

Studying the Effect of Different Water Disinfecting Agents on Liver and Kidney Ultrastructure in Mice

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ABSTRACTS

Disinfection for drinking water reduces the risk of pathogenic infection but may pose chemical threat to human health due to disinfection residues and their by-products when the organic and inorganic precursors are present in water.

Administration of huwa-san through drinking water or by oral injection caused alterations in the biochemical functions of the liver and kidney more than chlorine. The lipid profile showed that both of chlorine and huwa-san caused disturbances in the lipids. This was represented by elevation in the triglycerides. So, the lipid became more susceptible to be oxidized by the free radicals generated by chlorine and huwa-san.

The ultrastructure showed that administration of chlorine and huwa-san caused structural abnormalities in the liver and kidney surfaces. The chlorine administration caused more severe damages in the liver and kidney than huwa-san at the same dose and by the same administration way.

The administration of huwa-san caused more alterations in the biochemical liver and kidney functions than chlorine. The chlorine caused changes in the liver and kidney ultrastructure than huwa-san.

Key words: Chlorine, Huwa-san, Biological functions, Liver and kidney ultrastructure.

Introduction

Disinfection for drinking water reduces the risk of pathogenic infection but may pose chemical threat to human health due to disinfection residues and their by-products (DBPs) when the organic and inorganic precursors are present in water (Sadiq and Rodriguez, 2004).

Chlorine reacts with naturally occurring organic materials in raw water to produce a variety of disinfection by-products, including trihalomethanes, halogenated acetonitriles and halogenated acetic acids. These compounds deteriorate the water quality and lead to developing many types of cancers (Hussein *et al.*, 2012).

Several epidemiological studies have revealed that there is an association between health effects and exposure to disinfection by-products. It was noted a relationship between trihalomethanes and adverse birth outcomes (King *et al.*, 2000 ; Wright *et al.*, 2004).

It was identified that there was an association between trihalomethanes and the risk of bladder and colon cancer, respectively. Also, it was found that exposure to dichloroacetic acid and trichloroacetic acid was linked to a risk of growth reduction in infants (Hinckley *et al.*, 2005).

Huwa-San was produced as a disinfectant for biofilm removal. The previous study was restricted to evaluate with respect of Huwa-San capacity to remove the biofilm on pipe systems of drinking water (Liberti *et al.*, 2000). It is based on silver stabilised hydrogen peroxide. Combination of hydrogen peroxide and silver resulting in products showing a synergistic biocidal activity (Pedahzur *et al.*, 1995 ; Pedahzur *et al.*, 2000).

The recent studies compared between effect of chlorine and huwa-san added during water treatment on removal of the different heavy metals in addition to their effects on the other inorganic measurements. The current experiment studied effect of chlorine and huwa-san on the biochemical liver and kidney functions in addition to their effect on the tissue ultrastructure.

Material 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.

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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. (Meyer, *et al.* 1968).

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.

Biochemical measurements

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

Liver functions

Serum alanine transaminase (ALT) level and Serum aspartate transaminase (AST) level (Bergmeyer *et al.*, 1986).

Kidney functions

Urea level (Sampson *et al.*, 1980).

Creatinine level (Bartels and Böhmer, 1971).

Lipid profile

Cholesterol level (Trinder, 1969 ; Allain *et al.*, 1974).

Triglycerides level (T.Gs) (Fossati and Prencipe, 1982 ; McGowan *et al.*, 1983).

Statistics

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

Microscopic examination

This examination was carried out in part of the liver and kidney isolated from one mice selected from each group by using the scanning electron microscope depending on Gores *et al.* (1986) ; Tánaka, (1989). This method was applied by preservation of the liver and kidney specimens as mentioned by Karnovsky, (1965) and Kiernan, (2000) in glutaraldehyde purchased from Gpr Chemicals Co. The glutaraldehyde represents suitable fixative for keeping the tissues for long period. The tissue preserved, passed through series of the dehydration steps by placing it in ethyl alcohol obtained from Alpha Chemicals Co. The dehydration process was carried out by using different alcohol concentrations beginning with 30, 50, 70 then 90 % for 30 min in each concentration. After finishing the dehydration steps, the tissue should be incubated for 15 min then coated with the golden atoms to be ready for the electron microscopic examination.

Results

The experiment revealed to study effect of chlorine and huwa-san by mean of injection or through drinking water on the liver and kidney ultrastructure in addition to effect of these disinfecting agents on the liver and kidney functions and also on the lipid profile.

