

## Attenuation of Oxidative Stress and Cytokines in Rabbits with Experimentally Induced Hyperthyroidism by Ethanolic Red Cabbage Extract

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### ABSTRACT

The protective effects of red cabbage extract (RCE) on oxidative damage in rabbits with experimentally induced hyperthyroidism were studied on forty male New Zealand rabbits. The experimental animals were randomly divided into 4 groups : (healthy as (control), hyperthyroidism, hyperthyroidism treated with vitamine E and hyperthyroidism treated with RCE .Hyperthyroidism was induced by oral administration of 20 µg/kg b.w/day L-thyroxine for 4 weeks. At the end of the experimental period, blood samples were taken from heart puncture of all animals for the determination of oxidant/antioxidant biomarkers: lipid peroxidation marker malonaldehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), Catalase (CAT) and superoxide dismutase (SOD) ,triiodothyronine (T3), thyroxine (T4) , thyroxine-stimulating hormone (TSH),interleukin 6 (IL-6),interleukin 10 (IL-10) and tumor necrosis factor alpha(TNF-α) levels. Our results indicate that levels of MDA, T<sub>3</sub> , T<sub>4</sub> ,IL-6, IL-10 and TNF-α were significantly increased(P<0.05) in sera of the hyperthyroid animal group, while SOD; GPx, CAT, GSH and TSH levels were decreased significantly (p<0.05) compared to the control group. Hyperthyroid rabbits treated with 100 mg/kg.b.w daily of RCE for 4 weeks decreased significantly the elevated MDA, T<sub>3</sub> ,T<sub>4</sub> ,IL-6, IL-10 and TNF-α levels and restore levels of GSH, GPx, SOD, CAT and TSH to control value. In conclusion, results indicated that RCE was beneficial as a protective agent against oxidative stress induced by hyperthyroidism and it had antihyperthyroid activity for the first time.

**Keywords:** Thyroid hormones, Oxidative stress, Cytokines, Red cabbage extract

خفض مستويات الاجهاد التاكسدي وبعض السايٲوكينات في الارانب المصابة بفرط الدرقية المستحدث بالتايروكسين باستعمال المستخلص الكحولي للملفوف الاحمر

### الخلاصة

درس التاثير الوقائي لمستخلص الملفوف الاحمر على حالة الاجهاد التاكسدي وبعض السايٲوكينات في الارانب المصابة بفرط الدرقية المستحدث بالتايروكسين ,وقد اجريت الدراسة على 40من ذكور الارانب النيوزيلندية اذ قسمت حيوانات التجربة بصورة عشوائية الى اربع مجموعات

الاولى هي مجموعة الارانب الصحية (السيطرة) والمجموعة الثانية هي الارانب المعاملة بالتاثيروكسين، اما المجموعة الثالثة فشملت الارانب المعاملة بالتاثيروكسين مع فيتامين E بينما المجموعة الاخيرة فشملت الارانب المعاملة بالتاثيروكسين بالاضافة الى مستخلص الملفوف الاحمر . تم استحداث حالة فرط الدرقية وذلك بتجريع الحيوانات 20 مايكروغرام/كيلوغرام من وزن الحيوان يوميا وعن طريق الفم هورمون التاثيروكسين ولمدة اربعة اسابيع . اخذت عينات الدم بعد انتهاء التجربه من جميع الحيوانات وتم قياس قياس مستويات هرمونات الغدة الدرقية ومستويات السايبتوكينات مثل الانترلوكين 6 و 10 وعامل نخر الورم ومؤشرات الاجهاد التاكسدي حيث شملت المالنوالديهيد و الكلوتاثيون المختزل و انزيم السوبر دايميوتيز و الكلوتاثيون بيروكسيدز و الكاتيليز . بينت النتائج ان هنالك زيادة معنوية في مستويات هرموني الترايودوثايرونين و التاثيروكسين و المالنوالديهيد و السايبتوكينات في مجموعة الارانب المعاملة بالتاثيروكسين فقط في حين وجد هنالك انخفاض معنوي في مستويات كل من هرمون المحفز للغدة الدرقية و الكلوتاثيون و انزيمات السوبر دايميوتيز و الكلوتاثيون بيروكسيدز و الكاتيليز . ان تناول 100 ملغم /كيلو غرام من وزن الحيوان من مستخلص الملفوف الاحمر الكحولي ادى الى انخفاض مستويات هرموني الترايودوثايرونين و التاثيروكسين بالاضافة الى تاثيره الواضح على تقليل مستويات السايبتوكينات و المالنوالديهيد بالاضافة الى ارجاع مستويات كلا من الكلوتاثيون المختزل وفعالية انزيمات و السوبر دايميوتيز و الكلوتاثيون بيروكسيدز و الكاتيليز الى مستوياتها الطبيعية مقارنة بمجموعة السيطرة. نستنتج من هذه النتائج ان للمستخلص دور وقائي مهم لحالة الاجهاد التاكسدي الحاصله بفعل تناول التاثيروكسين، بالاضافة الى انه لأول مره تم ملاحظة دور المستخلص في كبح نشاط فرط الدرقية الذي ربما يعود الى عدة اليات تتضمن امتلاكها خاصية كبح الجنور الحرة وتقليل بيروكيدات الدهون غير المشبعة وزيادة مستوى مضادات الاكسدة بالاضافة الى تثبيط فعالية انزيم ازالة اليود.

## INTRODUCTION

Oxidative stress accompanying hyperthyroidism is caused by increased synthesis of reactive oxygen species (ROS) and changes in the antioxidant defence system (1-3). ROS have a high reactivity potential, therefore they are toxic and can lead to oxidative damage in cellular macromolecules such as proteins, lipids and DNA (4-5). In fact, the cell contains a variety of substances capable of scavenging the free radicals, protecting them from harmful effects. Among the enzymatic antioxidants, are GRx, GPx, CAT, SOD while the non-enzymatic antioxidants are GSH, vitamin E, vitamin C,  $\beta$ -carotene, and flavonoids (6). When ROS generation exceeds the antioxidant capacity of cells, oxidative stress develops (2,7). This phenomenon has been related to many pathological conditions and it has also been suggested that some complications of hyperthyroidism are due to  $T_3$  induced oxidative stress in target tissues (8-10). Thyroid hormones are the most important factors involved in the regulation of the basal metabolic state, as well as in the oxidative metabolism (11). Experimental studies and epidemiological data suggest that hyperthyroidism is associated with a general increase in tissue oxidative stress (12-15). Great controversy exists as to whether hyperthyroidism is associated with an increase or a decrease in the activities of antioxidant enzymes (16). Vitamin E is a potent lipid soluble

antioxidant in biological systems with the ability to directly quench free radicals and function as membrane stabilizer (17).

Antioxidants treatments might be helpful in reducing the oxidative damage due to hyperthyroidism. Several studies have demonstrated that various agents, including vitamin E (17) melatonin (18), and Selenium (19) can prevent oxidative damage in hyperthyroidism. The antioxidant protection of natural plants is a promising therapeutic remedy for free radical pathologies (20). Among myriad natural plants, red cabbage (*Brassica oleracea* var *capitata*) and other *Brassica* vegetables, have been found to have antioxidant, antihyperglycemic (21), anticancer (22-26), hypolipidemic (27). The principle constituents of RC are isothiocyanates (glucosinolate), vitamin A, B, C and Anthocyanins (28). Anthocyanins, a group of phenolic natural pigments present in RC, were found to have the strongest antioxidizing power of 150 flavonoids (29). There are some review articles on the general biochemical, cellular and medicinal properties of anthocyanins (30-31), but no detailed mechanisms of its action has yet been published. To date there is no study on the protective effect of RCE, a known potent antioxidant and free radical scavenger on hyperthyroidism. This study aims to investigate oxidative stress parameters, antioxidant status markers, some cytokine levels in hyperthyroid rabbits induced by thyroxine and their response to RCE supplementation compared with vitamin E as standard antioxidant.

## **MATERIALS AND METHODS**

### **Preparation of Red Cabbage Extract**

Red cabbage leaves were sliced into small pieces and oven-dried at 50°C. Dried plants (800 g) were extracted in 8000 ml of 70% aqueous ethanol using ultrasonic treatment at an intensity of 70 W/cm<sup>2</sup> and oscillation frequency at 20 kHz for 5 min. The use of an ultrasound extraction method has been shown to diminish the danger of thermal degradation (32). Ultrasound also provides a greater penetration of solvent into cellular materials, via cavitation, and improves the release of cell contents into the bulk medium. Ultrasonic irradiation was applied by means of a Branson 450 digital sonifier (20 kHz, 450 W) equipped with a cylindrical titanium alloy probe (12.70 mm in diameter). The use of dry plants can be effective to minimize enzymatic degradation of phenolic compounds inside plant tissues. After overnight maceration, the extract was filtered through gauze and ethanol was evaporated under reduced pressure at 50°C by using a rotary evaporator. The remaining water extract was dried using a freeze dry system under reduced pressure. The dried extract was dissolved in distilled water to a concentration of 1 g/ml before administration to normal and diabetic rats. The extraction yield for RC was about 12 %.

