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# Antiparasitic and Antibacterial Activities of Some Chemical Compounds against Contaminated- Water of Rafha City, Northern Borders, Saudi Arabia

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**The present chemical studies include applicable work to reveal activity of two anti-parasitic and anti-bacterial-compounds against resulted contaminators; including species of protozoan-parasites and coliform bacteria, that isolated from Rafha City. The present resulted protozoan parasites including cysts of *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba coli* whereas isolated coliform bacteria-species are *Proteus mirabilis, Escherichia coli* and *Enterobacter aerogenes*. The analysis of well and tap water to score and identifying the target protozoan parasites and coliform bacterial-species had been achieved before and after treatment with anti-parasitic and anti-bacterial compounds, that including chlorine and chloramines compounds, to reveal the effectiveness of these compounds against these contaminators, and from another view to ensure safety of these compounds to human health. After purifying the target water, or at least reducing it, from the present contaminators, field-application of chloramines must achieve instead of chlorine in the Public water tank and water pipe of Rafha City, in cooperation with Water Company in Rafha, to connect drinking water devoid of protozoan parasites and coliform bacteria and conform with specifications of the WHO to Raffia's citizens.**

**Keywords:** Protozoan parasites, Contaminated drinking water, Coliform bacteria, chlorine, chloramines.

Drinking water sources become contaminated when feces containing the parasites and bacteria are deposited into water. If treatment is inadequate, drinking water may contain sufficient numbers of parasites and bacteria to cause illness[1]. Infection commonly results during bathing, washing, drinking or consumption of contaminated food[2].

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Concerning protozoan parasites, recent research indicates that ultraviolet light will inactivate *Giardia* or *Cryptosporidium*. Moreover, certain types of filters can remove these parasites. Local drinking water treatment providing filtration and disinfection with chlorine can reduce the risk of giardiasis and cryptosporidiosis. Chlorine by itself is not effective against *Cryptosporidium* but can inactivate *Giardia*[3-4]. Anti-diarrheal drugs used to treat *G. lamblia* infection whereas there is no reliable treatment for *Cryptosporidium* [5]

Chlorine was effective in removing the coliform bacteria from water, including *Escherichia*

*coli* and *Enterobacter aerogenes*[6]. If your water is found to be contaminated with coliform bacteria, the best treatment is disinfection or filtration and other options involve UV-irradiation and ozonation[7].

Chloramines exist as three different forms: mono-chloramine, di-chloramine and tri-chloramine. They convert into each other dependent on pH, temperature, turbulence and chlorine to ammonia ratio[8].

The difference is that chlorine forms many byproducts, including trihalomethanes (THM) and haloacetic acids (HAA), where as chloramines forms a significantly lower amount of THMs and HAAs but also forms N-nitrosodimethyl amine (NDMA)[9].

## MATERIALS AND METHODS

### Water sampling

The present studies carried out from October (2015) to March (2016), in Rafha City, Northern Borders, Saudi Arabia. The water-samples had been collected, three times, from wells and tap water sources. Aliquots of 100 ml from each water-sample were collected in sterilized conical flasks, provided with silica gel to keep dryness.

### Preparation of chlorine and chloramines

### Preparation of chlorine

According to WHO-recommendation[10], safe dose of chlorine had been prepared (3 mg/L) from sodium hypochlorite (5.4 mL/100 ml) (ALPHA CHEMICA), which is the source of it, from which it released, and added to the present target samples, five wells and five areas of tap water.

### Preparation of chloramines

Chloramines are the products of a reaction between ammonia and chlorine. Chloramines (3 mg/L) had been prepared by mixing 11.4 mL of ammonium hydroxide solution (33 %) to 5.4 mL of sodium hypochlorite, and then adding this mixture to 100 ml of each water-sample. WHO recommended that the maximum acceptable concentration for chloramines in drinking water is 3 mg/L [10].

### Enumeration of protozoans and Coliform bacteria

### Protozoan parasites

Well and tap water-samples had been preserved in 4% neutralized formalin solution, left to settle[11]. Then, supernatants were collected, filtered through 20 ìm net mesh and then fixed with Lugol's solution. The protozoans were then counted by Sedwgwish Rafter counting method and identified (Cells/100ml), using Olympus binocular compound microscope[12].

### Coliform bacteria

Coliform bacteria were determined by incubation of samples into lactose broth, as presumptive test. The test tubes were placed at 35ºC for 24 hours for gas production. To confirm the presence of coliform bacteria, gas produced in incubation into Brilliant Green Bile broth at 35ºC for 24 hours[13]. Water quality analysis was based on the most probable number of Cells/100 ml. The test had been repeated three times.

### Statistical Methods

Average count per 100 ml of each water-sample as well as frequency % had been statistically evaluated. Finally, analysis of variance "ANOVA" of the two categories, protozoan parasites and coliform bacteria had been achieved to obtain significant differences, using the statistical SPSS-program.

## RESULTS

Rafha city-citizens essentially obtained their tap-water from treated well-water which stored in public tanks. However, they are usually avoid using tap water as a source of drinking water, owing to their dissatisfaction of taste, odor and color of this water. Instead, they prefer either drinking water consumption of desalinated groundwater obtained from some desalinating private companies, or drink industrially bottled-water.

