reported in hepatocellular disease (Mathur, *et al.* 2003). Increased activity of serum enzymes have been reported in conditions of tissue damage due to such disease conditions and from the use of several chemicals and drugs (Hanley *et al.* 1986).

Table (4): Effect of IWW on organ-to-body weight ratios (relative organ weights (%)) in rats.

Treatment	Heart%	Liver%	Kidney%	Spleen%	Lung%	Brain%	
Control	One month	0.46±0.055	4.62±0.25	0.95±0.10	0.62±0.19	0.78±0.16	0.83±0.10
	Two month	0.40±0.053	3.34±0.35	0.73±0.060	0.40±0.069	0.68±0.095	0.89±0.069
	Three month	0.37±0.012	3.99±0.24	0.73±0.060	0.37±0.057	0.65±0.122	0.62±0.14
I W W 25%	One month	0.44 ^{ns} ±0.038	4.39 ^{ns} ±0.38	0.92 ^{ns} ±0.072	0.57 ^{ns} ±0.12	0.71 ^{ns} ±0.062	0.95 ^{ns} ±0.044
	Two month	0.36 ^{**} ±0.036	2.98 ^{ns} ±0.50	0.67 ^{ns} ±0.069	0.38 ^{ns} ±0.066	0.74 ^{ns} ±0.079	0.77 ^{ns} ±0.11
	Three month	0.39 ^{ns} ±0.086	3.08 ^{ns} ±0.23	0.67 ^{ns} ±0.069	0.33 ^{ns} ±0.043	0.74 ^{ns} ±0.11	0.56 ^{ns} ±0.054
I W W 50%	One month	0.40 ^{ns} ±0.054	4.00 ^{ns} ±0.30	0.84 ^{ns} ±0.055	0.71 ^{ns} ±0.22	0.77 ^{ns} ±0.12	0.84 ^{ns} ±0.040
	Two month	0.38 [*] ±0.031	3.10 ^{ns} ±0.097	0.73 ^{ns} ±0.037	0.43 ^{ns} ±0.13	0.74 ^{ns} ±0.13	0.84 ^{ns} ±0.14
	Three month	0.41 ^{ns} ±0.061	3.43 ^{ns} ±0.27	0.73 ^{ns} ±0.037	0.35 ^{ns} ±0.027	0.74 ^{ns} ±0.12	0.67 ^{ns} ±0.096
I W W 100%	One month	0.40 ^{ns} ±0.039	3.78 ^{ns} ±0.36	0.83 ^{ns} ±0.040	0.55 ^{ns} ±0.17	0.69 ^{ns} ±0.11	0.82 ^{ns} ±0.11
	Two month	0.39 ^{ns} ±0.026	3.40 ^{ns} ±0.41	0.74 ^{ns} ±0.043	0.37 ^{ns} ±0.034	0.68 ^{ns} ±0.055	0.80 ^{ns} ±0.11
	Three month	0.38 ^{ns} ±0.088	4.06 ^{ns} ±0.38	0.74 ^{ns} ±0.043	0.39 ^{ns} ±0.042	0.82 ^{ns} ±0.15	0.76 ^{ns} ±0.046

Values are means ± (S.E.), Number of rats per group = 5, ns = non-significant difference, * Significant difference (p<0.05), ** highly significant difference (p<0.01).

It is well known that the transaminases play a paramount role in amino acid metabolism and in providing necessary intermediates for gluconeogenesis. It has also been reported that these enzymes assist in differential diagnosis of cardiac

diseases. On the other hand, the significant increase in activities of the two enzymes in the serum suggest that there may be a leakage of the enzymes from the liver to the serum which may be interpreted to mean damage to the liver (Hanley et al. 1986).

Table (5): Serum aminotransferase activities (ALT) and (AST) in rats treated with various concentrations of IWW.