### **Animals and Experimental design**

Male New Zealand rabbits (1.400–1.5 kg) weights were obtained from the Animal house at the biological control center, Ministry of Health. Rabbits were maintained on standard pellet diet and tap water *ad libitum*. They were kept in plastic cages under a 12 h light/dark cycle and room temperature 22–24°C and were acclimatized to the environment for 2 weeks prior to experimental use. Procedures for the care and use of research animals at Iraq meet or exceed all applicable local,

national and international laws and regulations. The forty animals were randomly divided into four groups of ten animals in each group. The control rabbit's group one, received only vehicle. The animals in the experimental group two were orally administered L-thyroxine ( $20\mu\text{g}/\text{kg}$  b.w) in 0.25 ml sterile physiological saline. Animals in experimental group three received thyroxine ( $20\mu\text{g}/\text{kg}$ .b.w) and vitamine E intramuscularly at a dose of  $20\text{ mg}/\text{kg}$  b.w per day for 4 weeks. The animals in the experimental group four, received L-thyroxine ( $20\mu\text{g}/\text{kg}$ .b.w) and RCE ( $10\text{ mg}/\text{kg}$ .b.w)for4 weeks.RCE was administered at the same time each day. At the end of the experimental period, the blood samples were collected through heart puncture after overnight fasting in test tubes without anticoagulant and blood samples were centrifuged at  $2500\text{ g}$  for 15 minutes at room temperature to obtain serum which used to estimate thyroid function tests: TSH,  $T_3$ , and  $T_4$ , oxidative stress markers MDA, GSH, SOD, GPx, CAT and some cytokines level (IL-6, IL-10 and TNF- $\alpha$ ).

#### Assay of thyroid function tests

Quantitative determination of rabbit  $T_3$  hormone was performed using rabbit triiodothyronine hormone,  $T_3$  ELISA Kit Catalog No: E0453Rb from ELA Ab,company.Quantitative detrmiation of rabbit thyroxine hormone was performed using rabbit thyroxine hormone,  $T_4$  ELISA Kit Catalogue Number: E04T0040 from ShangHai Blue Gene Biotech CO., LTD. Quantitative determination of rabbit thyroid stimulating hormone was performed using rabbit thyroid stimulating hormone, TSH ELISA Kit Catalog No: E0463Rb from ELA Ab.The principle depending on the microtiter plate provided in this kit has been pre-coated with an antibody specific to  $T_3$ . The principle depending on the microtiter plate provided in this kit has been pre-coated with an antibody specific to  $T_3$ . Standards or samples are then added to the appropriate microtiter plate wells with biotin-conjugated polyclonal antibody preparation specific for  $T_3$  and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain  $T_3$ , biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of  $T_3$  in the samples is then determined by comparing the O.D. of the samples to the standard curve. Quantitative determination of rabbit thyroxine hormone was performed using rabbit thyroxine hormone,  $T_4$  ELISA Kit Catalogue Number: E04T0040 from ShangHai Blue Gene Biotech CO., LTD Quantitative determination of rabbit thyroid stimulating hormone was performed using rabbit thyroid stimulating hormone, TSH ELISA Kit Catalog No: E0463Rb from ELA Ab as the same principle

#### Measurement of MDA

Lipid peroxidation (LPO) is frequently investigated in biomedical research, and the assays for thiobarbituric acid-reactive substances (TBARSs) are more widely used than any other index of LPO in biological samples. Thiobarbituric acid reacts with LPO aldehydes, such as MDA.Therefore, assessment of TBARS is a useful index of oxidative deterioration and LPO determination in body fluids.

MDA levels were determined at 532 nm by the method of Ohkawa (33). MDA formed a colored complex in the presence of thiobarbituric acid, which was detectable by measurement of absorbance at 532 nm. Absorbance was measured with Shimadzu UV-160 spectrophotometer. 1, 1, 3, 3 THP was used as a standard. Levels were calculated as nmol/dl.

#### **Measurement of reduced GSH**

Total reduced GSH content was measured according to the method of Tietze (34). In brief, 0.5mL sample or standard solution was mixed with 0.25 ml of 1mol/L sodium phosphate buffer (pH 6.8) and 0.5 mL 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.8 g/L in the phosphate buffer) for 5 minutes. Then, the absorbance was measured at 412 nm using a Shimadzu UV-160 spectrophotometer. The GSH levels were determined using standard aqueous solutions of reduced GSH. Results were expressed as  $\mu\text{mol/dl}$ .

#### **Measurement of SOD activity**

Superoxide Dismutase (SOD), which catalyzes the dismutation of the superoxide anion ( $\text{O}_2^-$ ) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In order to determine the SOD activity, several direct and indirect methods have been developed. Among these methods, an indirect method using nitroblue tetrazolium (NBT) is commonly used due to its convenience and ease of use. However, there are several disadvantages to the NBT method (35), such as poor water solubility of the formazan dye and the interaction with the reduced form of xanthine oxidase. The K-ASSAY SOD Assay kit Cat. No. KT-019 allows very convenient SOD assaying by utilizing water soluble tetrazolium salt, 1(2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium,Na salt that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with  $\text{O}_2^-$  is linearly related to the xanthine oxidase activity, and is inhibited by SOD. Therefore, 50% inhibition activity of SOD or SOD-like materials) can be determined.

#### **Determination of GPx activity**

Serum glutathione peroxidase was determined according a sandwich enzyme immunoassay method for the in vitro quantitative measurement of rabbit GP<sub>x</sub> in serum, plasma and other biological fluids (ELISA Kit for Rabbit Glutathione Peroxidase (GP<sub>x</sub>) Cat. No.: E0295Rb Usen Life Science Inc. Wuhan company). The microtiter plate provided in this kit has been pre-coated with an antibody specific to GP<sub>x</sub>. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for GP<sub>x</sub>. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain GP<sub>x</sub>, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured at a wavelength 450 nm spectrophotometrically. The concentration of GP<sub>x</sub> in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve.

**Determination of CAT activity**

Serum glutathione peroxidase was determined according a Rabbit Catalase ELISA kit Catalogue Number: E04C0086. This CAT enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for CAT. Standards or samples are then added to the microtiter plate wells and CAT if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of CAT present in the sample, a standardized preparation of horseradish peroxidase conjugated polyclonal antibody, specific for CAT are added to each well to “sandwich” the CAT immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, A and B substrate solution is added to each well. The enzyme and substrate are allowed to react over a short incubation period. Only those wells that contain CAT and enzyme-conjugated antibody will exhibit a change in color. The enzyme reaction is terminated by the addition of a sulphuric acid solution and the color change is measured colorimetrically using UV/Vis spectrophotometer at a wavelength of 450 nm.

**Determination of IL-6 levels**

Quantitative determination of rabbit IL-6 was performed using a sandwich enzyme immunoassay kit Rabbit Interleukin 6, IL-6 ELISA Kit, Catalog No: E0079Rb (EIA- AB company). The microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-6. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-6. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain IL-6, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of IL-6 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

**Determination of IL-10 level**

Quantitative determination of rabbit IL-10 was performed using a sandwich enzyme immunoassay kit Catalog No. E0056Rb (EIA-AB Company)

**Determination of TNF- $\alpha$  level**

Quantitative determination of rabbit TNF $\alpha$  was performed using a sandwich enzyme immunoassay kit Catalog No E90133Gu from Usnc Life Science Inc.

**Statistical analysis**

The data analysis was performed with SPSS soft ware package (Version 10). Data are presented as mean values (Mean $\pm$  S.E.M). Data were statistically analysed using a one-way analysis of variance (ANOVA) followed by a Student-t test to compare the groups. Linear regression analysis was used with thyroid function as the dependent variable. A value of  $P < 0.05$  was accepted as significant.

## RESULTS

### Effect of RCE on thyroid hormones levels

Serum T<sub>3</sub>, T<sub>4</sub> and TSH concentrations in the control and experimental groups are given in table 1 and Figure (1-3). S.T<sub>3</sub> and T<sub>4</sub> concentrations were significantly increased  $1.99 \pm 0.47$  ng/ml and  $16.5 \pm 2.22$  µg/dL respectively in rabbits with hyperthyroidism compared to controls  $1.14 \pm 0.37$  ng/ml and  $9.82 \pm 2.33$  µg/dl respectively. Co-administration of RCE with L-thyroxine significantly decreased the elevated T<sub>3</sub> levels ( $P < 0.01$ ) to  $1.24 \pm 0.37$  ng/ml compared with  $1.99 \pm 0.47$  ng/ml in hyperthyroid group without extract treatment as in group (3). Similar data was observed in the levels of serum T<sub>4</sub>. On the other hand RCE treatment increased TSH concentration in hyperthyroid group (G4) to  $1.04 \pm 0.54$  mIU/L compared with  $0.66 \pm 0.12$  mIU/L before treatment with extract.