Parasitological studies had been achieved in the present work through screening the protozoan parasites in water-samples from wells and tap water before and after treating water with 3 mg/L of both chlorine that released from sodium hypochloride.

The present work revealed three species protozoan parasites, as cysts; *Giardia lamblia* (Diplomonadida, Hexamitidae), *Cryptosporidium parvum* (Eucoccidiorida, Cryptosporidiidae) and *Entamoeba coli* (Archamoebae). The average counts of them in table (1) display *G. lamblia* only in control well and tap-samples and is absent after treating the target water with both chlorine and

chloramines, whereas average count of *C. parvum* and *E. coli* after treating with chlorine and chloramines revealed that chloramines has higher antiparasitic activity than chlorine in both well and tap water-samples.

Concerning coliform bacteria, table (1) shows that *P. mirabilis* and *E. aerogenes* is absent in well and tap-water after treating with chloramines, in spite of *E. aerogenes* was scored after chlorine-treating. Concerning *E. coli*, average count of it is apparently reduced after treating with chloramines in relative with chlorine.

Regarding frequency% of protozoan parasites, table (2) revealed that frequency% of *G. lamblia* is (0%) after treating the target water with chlorine and chloramines, *C. parvum* recorded (0%) after treating tap water with chloramines and *E. coli* in treated well- and tap-water with chloramines shows relatively lower % than chlorine.

Concerning frequency% of coliform bacteria, table (3) shows that *P. mirabilis* and *E. aerogenes* have frequency 0% in well and tap-water after treating with chloramines whereas *E. coli* revealed reduced % after chloramine-treating in relative with chlorine.

Statistically, analysis of variance "ANOVA", between control and treated well water with chlorine and chloramine, revealed highly significant differences ($P<0.001$) in the present

**Table 1.** Average counted-number of protozoan parasites and coliform bacteria (mean count/100 ml) which obtained from wells and tap water of Rafha City, Saudi Arabia

| Species Number | Isolated species | Average count, cysts or cells/100 ml water | | | | | |
|---|---|---|---|---|---|---|---|
| | | Wells | | | Tap water | | |
| | | | Treated water | | | Treated water | |
| | | Control | Chlorine | Chloramines | Control | Chlorine | Chloramines |
| I.Protozoan parasites "as: cysts": | | | | | | | |
| 1 | *Giardia lamblia* | 04.80 | 00.00 | 00.00 | 03.40 | 00.00 | 00.00 |
| 2 | *Cryptosporidium parvum* | 17.00 | 05.80 | 04.20 | 12.80 | 03.00 | 00.00 |
| 3 | *Entamoeba coli* | 10.60 | 05.00 | 03.20 | 07.80 | 02.00 | 01.60 |
| II. Coliform Bacteria: | | | | | | | |
| 1 | *Proteus mirabilis* | 64.00 | 34.00 | 00.00 | 00.00 | 00.00 | 00.00 |
| 2 | *Escherichia coli* | 102.0 | 54.00 | 04.00 | 144.0 | 06.00 | 03.00 |
| 3 | *Entrobacter aerogenes* | 82.00 | 36.00 | 00.00 | 46.00 | 00.00 | 00.00 |

**Table 2.** Frequency % of protozoan parasites of control and treated-well and tap water, Rafha City, Northern Borders, Saudi Arabia.

| Types of water | *Giardia lamblia* | | | *Cryptosporidium parvum* | | | *Entamoeba coli* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Chlorine | Chloramines | Control | Chlorine | Chloramines | Control | Chlorine | Chloramines |
| Well water | 100 % | 0 % | 0 % | 62.96 % | 21.48 % | 15.56 % | 56.38 % | 26.60 % | 17.02 % |
| Tap water | 100 % | 0 % | 0 % | 81.01 % | 18.99 % | 0 % | 68.42 % | 17.54 % | 14.04 % |

**Table 3.** Frequency % of Coliform Bacteria of control and treated-well and tap water, Rafha City, Northern Borders, Saudi Arabia

| | Isolated Coliform Bacteria "Negative gram" | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Types of water | *Proteus mirabilis* | | | *Escherichia coli* | | | *Entrobacter aerogenes* | | |
| | Control | Chlorine | Chloramines | Control | Chlorine | Chloramines | Control | Chlorine | Chloramines |
| Well water | 65.31% | 34.69% | 0 % | 63.75 % | 33.75 % | 02.50 % | 69.49% | 30.51% | 0 % |
| Tap water | 0 % | 0 % | 0 % | 94.12% | 03.92% | 01.96% | 100 % | 0 % | 0 % |

three species of protozoan parasites, *G. lamblia* and *C. parvum* and *E. coli*, as shown in table (4). Contrariwise, ANOVA revealed that *P. mirabilis* and *E. coli* show slightly significant differences (P<0.1) while *E. aerogenes* shows moderately significant differences (P<0.002).

In addition, ANOVA, between control and treated tap water with chlorine and chloramine, had also revealed highly significant differences (P<0.001) in the present three species of protozoan parasites, as shown in table (5). On the contrary, *E. coli* and *E. aerogenes* show non-significant differences (N.S).