It was found that the injection with chlorine and huwa-san caused no obvious effect on urea level while administration of chlorine through drinking water and huwa-san by injection caused significant increase in urea level. Administration of chlorine by injection or through drinking water decreased creatinine level while administration of huwa-san by any way caused significant increase in creatinine level (Table 1 and Figs. 1 & 2).

The data in table 2 and illustrated in fig. 3 showed that administration of chlorine by injection or through drinking water caused no obvious effect on GPT and GOT levels. While administration of huwa-san by any way caused significant increase in levels of these enzymes. The Huwa-san by injection caused elevation of these enzymes more than that through drinking water.

Free radical mechanisms initiated by chlorinated hydrocarbons and the hydroxyl radicals are well known as causes for lipid peroxidation. The malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and it is able to form DNA adducts and causes oxidative DNA damages especially in liver tissues (Marnett, 1999). It was found that all treated waters led to an increased MDA formation indicating oxidative stress caused

by treated drinking water. Accumulation of MDA reflects the extent of oxygen-derived free radical induced cell damage, as we observed that the level of DNA damage increased parallel to the MDA level (Yuan *et al.*, 2005).

The H₂O₂ diffuses to the cytosol. H₂O₂ may then be either reduced to water and oxygen by catalase and glutathione peroxidase or converted to the extremely reactive hydroxyl radicals which accelerate production of reactive oxygen species (Starke and Farber, 1985).

Table 1: Effect of Chlorine and Huwa-san through injection or drinking water on Urea and Creatinine levels in mice.

	C		Chl. Inj.		Chl. Drink		Hu. Inj.		Hu. Drink	
	Urea	Creat.								
Value	58.60± 4.07	0.60± 0.14	53.50± 6.85	0.53± 0.05	62.00± 2.27	0.40± 0.07	70.00± 8.65	0.68± 0.08	53.20± 8.31	0.98± 0.12
T-test	—		0.673	0.452	-0.676	1.16	-1.19	-0.492	0.584	-2.05

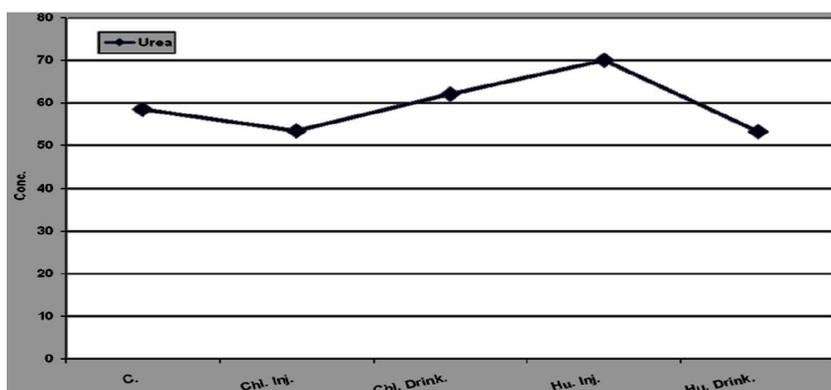


Fig. 1: Effect of Chlorine and Huwa-san through injection or drinking water on Urea and Creatinine levels in mice.

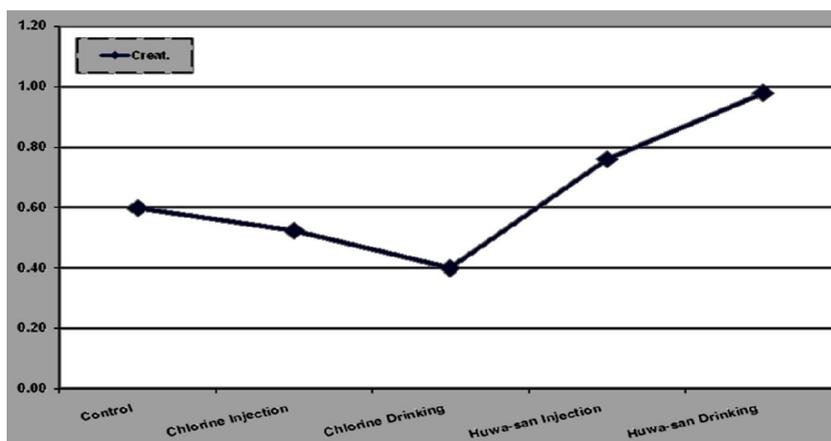


Fig. 2: Effect of chlorine and Huwa-san through injection or drinking water on Urea and Creatinine levels in mice.