Data on the effects of vitamin E supplementation on thyroid hormone levels show that vitamin E has a thyroid function suppressing action. The S.T<sub>3</sub> and S.T<sub>4</sub> values in the L-thyroxine and vitamin E intake animals as in group 3 were significantly decreased to  $1.43 \pm 0.13$  and  $12.65 \pm 1.52$  µg/dl respectively in respect to those of the L-thyroxine only administered group (G2) as shown in Table 1. This data agree well with the results of Petrulea *et al.*, (2009) who found a decrease in T<sub>4</sub> and T<sub>3</sub> levels in vitamin E-supplemented hyperthyroid rats and suggested that Vitamin E intake in the hyperthyroid state decreases either T<sub>4</sub>/T<sub>3</sub> synthesis or T<sub>4</sub> T<sub>3</sub> conversion.

### Effect of RCE on cytokine levels

Serum levels of IL-6, IL-10 and TNF-α were significantly enhanced after L-thyroxine administration ( $P < 0.01$ ) as shown in table 2 & figure 4-6. In the hyperthyroid rabbits, serum level of IL-6 was significantly higher than that in the healthy euthyroid rabbits, also a significant positive correlation between serum IL-6 and S.T<sub>3</sub> levels (Pearson correlation;  $r = + 0.98$ ,  $P < 0.01$  was observed (Data not shown). Meanwhile there was also a significant correlation between serum IL-6 and total T<sub>4</sub> levels ( $r = + 0.88$ ,  $P < 0.01$ ). Similarly IL-10 correlated with T<sub>3</sub> ( $r = + 0.98$ ,  $P < 0.01$ , and with T<sub>4</sub> but not with TSH. Interestingly, there was a significant correlation between IL-6 and IL-10 ( $r = 0.99$ ,  $P < 0.01$ ). By linear regression analysis, IL-6 was found to be a potential risk factor that could predict the observed higher circulating level of T<sub>3</sub> ( $P < 0.01$ ), in addition to IL-10 was another a predictor ( $P < 0.01$ ). Importantly, IL-6 and IL-10 were potential predictors for the higher thyroid hormone concentrations. Co-administration RCE with L-thyroxine significantly decreased the elevated IL-6 levels ( $P < 0.01$ ) to  $300.33 \pm 32.92$  pg/L compared with  $385.99 \pm 109.20$  pg/L in hyperthyroid group without extract treatment as in group (2). Similar data was observed in the levels of serum IL-10 and TNF-α.

### EFFECT OF RCE ON OXIDATIVE STRESS MARKERS

As expected, lipid peroxidation was significantly higher in the plasma of hyperthyroid group compared to the control group  $4.62 \pm 0.12$  mol/dL and  $2.23 \pm 0.08$  mol/dL, respectively. It was decreased after treatment with RCE in the hyperthyroid group ( $P < 0.01$ ). When the sera were used to measure MDA concentrations as index of lipid peroxidation, the result indicated a significant increase in sera of hyperthyroid group compared to the control group. On the contrary, when hyperthyroid rabbits were concurrently treated with RCE the result

demonstrated a significant reduction in MDA concentrations (Table 3 and figure 7). Serum GSH level was significantly lower ( $P < 0.01$ ) in the hyperthyroid group  $34.43 \pm 0.19 \mu\text{mol/dL}$  compared to the control group  $44.32 \pm 0.13 \mu\text{mol/dL}$  as shown in figure 8, while GSH level increased after treatment with RCE in the hyperthyroid group to  $47.57 \pm 0.16 \mu\text{mol/dL}$  and this increase was restored by the concurrent administration of RCE. The redox status (GSH/GSSG ratio), an oxidative stress indicator, was found to be significantly reduced ( $P < 0.05$ ). These decreases were restored to normal level by concurrent treatment with RCE.

#### EFFECT OF RCE ON ENZYMATIC ANTIOXIDANT ACTIVITY

A significant decrease in the activity of serum GPx, SOD and CAT were observed in the hyperthyroid group compared to the control group as shown in table 4 and figures 9-11). For GPx activity it reduced to  $8.25 \pm 0.14$  compared with  $18.70 \pm 0.13 \text{ mmol/dL}$  in control. After hyperthyroid rabbits treatment with RCE, GPx activity increased significantly and return to normal level  $17.25 \pm 0.22 \text{ mmol/dl}$  compare to the control group  $18.70 \pm 0.13 \text{ mmol/dL}$  (Figure 9). On the other hand in hyperthyroid groups, the CAT activity reduced significantly  $42.15 \pm 0.60 \text{ mmol/dL}$  compared with control animal group (figure 10), while treatment with RCE the activity increased to the mean value near normal. Thus, the marked increase in the oxidative stress was found in hyperthyroid groups as indicated by decrease in CAT activity, whereas treatment with RCE showed decrease in oxidative stress as indicated by the increased CAT activity as compared to hyperthyroid groups. The same pattern change was happen for SOD in hyperthyroid groups, the SOD activity reduced significantly compared with control animal group  $55.45 \pm 0.19$  and  $95.75 \pm 0.22 \text{ U/dl}$  respectively (figure 11), while treatment with RCE the activity increased to the mean value near normal. Thus, the marked increase in the oxidative stress was found in hyperthyroid groups as indicated by decrease in SOD activity, whereas treatment with RCE showed decrease in oxidative stress as indicated by the increased SOD activity as compared to hyperthyroid groups. The present finding revealed decrease in MDA level and increase in levels of GSH, GPx, SOD and CAT activity by the treatment of RCE, indicating reduction in oxidative stress in hyperthyroid state. Thus, it was concluded that the red cabbage extract had antioxidant property due to reduction in all oxidative stress markers .

#### DISCUSSION

Hyperthyroidism accelerates ROS generation and produces changes in the antioxidant systems of various tissues (2, 10). In the present study, increased plasma  $T_3$  and  $T_4$  levels and decreased in TSH levels were observed in the hyperthyroid group, while treatment with red cabbage extract or vitamin E restore levels near control healthy group. The mechanisms behind the RCE-induced reduction in thyroid hormone may be related to glucosinolate active compound in red cabbage extract. Possibilities include RCE induced modulation in deiodination system, which affects deiodinase activity through its antioxidant properties. Based on the results obtained, it can be concluded that the hyperthyroid group, which received RCE, shows a significantly different decrease of plasma  $T_3$  and  $T_4$  concentrations and significantly different increase of TSH levels. Pharmacological

antioxidants may have an effect on the peripheral conversion of thyroid hormones by way of deiodination and/or mechanism of cell membrane defence, the integrity of which may have an effect on the activity of deiodinases (36). On the other hand in this study, a substantially high level of the proinflammatory cytokine, IL-6 was detected in animals induced by thyroxine supporting its possible role as an endocrine cytokine with a regulatory effect on many endocrine systems including the thyroid gland (37-38), in addition to a considerably high level of the anti-inflammatory cytokine, IL-10 in the L-thyroxine treated animals was noticed too. Interestingly, a direct association between IL-6 and IL-10 was indicated which could be attributed to the fact that secretion of both Interleukins is stimulated by the same cytokines such as TNF- $\alpha$  (39). The findings of this study suggest that induced hyperthyroidism in animals by oral administration of L-thyroxine stimulates firstly the oxidative stress response and the production of inflammatory cytokines (IL-10, IL-6 and TNF- $\alpha$ ), secondly there is a relationship between oxidative stress parameters and inflammatory cytokines, thirdly red cabbage extract prevents the increase in T<sub>3</sub> and T<sub>4</sub> caused by chronic T<sub>4</sub> administration, and finally RCE based antioxidative treatment inhibits thyroid hormone induced increases in MDA, GSH, and TNF- $\alpha$  levels. Meanwhile, to our knowledge, the latter observation, which RCE acts on interleukins and TNF- $\alpha$  level, seems novel. We hypothesized that reactive oxygen intermediates, which were induced by hyperthyroidism, act as a signal for the release of cytokines like IL-6, IL-10, and TNF- $\alpha$ . The cellular GSH plays an important role as biological antioxidant defence systems, which act as protective mechanisms against oxidative damage (40), therefore, the decreased level of GSH may be due to overproduction of free radicals and increased lipid peroxidation in hyperthyroidism (7). In this study, serum GSH levels were decreased in hyperthyroid animals as compared to control animals, possibly secondary to increased ROS generation. RCE treatment caused normalization of GSH levels in hyperthyroid animals. The ratio of GSH to GSSG is considered an important marker of oxidative stress (16). The decrease in the ratio of GSH after hyperthyroid state suggests induction of oxidative stress in the animals. Furthermore, increase in the GSH levels of the RCE treated hyperthyroid rats suggests that RCE as a regulatory effect on the antioxidant system. Increased oxidative stress is a well-known phenomenon in the hyperthyroid state. Hyperthyroidism is believed to accelerate free radical generation that leads to oxidative damage of lipids (7).