Treatments		ALT (U/L)	AST (U/L)
Control	One month	42.76±2.07	148.5±4.03
	Two month	25.41±1.25	113.6±13.41
	Three month	43.24±1.52	94.66±2.61
I W W25%	One month	44.34 ^{ns} ±0.06	123.4 ^{ns} ±4.16
	Two month	31.21 ^{ns} ±7.79	151.3 ^{ns} ±14.77
	Three month	25.83 ^{**} ±0.63	103.0 ^{**} ±16.22
I W W50%	One month	52.31 ^{**} ±6.81	128.5 ^{ns} ±20.38
	Two month	52.37 ^{***} ±7.52	162.6 ^{***} ±7.79
	Three month	23.82 ^{**} ±0.32	133.8 ^{**} ±16.84
I W W100%	One month	58.02 ^{***} ±0.62	121.8 ^{ns} ±3.30
	Two month	81.06 ^{***} ±0.88	163.3 ^{***} ±20.75
	Three month	17.54 ^{***} ±4.95	158.6 ^{ns} ±9.24

Values are means ± (S.E.), Number of rats per group = 5, ns = non- significant difference, * Significant difference (p<0.05), ** highly significant difference (p<0.01),*** Very highly significant difference (p<0.001).

6- Effect of IWW at different concentrations on serum lipid profile of tested rats:

Table (6) shows lipid profile in rats treated with various concentrations of IWW for different periods with control. Tabulated data showed that rats in control groups contain triglyceride (TG) of 147±13.01, 199±56.02 and 149±10.06 mg/dL for 1, 2 and 3 months, respectively. Oral administration of 25%, 50% and 100%

v/v IWW to rats in experiment groups caused significant decrease (p<0.05) in triglyceride (TG) of rat groups received 25% IWW for 2 and a very highly significant decrease (p<0.001) for 3 months and a very highly significant decrease (p<0.001) in the rest parameters in rats of experiment groups exposed to all IWW concentrations for all periods when compared with control.

A very highly significant increase ($p < 0.001$) in total cholesterol (TC) was noticed at all IWW concentrations for all experimental periods compared to control groups. Similarly, the levels of both HDL-c and LDL-c in all experimental rats were decreased very highly significant during the exposure of rats to different IWW concentrations at different periods compared with the control groups. Other workers also recorded similar elevations and reductions in these parameters when the fishes exposed to silk dye effluent along with control (Hanan *et al.* 2013). Kanthariya and Tank (2015) reported a significant decrease in serum triglyceride level in Wistar albino rats orally administered with textile effluent for 28 days. Elevation in these parameters particularly cholesterol is ascribed due to the mobilization of lipid either through oxidation or a process of gradual instauration of lipid molecules from the synthesis site for subsequent utilization. Similarly the VLDL fraction decrease very highly significantly ($p < 0.001$) since its concentration depends on the triglyceride fraction. Triglyceride-rich lipoproteins (VLDL) are believed to be the components of the innate, non-adaptive immune defense system thus their decline leads to immune suppression. Elevation in lipid profile is either due to disturbance in the

metabolism of lipids or may be due to impaired clearance from plasma which favors liver dysfunction (Kanthariya and Tank (2015). Very no work had carried out in laboratory based studies in rats pertaining effect of sugar cane industry wastewater on lipid profile.

7- Effect of IWW at different concentrations on serum urea and protein profile of tested rats

Impact of different industrial wastewater (containing heavy metals) has been observed on protein profile of different tissues on Swiss albino rats. Within the body albumin and globulin makes most of the proteins and alteration in quantities of these proteins occur due to which the A:G ratio gets disturbed when intoxicated with xenobiotics/heavy metals (Sharma, *et al.* 2007). In the present study highly significant increase ($p < 0.001$) was observed in the serum total protein in rats treated with IWW 100% v/v but no alterations in values were noticed due to administration of IWW 25 and 50% v/v concentrations when compared with control groups. For albumin levels, in rats treated with IWW 50% and IWW 100% v/v showed very highly significant increase across all exposure periods in comparison to rats of control groups. Globulin levels were not affected by IWW at all tested concentrations.

Table (6): Lipid profile in rats treated with various concentrations of IWW.