Table 2: Effect of Chlorine and Huwa-san through injection or drinking water on GPT and GOT levels in mice.

	C		Chl. Inj.		Chl. Drink		Hu. Inj.		Hu. Drink	
	GPT	GOT	GPT	GOT	GPT	GOT	GPT	GOT	GPT	GOT
Value	120.00± 4.07	118.40± 8.13	106.75± 7.74	110.25± 10.35	123.00± 10.66	109.25± 5.19	155.60± 18.10	139.40± 5.82	148.80± 13.82	124.80± 11.88
T-test	-		0.756	0.629	-0.161	0.890	-1.54	-2.1	-1.45	-0.445

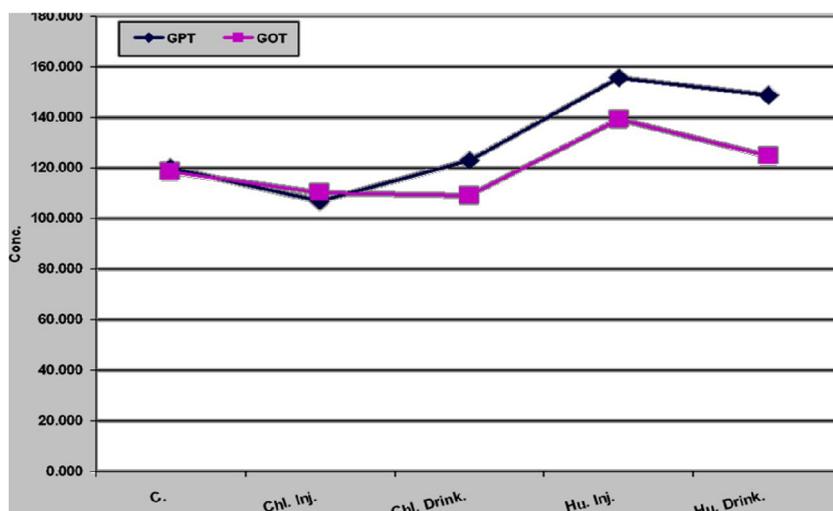


Fig. 3: Effect of chlorine and huwa-san through injection or drinking water on GPT and GOT levels in mice.

Administration of chlorine through drinking water and huwa-san by injection or through drinking water increased T.Gs level significantly (Table 3 and Fig. 4). This may refer to the stimulatory effect of chlorine and huwa-san on the lipogenesis and on the enzymes catalyzing this process.

It was found that administration of chlorine and huwa-san by oral injection was safe and caused no obvious effect on cholesterol level, while administration of chlorine by injection caused significant decrease in T.Gs level. Administration of chlorine through drinking water decreased the cholesterol significantly. On the other hand, huwa-san treated water caused non significant increase in the cholesterol. This may occur due to the inhibitory effect of chlorine on the key regulatory enzyme (hydroxyl methyl gluteryl COA reductase) in the mevalonate pathway (cholesterol biosynthesis).

The scanning electron microscope (SEM) has become a powerful tool for ultrastructural research (Tánaka, 1989). It is a useful tool to study intrahepatic surface structures, and its use may allow further correlations to be made between hepatic structure and function in both health and disease (Nopanitaya and Grisham, 1975). By means of SEM, it was possible to recognize a variety of aspects concerning the surface structure of hepatic cells and their supracellular organisation. In particular, it was focused on the sinusoids, hepatocellular plates, sinusoid endothelium, spaces of Disse, and intercellular spaces. The use of SEM for evaluating morphological changes during liver regeneration in the rat has been limited (Meyer *et al.*, 1981 ; Tomoyori *et al.*, 1983).

The liver ultrastructure of the control mice showed normal hepatic cord pattern, hepatic lobules and hepatocytes. This microscopic examination showed that liver of the mice associated chlorine and huwa-san administration by injection or through drinking water displayed fragmentation of hepatic cells showing difference in sinusoid diameter as a result of the severe damage occurred. The liver weight and volume of the huwa-san administrated mice increased in relation to original liver weight and volume as a result of enlargement of fenestrations in sinusoid endothelium associated with widening of the intercellular spaces and spaces of Disse (fig. 4).

Table 3: Effect of Chlorine and Huwa-san through injection or drinking water on Cholest. and T.Gs levels in mice.