The Serum MDA level was increased in the hyperthyroid rabbits as compared to the control rabbits. Our results are in accordance with Salwa, 2011 who reports a significant increase in MDA levels of hyperthyroidism. Increased plasma MDA concentrations in hyperthyroid rabbits suggest the role of free radicals in the pathogenesis of this lipid peroxidation and, thus, support the need for studies assessing the therapeutic role of antioxidants in hyperthyroidism. In support of the above statement, we found a highly significant decrease in the levels of serum MDA after treatment with RCE. The reduced MDA level by RCE likely indicates that RCE might be a novel agent to protect against oxidative damage induced by hyperthyroidism. It is also possible that the action of RCE is primarily a

normalization of thyroid function and secondarily a normalization of the oxidative stress markers.

Superoxide dismutase is an important intracellular oxygen radical-scavenging enzyme. It has been demonstrated that hyperthyroidism leads to accelerated free radical formation. Conversely, increased free radical formation enhances intracellular scavenging enzymes like SOD in experimentally hyperthyroid animals. We investigated the therapeutic value of RC against oxidative stress in hyperthyroid animals. These plants, members of the Cruciferae and genus Brassica such as RC, broccoli, kale and Brussels sprouts, contain anthocyanin pigments that are described as free-radical scavenging and antioxidant agents (42). Anthocyanin isolates and anthocyanin rich mixtures of bioflavonoid provide protection against myriad physiological failures such as LP, decreasing capillary permeability and fragility and membrane strengthening. RC extract contains vitamins A, B and C all of which have protective roles against oxidative damage. Our results indicate that RCE is beneficial as a protective agent against oxidative stress induced by hyperthyroidism in rabbits. The protection is probably due to multiple mechanisms involving free radical scavenger properties, attenuating lipid peroxidation and increasing the antioxidant status. However, further studies are essential to elucidate the exact mechanisms of protection by RCE.

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**Table (1) Effect of oral administration of red cabbage extract on serum T<sub>3</sub>, T<sub>4</sub>, TSH, levels**

(Mean ± SE) in normal and hyperthyroid rabbit's induced by L- thyroxine.

Parameters	Healthy	Hyperthyroid	Hyperthyroid treated with Vit. E	Hyperthyroid treated with RCE
T3( ng/ml)	1.14 ± 0.37	1.99 ± 0.47*	1.43 ± 0.13 *	1.24 ± 0.37*
T4( µg/dl )	9.82 ± 2.3	14.5 ± 2.8*	12.65 ± 1.52*	10.2 ± 2.7*
TSH( IU/ml)	2.04 ± 0.54	0.66 ± 0.12*	1.23 ± 0.63 *	1.04 ± 0.54*

\*Significantly different (p<0.05), \*\* significantly different (p<0.01),

**Table (2) Effect of oral administration of red cabbage extract on serum, IL-6, IL-10 and TNF-α levels**

(Mean ±SE) in normal and hyperthyroid rabbit's induced by thyroxine.

Parameters	Healthy	Hyperthyroid	Hyperthyroid treated with Vit. E	Hyperthyroid treated with RCE
IL-6( pg/L)	314.66 ± 26.28	385.99 ± 109.20*	323.33 ± 38.92*	300.33 ± 32.92*
IL-10(pg/L)	33.53 ± 10.03	61.99 ± 17.43 *	34.95 ± 4.61*	30.4 ± 6.33*
TNF (ng/ml)	0.73± 0.04	0.98 ± 0.10*	0.78± 0.05*	0.76± 0.04*

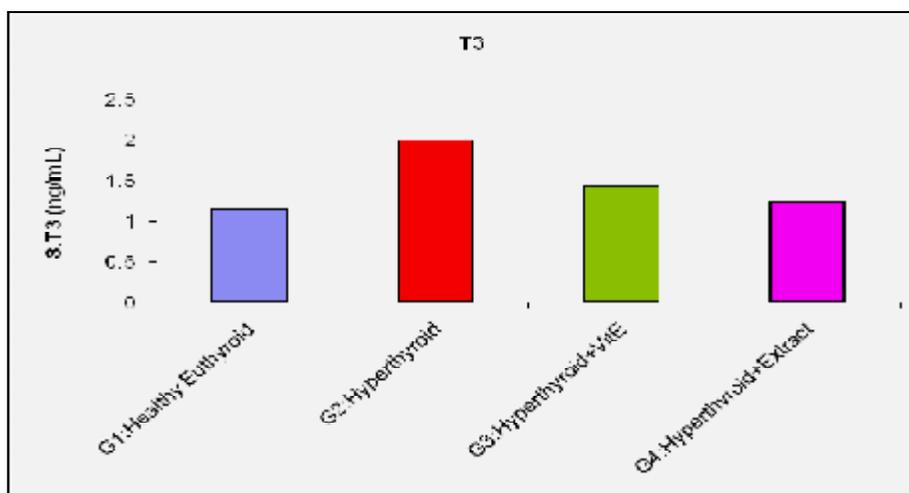
**Table (3) Effect of oral administration of red cabbage extract on serum MDA and GSH levels (M±SE) in normal and hyperthyroid rabbit's induced by thyroxine.**

Groups	MDA( µmol/dL)	GSH(µmol/dL)
Healthy	2.23±0.11	52.32±0.13
Hyperthyroid	4.62±0.13*	25.42±0.25**
Hyperthyroid + Vit E	2.45±0.02*	50.66±0.22**
Hyperthyroid + Extract	2.30±0.03*	47.57±0.16 **

**Table( 4) Effect of oral administration of red cabbage extract on serum activity of antioxidant enzymes (M±SE) in normal and hyperthyroid rabbit's induced by L-thyroxine**

Groups	GPX (mmol/dl)	SOD U/dl)	CAT(mmol/dl)
Healthy	18.70±0.13	95.75±0.22	67.00±0.56
Hyperthyroid	8.25± 0.14**	55.45±0.19**	42.15±0.60**
Hyperthyroid +Vit E	13.95±0.02*	86.66±0.16*	63.60±0.56*
Hyperthyroid + Extract	9.25±0.02**	68.61±0.10**	51.65±0.24**

\*Significantly different (p<0.05), \*\* significantly different (p<0.01),



**Figure (1) Effect of oral administration of red cabbage extract on serum T<sub>3</sub> levels in normal and hyperthyroid rabbit's induced by L-thyroxine.**

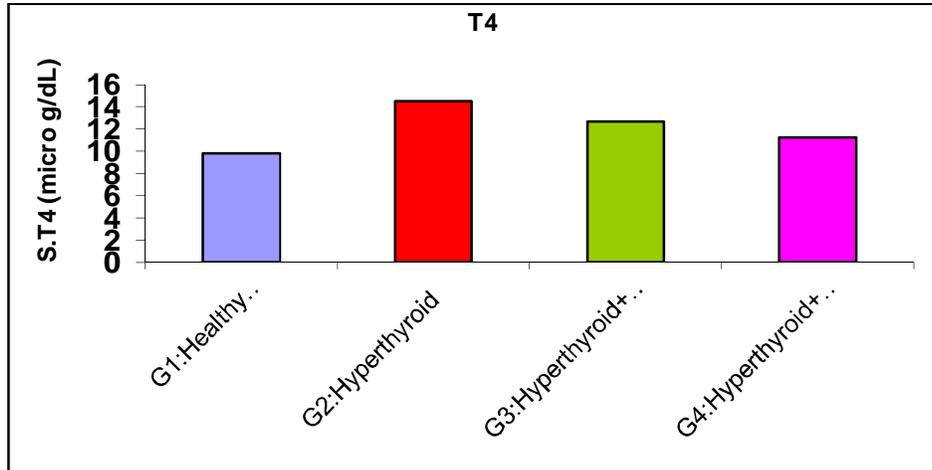


Figure (2) Effect of oral administration of red cabbage extract on serum T<sub>4</sub> levels in normal and hyperthyroid rabbit's induced by L- thyroxine