Treatment		TG(mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
Control	One month	147.6±13.01	175.5±1.88	83.01±9.40	30.66±1.26	69.86±1.57
	Two month	199.1±56.02	147.5±16.87	77.42±1.31	27.12±0.53	38.16±9.84
	Three month	149.0±10.06	191.4±8.92	77.50±1.80	29.81±2.01	84.07±6.25
IWW 25%	One month	141.9 ^{ns} ±16.91	210.2 ^{***} ±2.41	91.03 ^{ns} ±5.24	28.38 ^{ns} ±0.33	90.74 ^{ns} ±9.07
	Two month	135.6 [±] ±2.64	245.9 ^{***} ±4.40	94.10 ^{***} ±1.54	19.16 ^{***} ±1.05	124.7 ^{***} ±5.37
	Three month	118.0 ^{***} ±4.67	264.2 ^{***} ±16.0	95.13 ^{***} ±1.84	23.61 ^{***} ±0.94	145.4 ^{***} ±17.8
IWW 50%	One month	108.6 ^{***} ±1.76	255.1 ^{***} ±4.24	97.96 [±] ±2.06	21.73 ^{***} ±0.35	135.4 ^{***} ±6.39
	Two month	95.77 ^{***} ±5.28	274.9 ^{***} ±4.35	96.31 ^{***} ±1.93	14.16 ^{***} ±0.98	159.4 ^{***} ±4.89
	Three month	103.3 ^{***} ±4.19	292.4 ^{***} ±8.35	100.4 ^{***} ±4.11	20.66 ^{***} ±0.84	171.2 ^{***} ±6.52
IWW 100%	One month	95.95 ^{***} ±2.29	281.0 ^{***} ±10.2	100.5 ^{***} ±2.74	19.19 ^{***} ±0.46	161.2 ^{***} ±11.8
	Two month	70.82 ^{***} ±4.89	345.9 ^{***} ±31.9	100.5 ^{***} ±2.95	26.99 ^{***} ±2.1	231.3 ^{***} ±35.8
	Three month	91.62 ^{***} ±5.35	328.6 ^{***} ±2.85	106.5 ^{***} ±2.59	18.33 ^{***} ±1.07	203.9 ^{***} ±0.52

Values are means ± (S.E.), Number of rats per group = 5, ns = non- significant difference, * Significant difference (p<0.05), ** highly significant difference (p<0.01),*** Very highly significant difference (p<0.001).

3.7- Effect of IWW at different concentrations on serum urea and protein profile of tested rats

Table (7): Effect of IWW on serum urea, total protein, albumin (A) and globulin (G) levels and A: G ratio in rats.

Treatment		Urea(mg/dL)	Protein (g/L)	Albumin (g/L)	Globulin (g/L)	A : G
Control	One month	66.67±5.28	5.200±0.100	3.85±0.24	1.293±0.385	3.170±1.006
	Two month	62.17±1.04	5.183±0.057	3.33±0.11	1.850±0.121	1.807±0.177
	Three month	63.07±4.17	5.067±0.076	4.07±0.12	0.943±0.134	4.397±0.812
IWW 25%	One month	64.83 ^{ns} ±5.39	5.640 ^{ns} ±0.134	4.95 ^{ns} ±0.53	1.107 ^{ns} ±0.150	4.155 ^{ns} ±0.609
	Two month	62.17 ^{ns} ±0.58	5.867 ^{ns} ±0.775	4.08 ^{***} ±0.050	1.78 ^{ns} ±0.741	2.537 ^{ns} ±0.905
	Three month	49.03 ^{**} ±0.50	5.483 ^{ns} ±0.175	4.15 ^{ns} ±0.41	1.270 ^{ns} ±0.196	3.337 ^{ns} ±0.620
IWW 50%	One month	65.18 ^{ns} ±1.64	6.357 ^{ns} ±0.628	4.95 ^{**} ±0.53	1.187 ^{ns} ±0.174	4.207 ^{ns} ±0.515
	Two month	62.17 ^{ns} ±1.16	6.933 ^{ns} ±0.621	4.94 ^{***} ±0.065	1.99 ^{ns} ±0.678	2.657 ^{ns} ±0.762
	Three month	49.67 [±] ±3.69	6.710 ^{ns} ±0.494	5.16 ^{***} ±0.41	1.553 ^{ns} ±0.700	3.843 ^{ns} ±1.855
IWW 100%	One month	61.78 ^{ns} ±1.16	6.790 ^{***} ±0.7	5.75 ^{***} ±0.044	1.040 ^{ns} ±0.676	7.933 ^{ns} ±5.978
	Two month	60.23 ^{ns} ±1.25	8.217 ^{***} ±0.3	5.97 ^{***} ±0.045	2.233 ^{ns} ±0.362	2.723 ^{ns} ±0.474
	Three month	61.17 ^{ns} ±1.26	8.550 ^{***} ±1.45	5.93 ^{***} ±0.025	2.593 ^{ns} ±1.511	3.173 ^{ns} ±2.37

Values are means ± (S.E.), Number of rats per group = 5, ns = non- significant difference, * Significant difference (p<0.05), ** highly significant difference (p<0.01),*** Very highly significant difference (p<0.001).