	C		Chl. Inj.		Chl. Drink		Hu. Inj.		Hu. Drink	
	Cholest.	T.Gs	Cholest.	T.Gs	Cholest.	T.Gs	Cholest.	T.Gs	Cholest.	T.Gs
Value	120.00± 7.56	109.60± 6.77	111.50± 8.18	98.50± 3.09	86.00± 9.93	57.50± 3.40	115.60± 10.56	88.80± 5.00	134.80± 9.60	231.60± 12.19
T-test			0.760	1.36	2.78	6.33	0.339	2.47	-1.21	-8.75

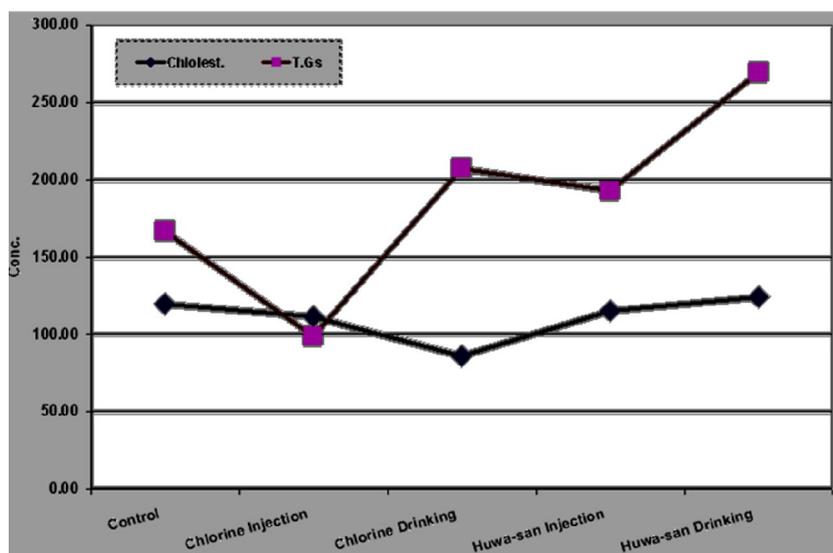


Fig. 4: Effect of Chlorine and Huwa-san through injection or drinking water on Cholest. and T.Gs levels in mice.

The SEM showed that chlorine and huwa-san caused microscopic lesions. These lesions may be consisted of loss of the parenchymal architecture and lysis of hepatocyte plasma membranes (Xu *et al.*, 2007). It was suggested that chlorine and huwa-san administration caused increase in level of the hepatocyte membrane lipid peroxidation products preceded liver morphological alterations.

The liver enlargement occurred after administration huwa-san through drinking water due to the severe degeneration of organelles, accumulation of glycogen particles, swollen mitochondria and broken endoplasmic reticulum in liver tissue. Sinusoid dilatation and enlargement of fenestrations were still evident and became markedly indistinguishable on the liver cell surfaces. The fenestrations in the sinusoidal endothelium and intercellular spaces showed a wide range of diameters. Large fenestrations in the sinusoid endothelial wall were seen and small pores were mostly arranged in sieve plates. The liver enlargement may indicate to the liver regeneration (Morsiani *et al.* 1995). The liver volume increased because the sinusoidal pressure is raised associated with dilatation of sinusoids and separation of the sinusoid endothelium from the underlying parenchymal cells with dilatation of the spaces of Disse. (Ogawa *et al.*, 1979).

The ultrastructural observations suggested that haemodynamic changes could play a role in liver regeneration, primarily by increasing sinusoid endothelial permeability to any circulating hepatotrophic substances. Raised sinusoidal pressure may further decrease cell-to-cell contacts by mechanically enlarging the intercellular spaces and contributing in this way to extracellular matrix degradation (Mars *et al.*, 1995).

The chlorine caused dose-dependent increase in product of the lipid peroxidation. The liver represents a target organ for chlorine-induced lipid peroxidation. The epidemiological study confirmed that lipid peroxidation by chlorinated water could cause liver tumors and mutagenicity in rats exposed to chlorinated water (Li *et al.*, 1992 ; Yuan *et al.*, 2005).

Due to mutagenic effect of chlorinated water on hepatocytes, it should be the goal of further studies to examine DNA damages in liver cells *in vivo*.

The exposure to chlorine and huwa-san was associated with kidney histological disorders and that could be attributed to the destruction and malfunction of kidney cells (fig. 5). This may be occurred due to the action of free radicals especially reactive oxygen species (Anderson *et al.*, 1997).