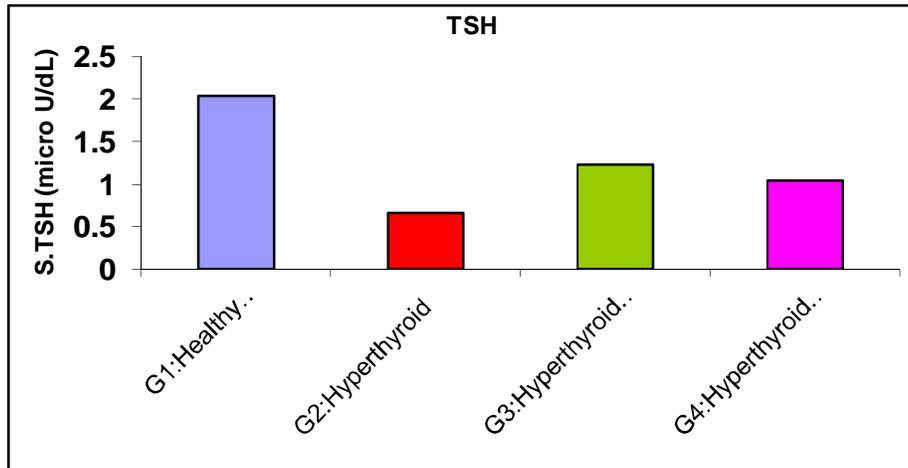
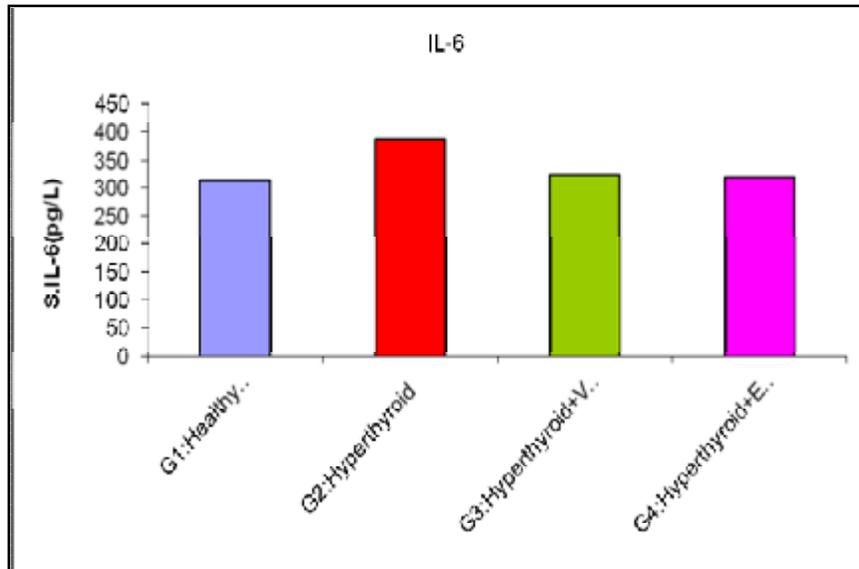


Figure (3) Effect of oral administration of red cabbage extract on serum TSH levels in normal and hyperthyroid rabbit's induced by L- thyroxine.



Figure(4) Effect of oral administration of red cabbage extract on serum IL-6 levels in normal and hyperthyroid rabbit's induced by thyroxine

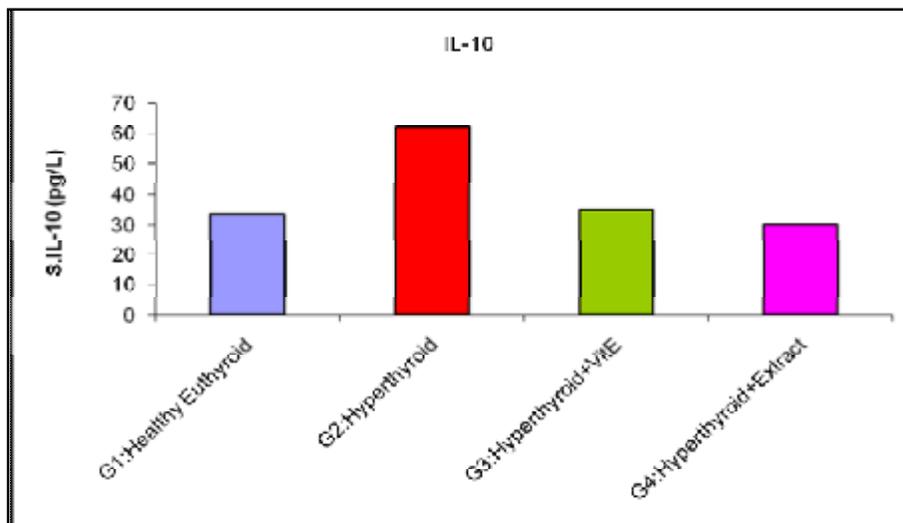


Figure (5): Effect of oral administration of red cabbage extract on serum IL-10 levels in normal and hyperthyroid rabbit's induced by thyroxine

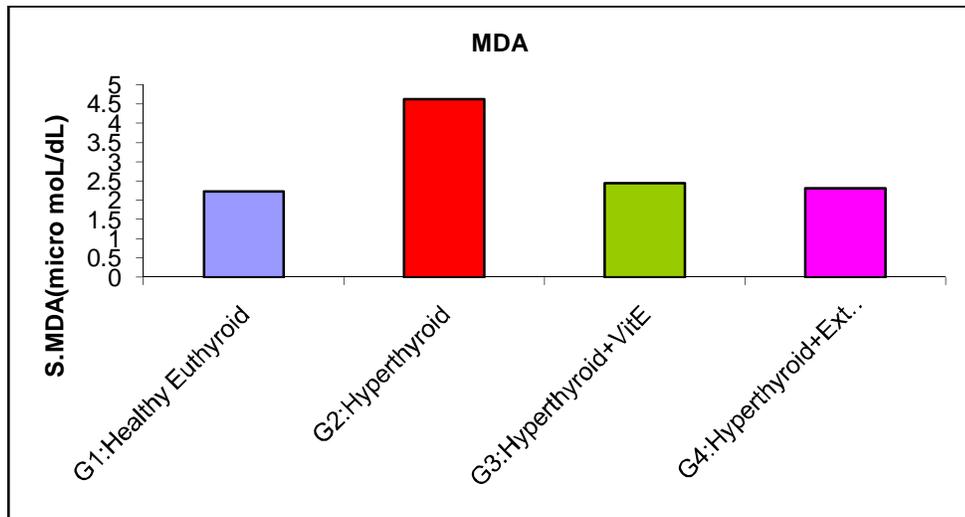


Figure (7) Effect of oral administration of red cabbage extract on serum MDA levels in normal hyperthyroid rabbit's induced by thyroxine

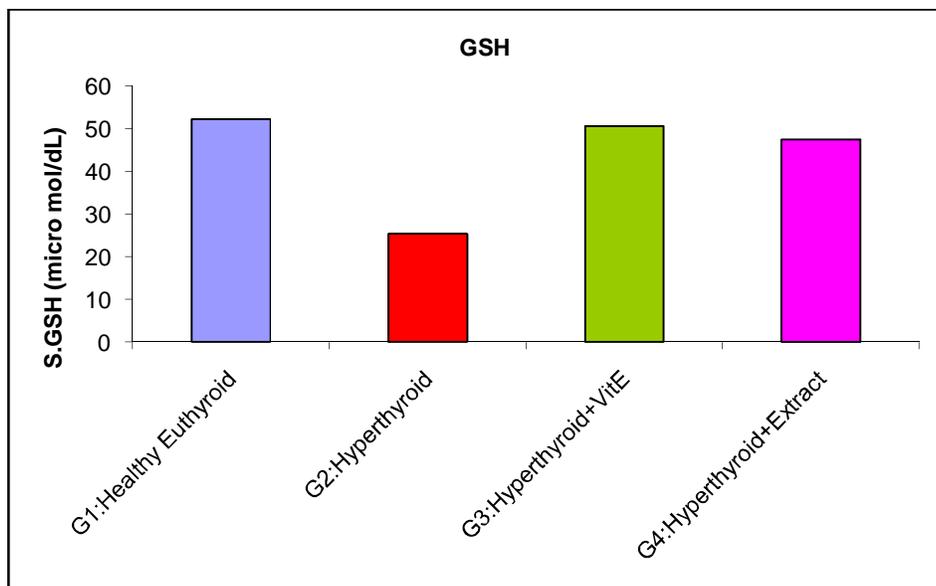


Figure (8) Effect of oral administration of red cabbage extract on serum GSH levels in normal and hyperthyroid rabbit's induced by thyroxine.