Thus A:G ratio decline as compared to (2007) observed increase in serum the control groups. Sharma, et al. total protein and albumin but decline

in globulin in Swiss albino rats when exposed to textile dye wastewater for 15 days. A:G is an index used to track changes in the composition of serum or plasma and its normal value lies between 0.8 and 2.0 reported for mammalian models. Changes in albumin and globulin protein levels can provide early and valuable diagnostic and prognostic information. Since albumin is entirely produced by the liver so an increase in albumin and total protein in the present study could be attributed toward the protein synthesis utilization to meet high energy demand, *e.g.* to overcome the stress conditions. Serum urea level in experimental rat groups was not affected throughout the experiment period when compared to control groups. No significant statistical differences of serum urea was recorded among the different experimental and control groups.

8- Effect of IWW at various concentrations on malondialdehyde (MDA) and catalase activity in tested rats:

As shown in Table (8), IWW caused concentration depending significant alterations in MDA levels and CAT activities. The levels of MDA, an end product of lipid peroxidation (LPO), in rats of the control groups were (125 ± 0.00 , 111 ± 24.25 and 69.22 ± 23.87 nmol/g) for one, two and three months,

respectively. Oral administration of various IWW concentrations (25%, 50% and 100% $\%_v$) caused elevation of lipid peroxides (MDA) with a concomitant reduction in catalase (CAT) activity. These alterations were increased as IWW concentrations increased across all different experiment periods. The highest increase in MDA level as presented in Table (8) was achieved in the groups that received IWW 100% (361 ± 24.25) followed by 319 ± 24.25 at IWW 50% and then 208 ± 0.00 at IWW 25% $\%_v$ for 3 months. The lowest value of MDA level (124.7 ± 41.50) was noticed in serum of rats that exposed to IWW 25% $\%_v$ for one month. Our findings indicated that oral administration of cane sugar wastewater IWW at different concentrations (*viz* 25, 50 and 100% $\%_v$) for 1-3 months caused highly significant ($p < 0.001$) increase in MDA levels with a concomitant highly significant reduction in catalase (CAT) activity specially for 3 months. MDA is a secondary product of lipid peroxidation and is known to cause damage to the cell by making cross linkage of membrane components containing amino groups and make the membrane fragile. Therefore, the assay of MDA could be a marker of cell damage. Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and

change the activity of membrane bound enzyme and receptors. The observed results on MDA were in accordance with findings of (Acworth *et al.* 1997).

The profile of CAT activities in Table (8) indicated that oral administration of IWW lowered very highly significant ($p < 0.001$) the catalase (CAT) activity when compared with the control groups. The maximum lowering of catalase activity was recorded in the groups that exposed to IWW 100% v/v for all exposure periods (11.56 ± 5.89 , 8.16 ± 0.00 and 11.59 ± 5.89) followed by the groups that received IWW 50% (35.99 ± 14.58 , 21.76 ± 5.89 and 21.56 ± 5.88) and then the groups that received IWW 25% v/v (69.39 ± 10.21 , 48.97 ± 10.21 and 42.17 ± 15.00). The observed results on MDA were in

accordance and that on catalase activity were not in accordance with those previously reported by Adeoye, *et al.* (2015) who found that pharmaceutical effluent caused significantly ($p < 0.05$) increase in CAT activities and MDA concentration in Wistar rats of groups exposure for 28 days compared to control. Li, *et al.* (2006) reported alterations in CAT activities with concomitant change in MDA concentrations in landfill leachate treated mice due to the toxic metals of leachate generated free radicals by autoxidation.

9- Histopathological analysis of Liver and Kidney in tested rats:

Microscopical examination of liver and kidney sections from control and tested groups are shown in Figs. (1 A-L and 2 A-L).

Table (8): Effect of IWW at various concentrations on malondialdehyde (MDA) and catalase activity in rats.

