International Journal of Environment 3(4): 238-244, 2014
ISSN: 2077-4508

Comparative Bioanalytical Study of Chlorine and Huwa-san during Water Treatment in Mice

[1]Mohammed A. Hussein, [1]Sherif A. M. Kamal,[3] Yasser H. Mohamed and [4]Wael M. Kamel

[1]Assosiated professor, Biochemistry Department, Faculty of Pharmacy, October 6 University, Egypt.
[2]Assistant professor of Public Work Engineering, Construction and Building Department, Faculty of Engineering, October 6th.University, Egypt.
[3]Organic Laboratory, Central Laboratory, Greater Cairo Drinking Water Company, Fustat water treatment plant.
[4]Department of Biochemistry, National Research Centre, Dokki, Giza, Egypt.

ABSTRACT

Chemical disinfectants are effective and required for killing harmful microorganisms in drinking water. Each disinfecting produces its own suite of disinfection byproducts in drinking water with overlapping constituents. Huwa-San was produced as a disinfectant for biofilm removal and without formation of byproducts and residual chlorine.

Administration of chlorine by injection or through drinking water increased the mortality percent while huwa-san caused no loss in the experimental animals.

Administration of chlorine and huwa-san by injection or through drinking water, caused significant decrease in the HB level. Huwa-san caused decrease in HB level with higher rate than chlorine. The chlorine, huwa-san injection and huwa-san treated water caused decrease in level of RBCs and hence decreased level of HCT. While the chlorine through drinking water only caused increase in the RBCs level and hence increased the HCT level. Both of chlorine and huwa-san have no significant effect on the blood measurements related to immune system.

It was showed that the chlorine administration by injection or through drinking water caused decrease in the protein profile. The administration of huwa-san only by injection caused decrease in the protein profile and it increased the protein when administrated through drinking water.

Administration of chlorine and huwa-san by injection and through drinking water caused decrease in the heart enzymes especially CK. and CK-MB. The huwa-san was more effective in decreasing these measurements than chlorine. While administration of huwa-san and chlorine by any way caused significant increase in the LDH level.

***Key words*: Chlorine, Huwa-san, Biological functions, Mice**

Introduction

Chemical disinfectants are effective and required for killing harmful microorganisms in drinking water. Chlorine, ozone, chlorine dioxide and chloramines are the most common disinfectants used today during water treatment. Each produces its own suite of disinfection byproducts in drinking water with overlapping constituents (Richardson, 1998).

Chlorination is one of the mainly used procedures for disinfection of raw water. The studies have shown that chlorinated water was mutagenic and induced genotoxic effect on mammalian cells (Athanasiou, and Kyrtopoulos, (1983); Park, *et al*., (2000). Epidemiological studies provided evidence that the consumption of chlorinated drinking water may be associated with increased incidence of some specific types of cancer (Li, *et al*., (1992). The hypochlorite destroyed DNA through oxidative damage and base modifications (Hayatsu, *et al*., (1971) ; Whiteman, *et al*., (2002).

It was found recently that the chlorinated drinking water enhanced the body burden with mutagenic and/or carcinogenic substances (Lua, *et al*., (2002). The previous studies showed that the decrease in oxidative metabolism, spleen weight and hypersensitivity reactions was reported in rats exposed to chlorine (Fidler, I.J. (1977); Exon, *et al*., (1987).

The recent study directed to search for new substance used for water disinfection and more suitable than chlorine. Huwa-San was produced as a disinfectant to remove the biofilm on pipe systems of drinking water (Liberti, *et al*., (2000). It is characterized that there is no by-products (Armon, *et al*., (2000).

It is based on silver stabilised hydrogen peroxide. Combination of hydrogen peroxide and silver resulting in products with higher disinfecting capacity (Pedahzur, *et al*., (1995); Pedahzur, *et al*., (2000).

Corresponding Author: Mohammed A. Hussein, Biochemistry Department, Faculty of Pharmacy, October 6 University, Egypt.
E-Mail: prof.husseinma@o6u.edu.eg

The current study directed to study the effect of huwa-san as alternative disinfecting agent on the different biochemical functions in mice.