## DISCUSSION

Kingdom of Saudi Arabia is located in a very harsh natural desert environment with no rivers or lakes. To meet the ever increasing water demand, KSA currently produces around Billion Cubic Meter of desalinated sea water per year[14]. Thereby, the present work selected chloramines as specialized chemical anti-parasitic and anti-bacterial compound to control the present resulting contaminators, including protozoan parasites and coliform bacteria, to gain safe level of drinking water, according to WHO- level, to reduce the risk of propagation of the present microorganisms.

1.8 million people die each year as a result of severe diarrhea as a result of drinking contaminated water[15].

Chloramines are considered as safe water-treatment chemicals when present at the safe concentrations, not more 3 mg/L. International guidelines for drinking water quality suggest that no effects have been associated with chloramines in chloraminated water, but it should be removed prior to processing drinking water; using either

**Table 4.** Statistical analysis of variance (ANOVA) of counts of protozoan parasites and coliform bacteria-species (CFU/100 ml water), from Well-water "Un-treated and treated with chlorine and chloramine" of Rafha City, Northern Borders, Saudi Arabia (N. S=non-significant & P<0.001=highly significant).

| No | Species | df | SS | MS | F | P-value |
|---|---|---|---|---|---|---|
| | i. Protozoan Parasites: | | | | | |
| 1 | *Giardia lamblia* | 14 | 06.80 | 00.57 | 40.66 | P<0.001 |
| 2 | *Cryptosporidium parvum* | 14 | 34.00 | 02.83 | 63.44 | P<0.001 |
| 3 | *Entamoeba coli* | 14 | 18.00 | 01.50 | 29.79 | P<0.001 |
| | ii. Coliform Bacteria: | | | | | |
| 1 | *Proteus mirabilis* | 14 | 11640 | 970 | 3.17 | P<0.1 |
| 2 | *Escherichia coli* | 14 | 26270 | 2189.17 | 3.29 | P<0.1 |
| 3 | *Entrobacter aerogenes* | 14 | 12800 | 1066.67 | 4.75 | P<0.002 |

**Table 5.** Statistical analysis of variance (ANOVA) of counts of protozoan parasites and coliform bacteria-species (Cells/100 ml water), from Tap-water "Un-treated and treated with chlorine and chloramine" of Rafha City, Northern Borders, Saudi Arabia (N. S=non-significant & P<0.001=highly significant)

| No | Species | df | SS | MS | F | P-value |
|---|---|---|---|---|---|---|
| | i. Protozoan Parasites: | | | | | |
| 1 | *Giardia lamblia* | 14 | 01.20 | 00.10 | 115.6 | P<0.001 |
| 2 | *Cryptosporidium parvum* | 14 | 26.80 | 02.23 | 60.20 | P<0.001 |
| 3 | *Entamoeba coli* | 14 | 12.00 | 01.00 | 36.12 | P<0.001 |
| | ii. Coliform Bacteria: | | | | | |
| 2 | *Escherichia coli* | 14 | 166620 | 13885 | 01.40 | N. S |
| 3 | *Entrobacter aerogenes* | 14 | 9720 | 810 | 02.18 | N. S |

reverse osmosis membrane system, granular-activated carbon treatment or adding ascorbic acid; in spite of the excess of chloramine excreted in the human urine[9]. Chloraminated water is safe to use for cooking and drinking. Chloramine is supposed to be a safer disinfectant than chlorine, where it does still produce many of chemical products as chlorine[8].

Chlorine by itself is not effective against *Cryptosporidium* but can inactivate *Giardia*[3-5]. This result reinforced the present counts of *Giardia* and *Cryptosporidium*-cysts in well and tap water, where *Giardia*-cysts disappeared after treatment with chlorine and chloramines in both well and tap water, but *Cryptosporidium*-cysts had been scores after treating with chlorine and chloramines in well water and chlorine only in tap water. In general, chloramines are relatively effective antiparasitic compound against the present resulted cysts of the three target species than chlorine.

From another angle, chlorine was effective in removing two species of coliform bacteria, isolated from Solan City of India, *E. coli* and *Enterobacter aerogenes*[16].

Concerning standard levels of protozoans in drinking water, EPA's MCLG recommended the proper way to control pathogenic protozoans is using an effective water treatment technique, such as reverse osmosis or ozonation[17].

In this respect, EPA MCL, coliform must be less than one/100 mL. In this concern, *E. coli* must be zero CPU count/100 ml of water sample[18].

The present investigation recommended that chloramines, concentration not more than 3 mg/L, are the suitable specialized anti-protozoan parasites and anti-coliform-bacterial chemical compounds that control, or at least reduce, the present resulting contaminators to gain considerable safe levels of drinking water, according to WHO.

After purifying the target water from the present contaminators, field-application of chloramines must achieve instead of chlorine in the Public water tank and water pipe of Rafha City, in cooperation with Water Company in Rafha, to connect drinking water devoid of protozoan parasites and coliform bacteria and conform with specifications of the WHO to Raffia's citizens.

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