Treatment		MDA (nmol/g)	Catalase (U/g)
Control TW 100%	One month	125±0.00	69.39±0.00
	Two month	111±24.25	72.79±5.88
	Three month	69.22±23.87	82.919±5.88
IWW 25%	One month	124.7 ^{ns} ±41.50	69.39 ^{ns} ±10.21
	Two month	194.3 ^{ns} ±63.61	48.97 ^{**} ±10.21
	Three month	208 ^{***} ±0.00	42.17 ^{***} ±15.00
IWW50%	One month	263.7 ^{**} ±63.61	35.99 ^{**} ±14.58
	Two month	277.7 ^{***} ±47.92	21.76 ^{***} ±5.89
	Three month	319 ^{***} ±24.25	21.76 ^{***} ±5.89
IWW 100%	One month	319.3 ^{***} ±63.61	11.56 ^{***} ±5.88
	Two month	375 ^{***} ±0.0	8.16 ^{***} ±0.00
	Three month	361 ^{***} ±24.25	11.59 ^{***} ±5.89

Values are means ± (S.E.), Number of rats per group = 5, ns = non- significant difference, * Significant difference ($p < 0.05$), ** highly significant difference ($p < 0.01$), *** Very highly significant difference ($p < 0.001$).

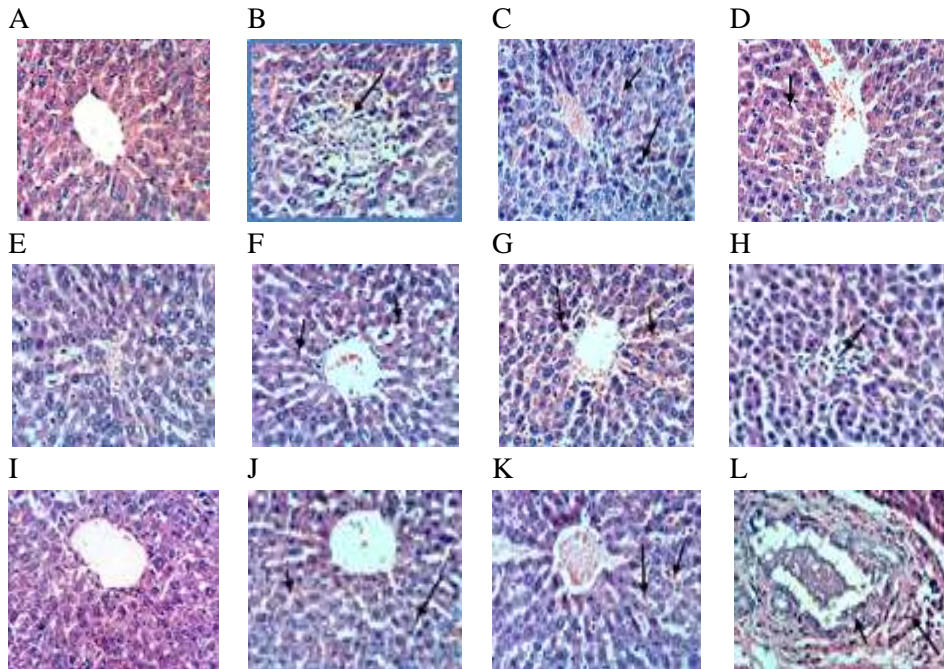


Fig. 1 A-L:

- A): Liver of rat from group control 1month showing the normal histological structure of hepatic lobule
- B): Liver of rat from group industry 25% 1month showing congestion of hepatic sinusoids and necrosis of sporadic hepatocytes
- C): Liver of rat from group industry 50% 1month showing focal necrosis of hepatocytes associated with inflammatory cells infiltration
- D): Liver of rat from group industry 100% 1month showing Kupffer cells activation
- E): Liver of rat from group control 2month showing no histopathological changes
- f): Liver of rat from group industry 25% 2month showing Kupffer cells activation
- G): Liver of rat from group industry 50% 2month showing congestion of hepatic sinusoids and necrosis of sporadic hepatocytes
- H): Liver of rat from group industry 100% 2month showing focal hepatic necrosis associated with inflammatory cells infiltration
- D): Liver of rat from group control 3month showing no histopathological changes
- J): Liver of rat from group industry 25% 3month showing slight congestion of hepatic sinusoids and Kupffer cell activation

K): Liver of rat from group industry 50% 3month showing slight congestion of hepatic sinusoids and Kupffer cell activation

L): Liver of rat from group industry 100% 3month showing hyperplasia of epithelial lining bile duct and fibroplasia in portal triad