Materials and methods

Animals

50 mice (20 – 25 g) were obtained from the Animal house of National Research Centre. The mice were divided into five groups (control, chlorine injected, huwa-san injected, chlorine water treated and huwa-san water treated). Each group is consisting of 10 mice. All animals were acclimated to the animal facility for 2 weeks before use in experiments.

Chlorine and Huwa-san dose

The beakers were filled with nile water. The concentration 4 ppm of chlorine and huwa-san added individually to each beaker. Concentration of chlorine and huwa-san solutions were 1 %. 1ml of chlorine or huwa-san solution added to 4 L of nile water to make concentration 4 ppm. The other chlorine doses added in the same manner. 30 ppm $Al_2(SO_4)_3$ added to each beaker. The contents in each beaker were mixed well at speed 120 rpm for 10 min. then mixed at speed 20 for 30 min. (Mark, (1986); Mackenzie, and Cornell, (1991).

The chlorine residues were measured by the DPD colorimetric method according to APHA, 1998.

Administration of chlorine and huwa-san dose

25 ml / Kg (0.5 ml per 20 mg mice) of 1 % chlorine and huwa-san was injected daily using stomach tube in the chlorine and huwa-san injected groups. Two groups drink 4 ppm chlorine and huwa-san representing chlorine and huwa-san water treated groups.

Haemtological measurements

All the haemtological measurements were estimated in the whole blood samples using heparin as anticoagulant. These measurements include :

Haemoglobin level (Drabkin and Austin, 1932).

Red blood cells manually by haemocytometer (Cheesbrough and MacArthur, 1976).

Biochemical measurements

All the biochemical measurements were estimated in the serum samples by using closed full automatic system (Cobas Integra 400 plus). They include:

Heart functions

Creatine kinase (CK) level (Hørder *et al*., 1989).

Creatine kinase-MB (CK-MB) level (Würzburg *et al*., 1976).

Lactate dehydrogenase level (LDH) (Zimmerman and Henry, 1984).

Protein profile

Total protein level (Doumas *et al*., 1981).

Albumin level (Doumas *et al*., 1971).

Statistics

The results reported are mean values ± standard error (S.E.). Student's t-tests (unpaired and paired) were carried out to calculate significance. All the groups were compared to control.

Results and Discussion

It was found that about 20 % of the chlorine injected group died after 3 days of injection. Another 20 % of this group died also after 14 days of injection. In the chlorinated water treated group, it was found that 20 % of the group died after 20 day of water drinking. No lose in the huwa-san water treated or injected group. This indicated that huwa-san was less toxic than chlorine. The exposure to chlorinated drinking water has the potential to adversely affect immune function (Stiehm *et al*., 1986).

As shown in table 1 and graphically illustrated in fig. 1, it was found that the treatment with chlorine and huwa-san by injection or through drinking water, caused significant decrease in the hemoglobin level (HB). Huwa-san caused decrease in HB level with higher rate than chlorine. The chlorine, huwa-san injection and huwa-san treated water caused decrease in level of RBCs and hence decrease in level of hematocrit (HCT). While the chlorine through drinking water caused increase in the RBCs level and hence increase in the HCT level. This was in agreement with Abdel-Rahman *et al*., (1984). This may occur due to decrease in blood glutathione and an increase in erythrocyte osmotic fragility in rats receiving a dose of 1 ppm chlorine in drinking water. The haemoglobin released and undergoes the degradation pathway after rupturing the RBCs. So, the HB decreased.

The fig. 2 showed that the chlorine and huwa-san have no significant effect on the blood measurements related to immune system. This was in agreement with French *et al*. (1998). The author suggested that the immune system is not a sensitive target organ for chlorine toxicity, whether mediated directly by chlorine or by its reaction by-products. The other studies showed that the exposure to chlorine in drinking water may suppress certain immune functions in laboratory animals.

Table 1: Effect of Chlorine and Huwa-san through injection or drinking water on Hemoglobin, Red blood cells and Hematocrit.