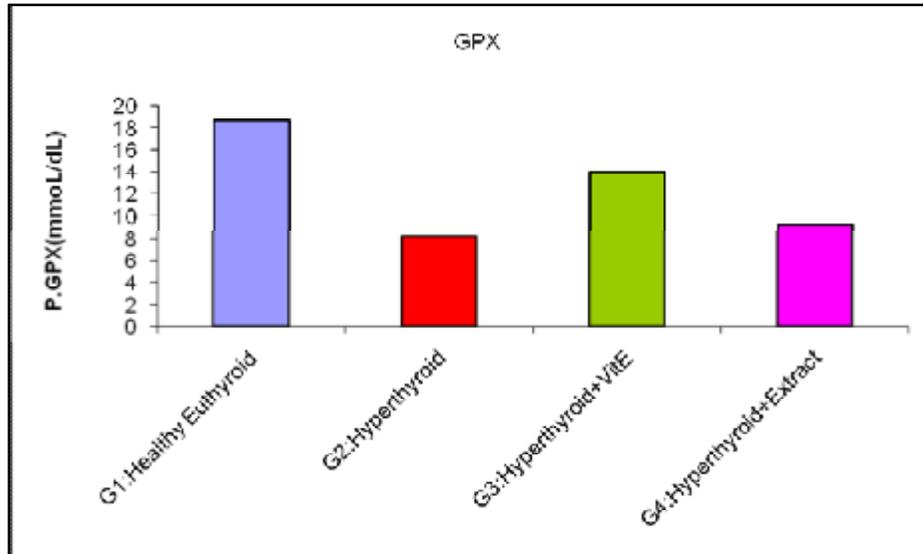


Figure (9) Effect of oral administration of red cabbage extract on serum GP<sub>x</sub> activity in normal and hyperthyroid rabbit's induced by thyroxine

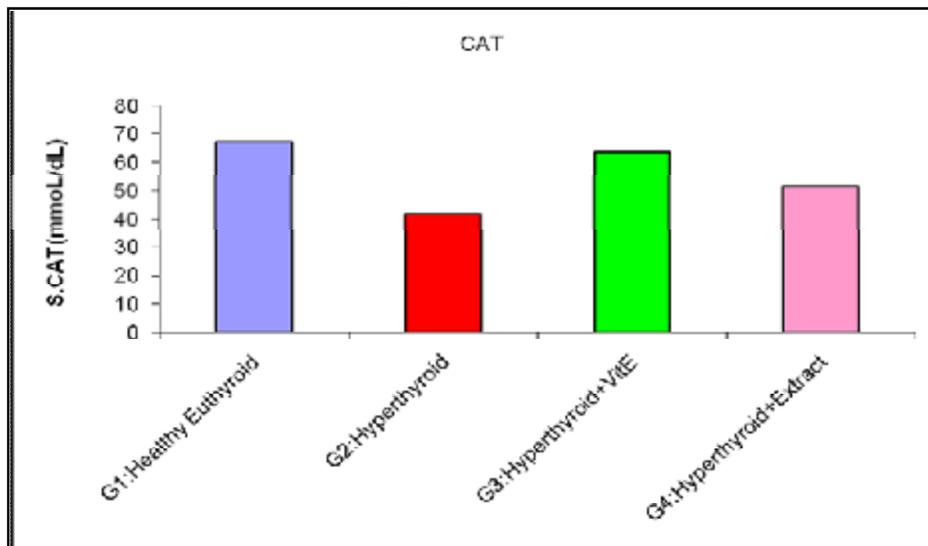


Figure (10) Effect of oral administration of red cabbage extract on serum CAT activity in normal and hyperthyroid rabbit's induced by T4 thyroxine

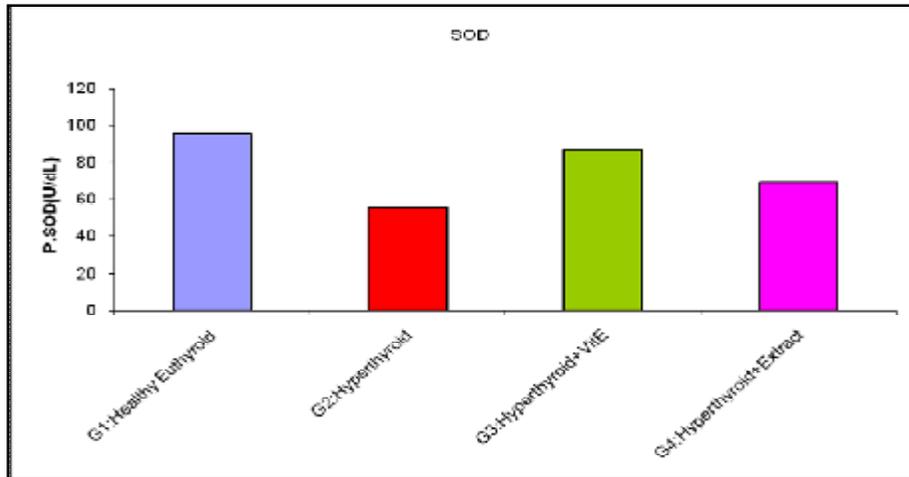


Figure (11) Effect of oral administration of red cabbage extract on serum SOD activity in normal and hyperthyroid rabbit's induced by T4

## Chlorine Removal with Activated Carbon Using Bubble Column

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### ABSTRACT

Bubble column slurry reactor was used for the measurements of gas adsorption and ion-exchange in this work. Adsorption of chlorine (dissolved in water) on activated carbon was carried out in the same reactor. The effect of gas flow rates  $u_G$  0.016 m s<sup>-1</sup>-0.027 m s<sup>-1</sup> which covers the boundaries of the following four regimes: bubbly flow, first transition, second transition, and coalesced bubble and solid concentrations were investigated. In addition of studying the pH which gives an indication for Hypochlorous Acid HOCl, the most active sanitizer form of Free Chlorine. These design guidelines provide a good starting point for system of removing chlorine from water at activated carbon concentration 20 gm/L, gas velocity 0.023 m/s and contact period of time more 17 minutes.

**Keywords:** Activated carbon; chlorine removal; slurry bubble column.

### ازالة الكلورين في عمود كاربون منشط

#### الخلاصة

استخدم العمود الفقاعي الثلاثي الاطوار لحساب امتزاز الغاز وكذلك تحول الايونات. وفيه يتم امتصاص الكلور المذاب بالماء بواسطة الكاربون المنشط المحمل داخل الانبوب. حسب كل من تأثير سرعة جريان الغاز (الهواء) والتي تغطي اطوار الجريان الفقاعي والانتقالي 1,2 وتكسر الفقاعات 0.016-0.027 م/ثا وتأثير كمية الصلب لكل لتر ماء بالاضافة الى حساب التغيير في قيمة الاس الهيدروجيني والذي يعتبر مؤشر لتحول ايونات OCl<sup>-</sup> الى HOCl. من خلال التجارب تقدم نقطة البداية لتصميم منظومة ازالة الكلور من المياه وذلك عند تركيز الكاربون المنشط 20 غم/لتر ، سرعة غاز 0,023 م/ثا وفترة زمنية اكثر من 17 دقيقة. **الكلمات المفتاحية:** كاربون المنشط، ازاله الكلور، العمود الفقاعي ذو الحبيبات الصلبة المعقدة.

### INTRODUCTION

Municipalities routinely began using chlorine to treat drinking water starting in 1908 with Jersey City, NJ. Its use has helped to virtually eliminate diseases like typhoid fever, cholera and dysentery in the US and other developed countries. Globally the World Health Organization (WHO) estimates that 3.4 million people in underdeveloped countries die every year from water-related diseases. Use of chlorine in water can produce an undesirable taste;

therefore, many consumers prefer to remove it. Disinfection by products (DBPs) may also unintentionally form when chlorine and other disinfectants react with natural organic matter that is in the water. To reduce DBP formation, many municipalities are switching to monochloramine [1].

Chloramine is a generic term describing the products from the reaction of ammonia and chlorine. While chlorine is an effective disinfectant for municipal water supplies, the use of chloramine compounds has increased recently. The main reason for this is the relative stability of chloramines over free chlorine. Free chlorine tends to react with organic compounds in water systems creating tri-halo-methanes (THM's) and other disinfection byproducts (DBP's).

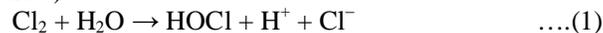
Chloramines are less reactive in water systems and therefore less likely to form these EPA regulated substances. In addition, chloramines tend to maintain a residual concentration throughout large distribution systems. They are also much more difficult to remove from water than free chlorine. This can become a problem in many applications [2].

With more and more plants installing membrane technology, chloramine removal has become a critical water treatment concern. Chloramines, just like any other oxidant, can react with certain compounds in the beverage to effect the taste and shelf life of the final product. Most public water utilities maintain a residual of 2.5 to 3.5 mg/L. Reverse osmosis (RO) and nanofiltration (NF) membranes that are made of cellulose acetate can easily handle these levels, but then the chloramine in the permeate must be removed before the water can be used for beverage and syrup making purposes. Membranes that are polyamide (PA) or thin film composite (TFC) will be damaged by strong oxidants, such as free chlorine, even in low concentrations. While chloramines are not as aggressive as chlorine, they are still capable of damaging the membranes and should be removed [3].