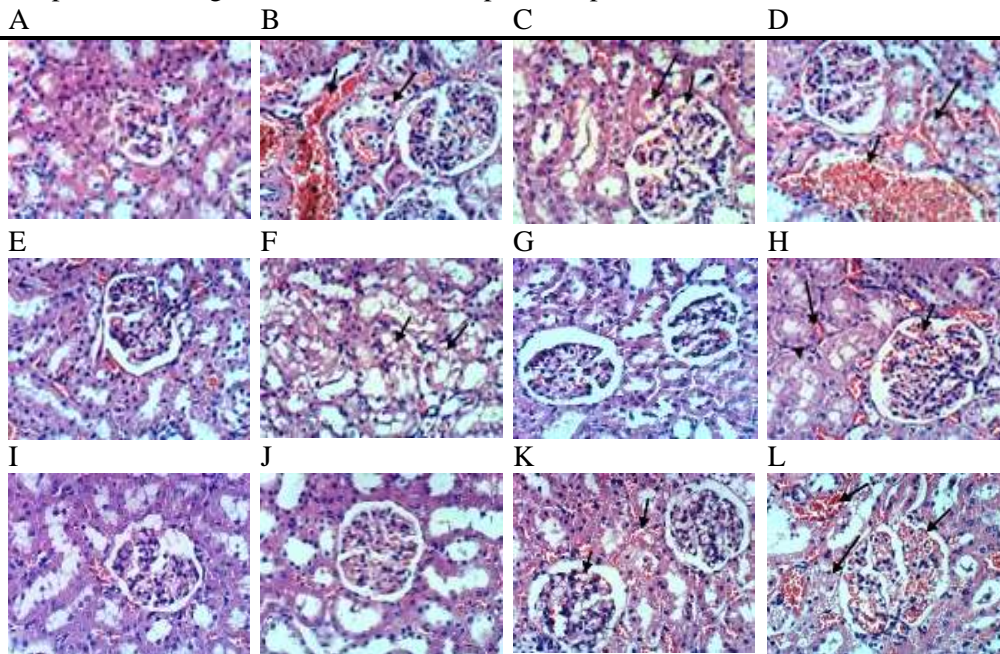


Fig. 2 A-L:

- A): Kidney of rat from group control 1 month showing the normal histological structure of renal parenchyma
- B): Kidney of rat from group industry 25% 1month showing congestion intertubular blood vessels and necrobiotic changes of renal tubular epithelium
- C): Kidney of rat from group industry 50% 1month showing hypertrophy and congestion of glomerular tuft as well as presence of protein cast in the lumen of renal tubules
- D): Kidney of rat from group industry 100% 1month showing congestion of intertubular blood vessels and vacuolation of renal tubular epithelium
- E): Kidney of rat from group control 2month showing no histopathological changes
- F): Kidney of rat from group industry 25% 2month showing vacuolation of epithelial lining renal tubules
- G): Kidney of rat from group industry 50% 2month showing no histopathological changes
- H): Kidney of rat from group industry 100% 2month showing congestion of glomerular tuft , congestion of intertubular blood vessels and vacuolation of renal tubular epithelium
- I): Kidney of rat from group control 3month showing no histopathological changes
- J): Kidney of rat from group industry 25% 3month showing no histopathological changes
- K): Kidney of rat from group industry 50% 3month showing slight vacuolation of endothelial lining glomerular tuft and congestion of intertubular blood capillaries
- L): Kidney of rat from group industry 100% 3month showing congestion of glomerular tuft and intertubular blood vessels as well as necrobiotic changes of renal tubular epithelium

CONCLUSION

Toxicity due to long term exposure of untreated cane sugar effluent IWW (25, 50 and 100%^{v/v}) of Abu-Qurqas cane sugar factory was studied on various hematological parameters, lipid profile, protein profile as well as histopathology of liver and kidney on male albino rats. From the findings, it was found that cane sugar effluent has a deleterious effect upon the blood profile and lipid profile of rats, which can lead to many metabolic and physiological disorders. Thus, present study revealed toxic effects of untreated cane sugar effluent, providing a basis for documental potential regarding clinical, pathological and biochemical assessment. This investigation may be a valuable step in the toxicity assessment of cane sugar effluent in male albino rats.