	C.			Chl. Inj.			Chl. Drink.			Chl. Inj.		
	HB	RBCs	HCT	HB	RBCs	HCT	HB	RBCs	HCT	HB	RBCs	HCT
Value	15.85 ± 0.31	4.25 ± 0.86	19.93 ± 4.22	13.07 ± 1.13	3.98 ± 0.55	18.23 ± 2.16	13.67 ± 0.61	5.05 ± 0.85	24.90 ± 4.78	12.33 ± 1.32	3.80 ± 0.53	17.83 ± 2.48
T - test	---			2.64	0.25	0.33	3.4	-0.66	-0.78	2.91	0.41	0.4

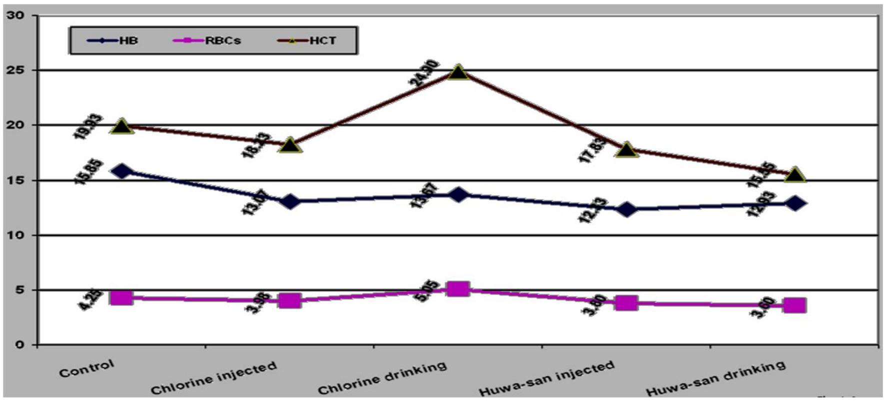

Fig. 1: Effect of Chlorine and Huwa-san through injection or drinking water on Hemoglobin, Red blood cells and Hematocrit.

Tap water typically contains 0.5 - 1.0 ppm residual chlorine, a concentration considered to be sufficient to prevent regrowth of organisms within the distribution system. In the present experiments, animals received high doses by stomach tube or through drinking water. Previous studies were reported the chlorine suppressed macrophage function in mice and altered activity or function of both macrophages and lymphocytes in rats (Fidler, (1977); Exon, *et al*., (1987).

It was found that concentration of the residual chlorine increased with increasing the chlorination dose in dose dependent manner. The residual chlorine represents chlorine which remains free but it is ready to undergo disinfection to any microbial activity. It is able to involve in alteration of the biological functions. The residual chlorine was less toxic than the chlorinated by-products. It was found that the disinfection by-products are more toxic than the disinfectant itself.

The data in table 2 and illustrated in fig. 3 showed that the chlorine administration by injection or through drinking water caused decrease in the protein profile (total protein and albumin). The administration of huwa-san only by injection caused decrease in the protein profile and it increased the protein when administrated through drinking water.

The chlorinated water increased oxidative damages and induced various biological effects in mammalian cells. It caused dose-dependent increases in the lipid peroxidation product (MDA) in livers of rats (Lu, *et al*., (2002). MDA is a naturally occurring product of lipid peroxidation. It may be caused as a result of the presence of chlorinated hydrocarbon. It is able to form DNA adducts and causes oxidative DNA damages (Marnett, (1999).

On the other hand, administration of chlorine and huwa-san by injection and through drinking water caused decrease in the heart enzymes especially CK. and CK-MB. The huwa-san was more effective in decreasing these measurements than chlorine. (Table 3 and fig. 4). While administration of huwa-san and chlorine by any way caused significant increase in the LDH level. (Fig. 5).

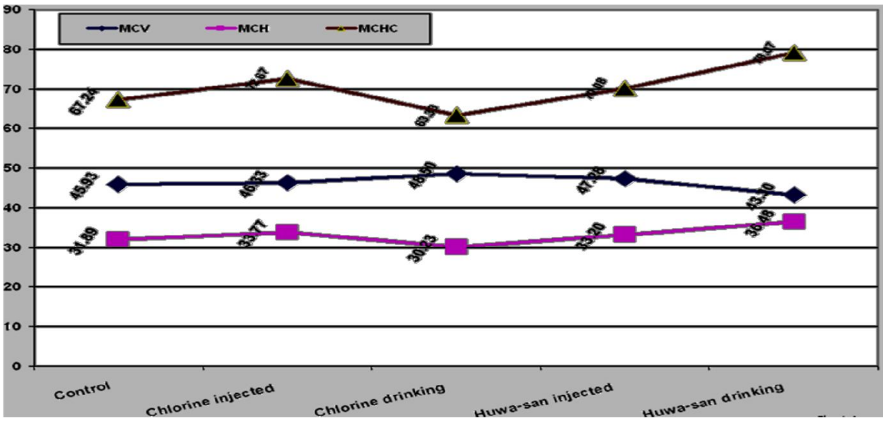

Fig. 2: Effect of Chlorine and Huwa-san through injection or drinking water on MCV, MCH and MCHC.