Tory L. Champlin *et. al* identify effective treatment methods for removing chloramines, both chlorine and ammonia, while reducing natural organic matter from the chloraminated water. Although possible technologies exist, including granular activated carbon (GAC), their effectiveness to remove chloramines (i.e., chlorine and ammonia) is not well documented. Essential for converting from chloramines to free chlorine is the removal of ammonia. If ammonia were to remain, chloramines would once again be formed by the addition of chlorine intended to provide free chlorine residual [4].

USEPA's maximum residual disinfection levels (MRDLs) are four mg/L for chlorine; however, chlorine may cause problems that activated carbon can help resolve. The addition of chlorine to disinfect water is accomplished by one of three forms: chlorine gas ( $\text{Cl}_2$ ), sodium hypochlorite solution ( $\text{NaOCl}$ ) or dry calcium hypochlorite,  $\text{Ca(OCl)}_2$  [5].

The addition of any of these to water will produce hypochlorous acid ( $\text{HOCl}$ ). This dissociates into hypochlorite ions ( $\text{OCl}^-$ ) to some degree. (The reaction is summarized below).



The ratio of hypochlorous acid and hypochlorite ion in water is dependent upon pH level and, to a much lesser degree, water temperature. The ratio of

hypochlorous acid and hypochlorite ion at various water pH and temperature is shown in Table 1 [6].

It is important to understand the ratio of hypochlorous acid and hypochlorite ion in water. First, it has been estimated that hypochlorous acid is almost 100 times more effective for disinfection than hypochlorite ion. Secondly, activated carbons more readily remove hypochlorous acid compared to the hypochlorite ion [6].

Chlorine concentrations greater than 0.3 ppm in water can be tasted. Activated carbon is very effective in removing free chlorine from water. The removal mechanism employed by activated carbon for dechlorination is not the adsorption phenomena that occur for organic compound removal [7].

Dechlorination involves a chemical reaction of the activated carbon's surface being oxidized by chlorine. There are reactions when hypochlorous acid and hypochlorite ion react with activated carbon (shown below).



C\*O represents the oxidized site of activated carbon after reacting with chlorine; the chlorine has been reduced to chloride ion (Cl<sup>-</sup>). These reactions occur very quickly [7].

**Table (1) Percentages of HOCl and OCl<sup>-</sup> [1]**

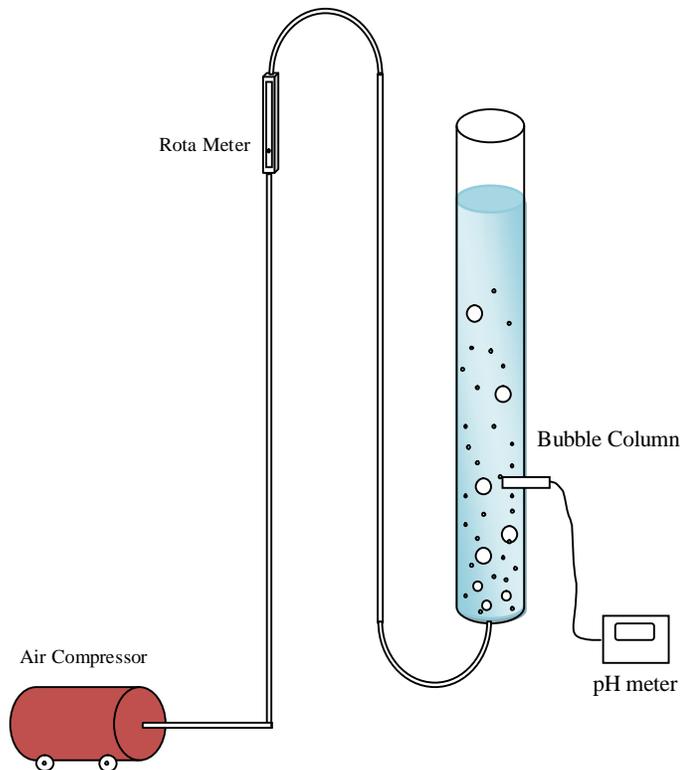
	% HOCl	% OCl <sup>-</sup>	% HOCl	% OCl <sup>-</sup>
pH	0°C	0°C	20°C	20°C
4	100.0	0.0	100.0	0.0
5	100.0	0.0	97.7	2.3
6	98.2	1.8	96.8	3.2
7	83.3	16.7	75.2	24.8
8	32.2	67.8	23.2	76.8
9	4.5	95.5	2.9	97.1
10	0.5	99.5	0.3	99.7
11	0.05	99.95	0.03	99.97

### EXPERIMENTAL WORK

Chlorine removal with activated carbon applied by using transparency Plexiglas bubble column in a 0.08 m I.D and 1.8 m high with liquid level 1.5 m. bubble column equipped with a porous distributor. Air was used as the gas, while taped water used as liquids Figure 1. Taped water with chlorine was aerated at pressures, P = 0.1-0.3 MPa and u<sub>G</sub> 0.016 -0.027 m s<sup>-1</sup> with ambient temperature 18-19 deg. C, the boundaries of the following four regimes were visually identified: bubbly flow, first transition, second transition, and coalesced bubble. As the pressure increases to P = 0.3 MPa in liquid, all four transition velocities shift to higher superficial gas velocity, u<sub>G</sub>. In addition, the existence of a chain bubbling regime was detected, whereas in solid liquid at P = 0.3 MPa and u<sub>G</sub> 0.027 m s<sup>-1</sup>, both laminar and turbulent chain bubbling subregimes were identified. It was found that in solid-liquid under ambient pressure, the transition velocities occur earlier than in liquid.

The pH sensor readings vary from 0 mV for a neutral pH of 7 to about -60 mV for a pH of 8. For the recommended pH of 7.4, the reading is about -25 mV. It should be recognized however that the pH readings vary slightly from electrode to electrode. This is why pH testers and controllers always include a pH calibration adjustment. Calibration can be made with a Phenol Red test kit.

A colorimetric kit supplied by Hach company was used to monitor dechlorination in the field. The kit can measure free or combined chlorine residuals at concentrations of 0 to 4.5 mg/L with a detection limit of 0.1 mg/L. In this method, a pre-measured amount of reagent is added to the water sample of 10 cm<sup>3</sup>, mixed well, and the sample analyzed for chlorine concentration. A liquid crystal detector indicates the chlorine concentration in solution based on the intensity of the color formed.



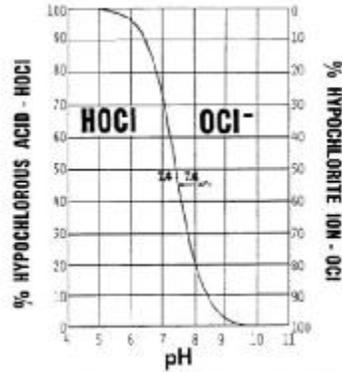
**Figure (1) Bubble column with activated carbon**

## RESULTS AND DISCUSSION

Activated carbon (AC) is generally used in water treatment for removing free chlorine and / or organic compounds. Removal of organics from potable water could be to prevent common organic acids such as humic or fulvic from reacting with chlorine to form trihalomethanes pH represents the acidity or basicity of the water on a logarithmic scale that represent the concentration of hydrogen ions  $H^+$  in the water. pH is very important to water chemistry because the hydrogen ions are small and very active and therefore affect most chemical reactions in water.

Most important, pH affects the dissociation, and therefore the effectiveness, of Hypochlorous Acid HOCl, the most active sanitizer form of Free Chlorine, as shown in curve Figure 2.

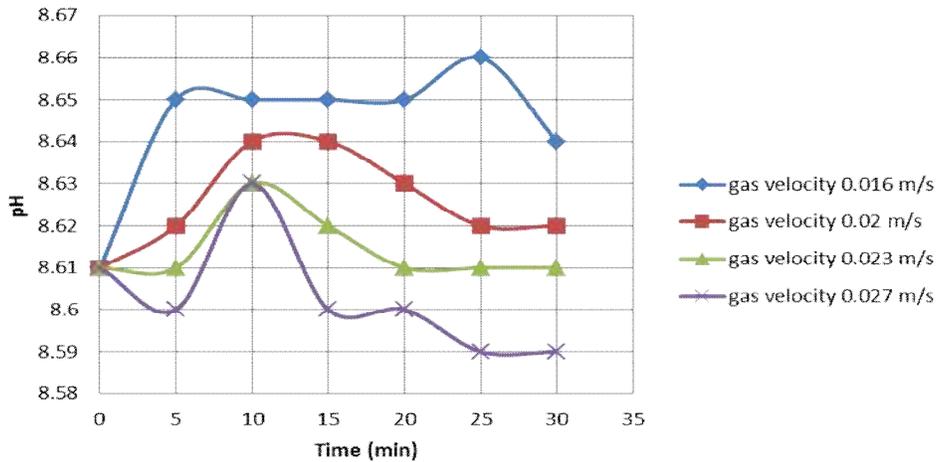
At a pH of 7.5, Free Chlorine is about 50% HOCl . At higher pH values, HOCl dissociates into the ionic form OCl<sup>-</sup> which is a less active sanitizer.