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الملخص العربي

تقييم تأثير مياه صرف صناعة سكر القصب علي المؤشرات البيوكيميائية والتشريحية في ذكور الفئران البيضاء

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أجري هذا البحث بمعامل قسم الكيمياء الزراعية بكلية الزراعة جامعة المنيا بهدف تقييم مياه الصرف الصناعي الناتجة من مصنع سكر القصب بمركز أبو قرقاص ودراسة تأثير ثلاث تراكيزات منها (25%، 50%، 100%) لمدة 30 يوم، 60 يوم، 90 يوم على ذكور الفئران البيضاء كما تم تحديد بعض الصفات الطبيعية والكيميائية لهذه المياه وكذلك لمياه الصنبور.

أظهرت التحليلات الكيميائية والطبيعية لمياه الصرف الصناعي المستخدمة بالبحث ومياه الصنبور أن لون مياه الصرف الصناعي كان أسود له رائحة المولاس ورقم pH (4.4) وذلك بالمقارنة WHO القياسى في حين كان لون ورقم pH لماء الصنبور طبيعى، تم تقدير العناصر الثقيلة في مياه الصرف الصناعي المستخدم في البحث (Fe, Mn, Cu, Pb, Hg, Mo, Co & Cd) ووجد أن كل التركيزات لهذه العناصر كانت أعلى من WHO القياسى وذلك باستثناء (Mn, Cd & Cu) كان تركيزهم أقل أما بالنسبة لماء الصنبور Fe, Mn, Co and Mo كان في التركيز الطبيعى مقارنة WHO القياسى في حين كان تركيز Pb عالى كما لم يوجد أى تركيز لكل من (Hg, Cd and Cu) ، كما أظهرت إختبارات (Hematology) فروق نقص معنوية ($p < 0.05$) في RBCs لمجموعات الفئران التى أعطيت ماء صرف صناعى بالتركيزات الثلاثة المستخدمة في البحث وذلك بالمقارنة بالكنترول في حين كانت هذه الفروق غير معنوية لهذه المجموعات في (WBCs, PCV and MCV).

كما لم تظهر النتائج أى زيادة معنوية في وزن الأعضاء بالنسبة لوزن الفأر لجميع التركيزات عند مقارنتها بالكنترول ولكن كان أعلاهم تركيز 100% لمدة 3شهور ، كما لوحظ أن زيادة تركيز ماء الصرف الصناعى يؤدى لزيادة معنوية في نشاط AST&ALT وكان أعلى نشاط لمجموعة الفئران تركيز 100% مياه صرف صناعى لمدة 3شهور، كما وجد أن الثلاث تركيزات لماء الصرف الصناعى المستخدمة بالبحث أعطت زيادة عالية جدا للمعنوية (أى عند مستوى 0.001) في TG بالمقارنة بالكنترول وبنفس اتجاه النتائج كان VLDL-c وكذلك الكولسترول الكلى، كما أوضحت النتائج أن في كل المجموعات المختبرة بالتركيزات الثلاثة أن الفروق عالية جدا للمعنوية (أى عند مستوى 0.001) لكل من HDL-c & LDL-c عند المقارنة بمجموعة الكنترول وكذلك وجد زيادة عالية جدا للمعنوية في البروتين الكلى للسيرم لمجموعة الفئران 100% ماء صرف صناعى وذلك بالمقارنة مع مجموعة الكنترول، كما أن مستوى الألبومين أظهر فروق عالية جدا للمعنوية في حالة المعاملة بصرف صناعى 50% أو 100% مقارنة بالكنترول، في حين أن مستوى الجليبولين لم يتأثر بتركيزات ماء الصرف الصناعى المستخدمة، في حين أن نسبة A:G زادت بالتدرج بزيادة تركيز ماء الصرف الصناعى أما مستوى اليوريا بالسيرم لم يحدث به أى فروق معنوية كما أن كل المجموعات المختبرة أظهرت زيادة معنوية عالية في مستويات MDA مصاحبة بإنخفاض عالى المعنوية في نشاط الكاتاليز (CAT) وخاصة بعد المعاملة لمدة 3شهور، وبذلك أظهرت نتائج البحث تغيرات في ملف الدم وملف الليبيدات تعزى إلي الإجهاد التأكسدي الذي تسببه العناصر الثقيلة بالإضافة إلي الضرر الذي لحق بالكبد والكلى وهذا ما أكده الفحص الهستولوجي، مما يشير إلي سمية مياه صرف صناعة سكر القصب قبل معالجتها على ذكور الفئران البيضاء .