The study indicated that consumption of chlorinated drinking water led to oxidative damage and the induction of various biological effects, i.e. mutations, chromosomal damages and DNA strand breaks. These biological effects may be caused at least partly by chlorination by-products, which were also analyzed (gas chromatography) in the chlorinated water tested (Li, *et al*., (1995); Park, *et al*., (2000), Liu, *et al*., (1999).

Table 2: Effect of Chlorine and Huwa-san through injection or drinking water on Total protein and Albumin level.

	C.		Chl. Inj.		Chl. Drink.		Hu. Inj.		Hu. Drink.	
	T. protein	Albumin	T. protein	Albumin	T. protein	Albumin	T. protein	Albumin	T. protein	Albumin
Value	6.76 ± 0.32	3.00 ± 0.11	6.40 ± 0.54	250 ± 0.51	6.20 ± 0.08	2.60 ± 0.27	6.20 ± 0.46	2.56 ± 0.28	7.08 ± 0.37	3.08 ± 0.14
T - test	-----		0.61	1.08	1.52	1.49	1.00	1.47	-0.653	-0.459

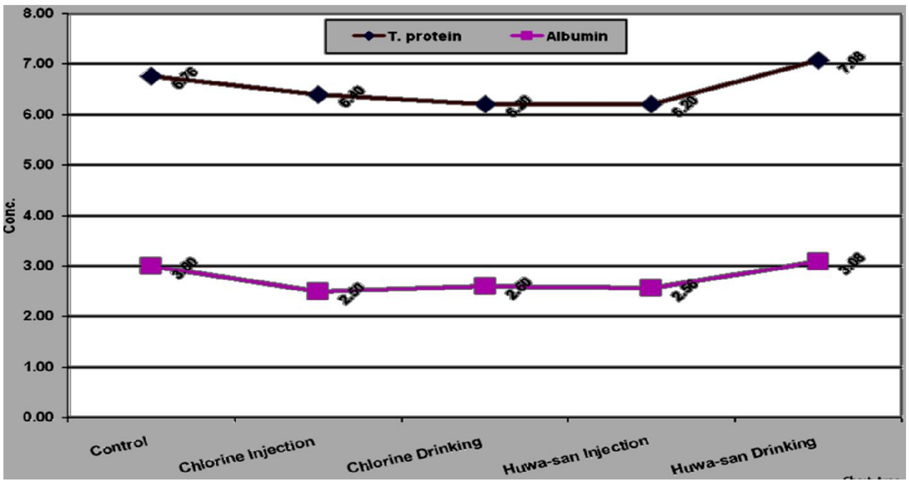

Fig. 3: Effect of chlorine and huwa-san through injection or drinking water on Total protein and Albumin level.

Table 3: Effect of Chlorine and Huwa-san through injection or drinking water on the Heart enzymes (CK., CK-MB and LDH).

	C.			Chl. Inj.			Chl. Drink.			Hu. Inj.			Hul. Drink.		
	CK.	CK. MB	LDH	CK.	CK. MB	LDH	CK.	CK. MB	LDH	CK.	CK.M B	LDH	CK.	CK. MB	LDH
Value	66.8 ± 10.46	66.8 ± 7.06	2106 ± 221.29	451.0 ± 4.44	39.0 ± 7.94	2760.5 ± 316.1	41.5 ± 6.60	41.5 ± 4.92	2814.5 ± 55.68	26.8 ± 5.89	41.2 ± 10.63	2799.6 ± 138.65	36.6 ± 3.82	23.2 ± 1.86	2951.6 ± 159.16
T - test	---			1.75	2.62	-1.75	1.92	2.78	-2.77	3.33	2.01	-2.66	2.53	5.97	-3.1