It was suggested that that the hypercellularity in kidneys especially in inflammatory conditions are associated with an increase in cell number linked to cellular proliferation of mesangial, endothelial or parietal epithelium cells. The administration of chlorine and huwa-san caused changes in the configuration and architecture of the kidney as a result of elevation of the lipid peroxidation product (Yuan *et al.*, 2005). The chlorine by injection or through drinking water caused more negative effect on the kidneys. This indicated that the free radicals generated from chlorine were more negatively effective than those from huwa-san. All components of the kidneys are affected by the drinking water including glomeruli, mesangium, blood vessels, tubular epithelium, and interstitium. This cause accumulation of exudates particularly fluid, proteins, and cells from local vessels unto the damaged part (Stevens and Lowe, 2000).

The present experiment was in agreement with the previous histopathological study in the mice which showed that liver and kidneys are target organs susceptible to toxicity and carcinogenicity as a result of water

chlorination process (Iarc, 1999). The chlorinated water induced a similar type of liver toxicity, such as increased liver weight and enhanced cell proliferation (Coffin *et al.*, 2000).

The previous study demonstrated that the formation of MDA was significantly enhanced in different organs (liver and kidney) of rats after oral application of chlorinated drinking water (Lu *et al.*, 2002).

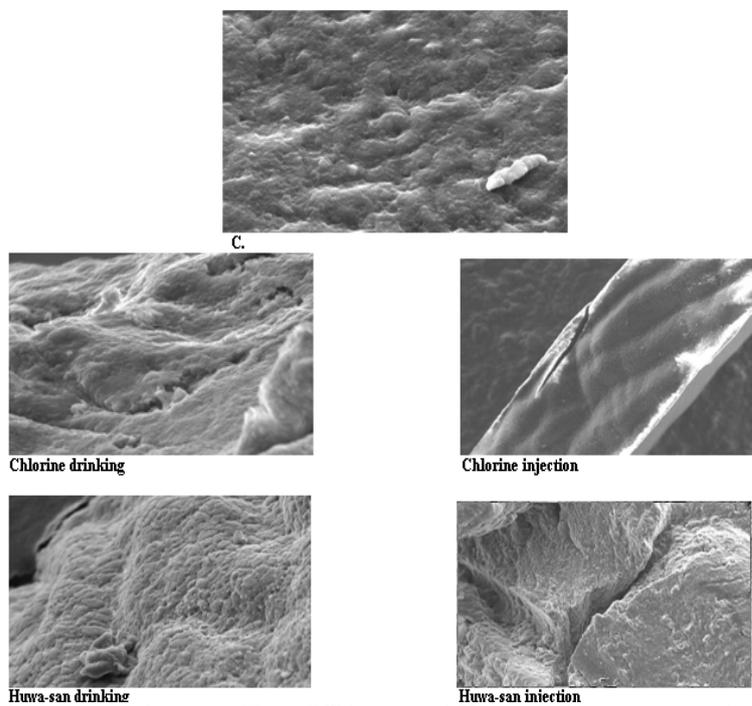


Fig. 5 : The liver ultrastructure showing effect of Chlorine and Huwa-san by injection or through drinking water on the liver tissue in mice.

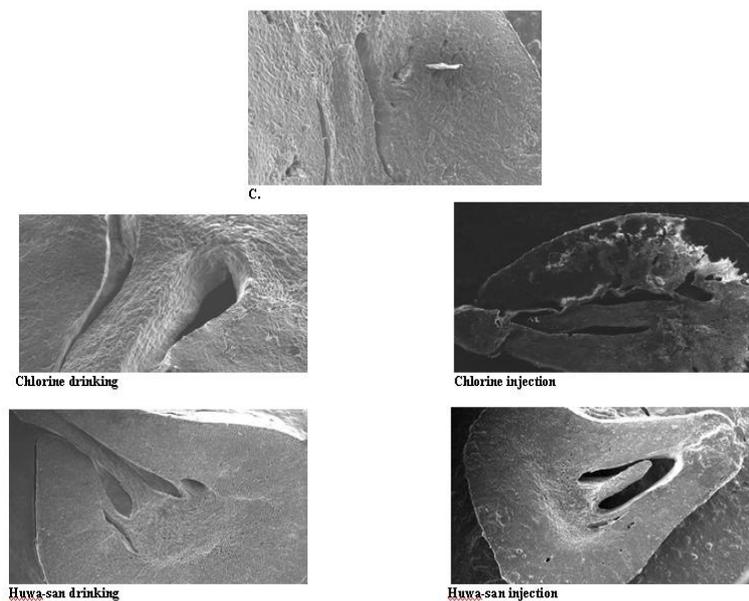


Fig. 6 : The kidney ultrastructure showing effect of Chlorine and Huwa-san by injection or through drinking water on the kidney tissue in mice.

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