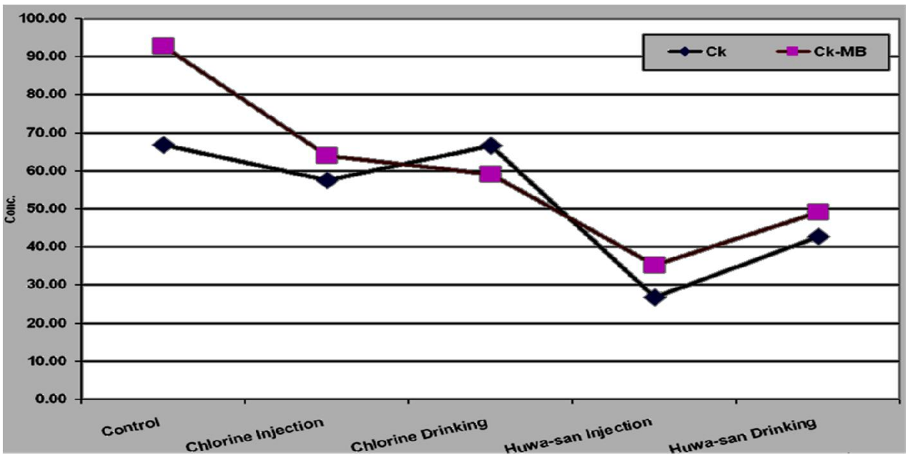

Fig. 4: Effect of Chlorine and Huwa-san through injection or drinking water on the Heart enzymes (CK. and CK-MB).

The chlorine and huwa-san increased LDH level and this may be due to rupturing the erythrocytes by mean of hemolysis and / or due to rupturing the cell membranes and hence releasing the cell contents including LDH by mechanism similar to that of the free radicals attack (Duthie, *et al*., (1997). Both of chlorine and huwa-san increased generation of the free radicals which cause changes in the membrane structure and fluidity resulting in enhancement of permeability hence altered cellular function. It was found that huwa-san was more effective to cause hemolysis more than chlorine. This may refer to generation of the hydroxyl radicals which were more effective and directed sharply to membranes of the RBCs.

Chlorine is highly reactive and essentially consumed in reactions with certain amino acids. A variety of reaction by-products are produced in the gastrointestinal tract, including chloroform, dichloroacetic acid, and trichloroacetic acid (Mink, *et al*., (1983).

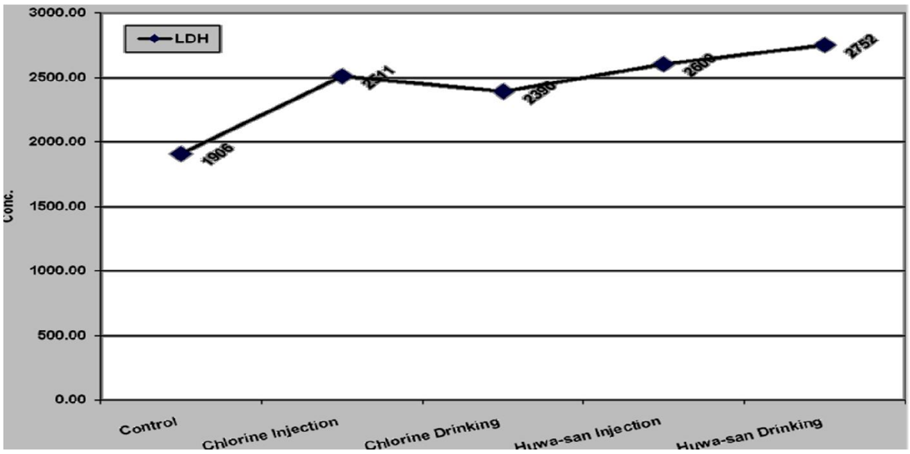

Fig. 5: Effect of Chlorine and Huwa-san through injection or drinking water on the LDH level.

Albumin represents the most abundant protein in the body. So, it used as important marker for disturbances of the protein profile. The decrease in the protein and albumin levels after administration of the chlorine and huwa-san may be occurred due to excretion of high amounts of protein and nitrogenous compounds with urine and this leads to decreasing in the protein level in the blood stream (Batshaw, (1984).

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