Figure(2) Ionization curve of HOCl as a function of pH [6]

**EFFECT OF GAS VELOCITY**

Figure (3) shows the effect of the gas velocity on the pH with time and zero concentration of activated carbon and Figure (4) shows the effect with presence of activated carbon (5 gm/L)



Figure(3) Effect of the gas velocity on the pH with time and zero concentration of AC.

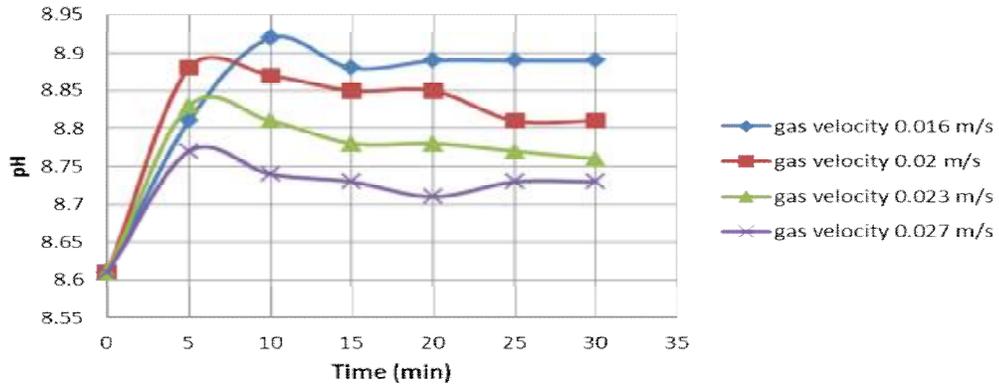
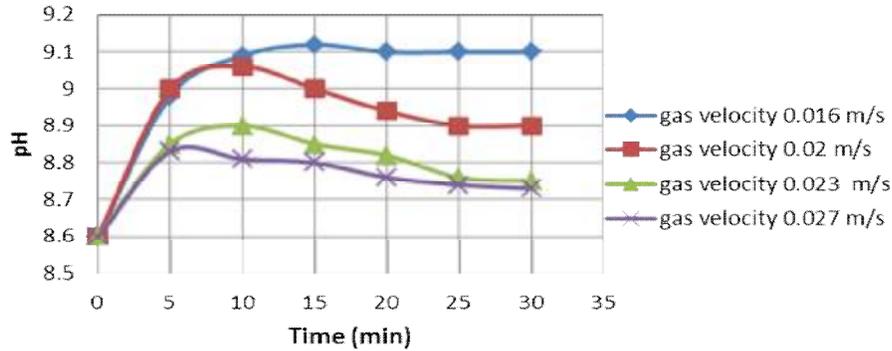
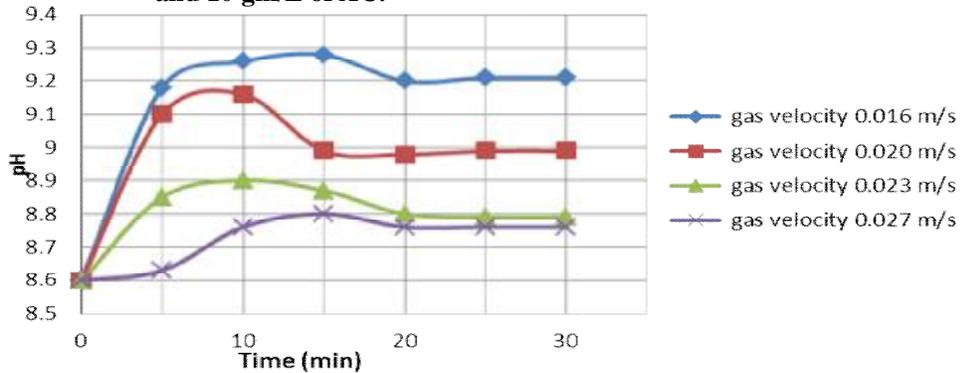


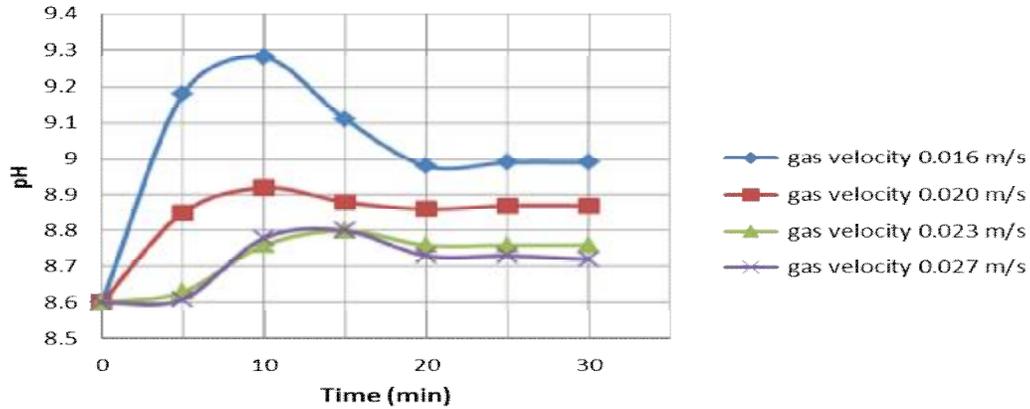
Figure (4) Effect of the gas velocity on the pH with time and 5 gm/L of AC.



Figure(5) Effect of the gas velocity on the pH with time and 10 gm/L of AC.



Figure(6) Effect of the gas velocity on the pH with time and 15 gm/L of AC.



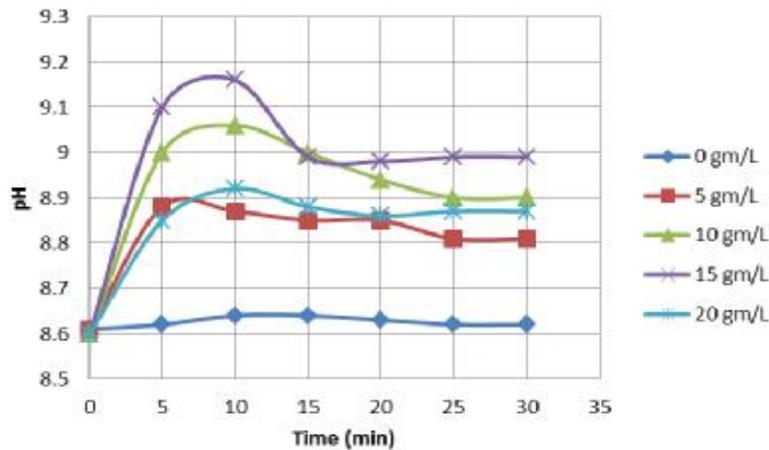
**Figure(7) Effect of the gas velocity on the pH with time and 20 gm/L of AC.**

Figures 3 through 7 showed that increasing then lowering in the pH of the water to an activated carbon bubble column could extend the time before breakthrough of chloramine occurs. Experimental results yielded breakthrough time after approximately 10 minutes then lowering the pH. This may prove valuable in the application of pH reduction to existing equipment.

However, when designing a new system for chloramine removal it may be more appropriate to design the equipment based on reaction kinetics. Because the reactions of monochloramine with activated carbon are overall catalytic ones, design of a carbon reactor using reaction kinetics should allow for a theoretically infinite bed life for the removal of monochloramine. The reaction between dichloramine and activated carbon is not catalytic meaning that eventually the carbon will lose its capacity to remove dichloramine and breakthrough will occur.

#### **EFFECT OF ACTIVATED CARBON CONCENTRATION**

Figure (8) shows the effect of the activated carbon concentration on the pH with time and taking constant selected velocity at 0.020 m/s.



**Figure( 8) Effect of the activated carbon concentration the PH with time at gas velocity 0.020 m/s.**

The pH of the water can increase due to the addition of alkaline (basic) chemicals such as liquid chlorine (Sodium Hypochlorite NaOCl), dry chlorine (Calcium Hypochlorite  $\text{Ca}(\text{OCl})_2$ ) or make-up water. It can decrease with the addition of Chlorine Gas ( $\text{Cl}_2$ ), body perspiration, acid rain or make-up water.

Acids are used to decrease the pH of the water, usually liquid muriatic acid (Hypochloric Acid HCl), dry acid (Sodium Bisulfate  $\text{NaHSO}_4$ ) or Carbon Dioxide  $\text{CO}_2$  gas. Bases like caustic soda (Sodium Hydroxide NaOH) or dry soda are used to raise the pH.

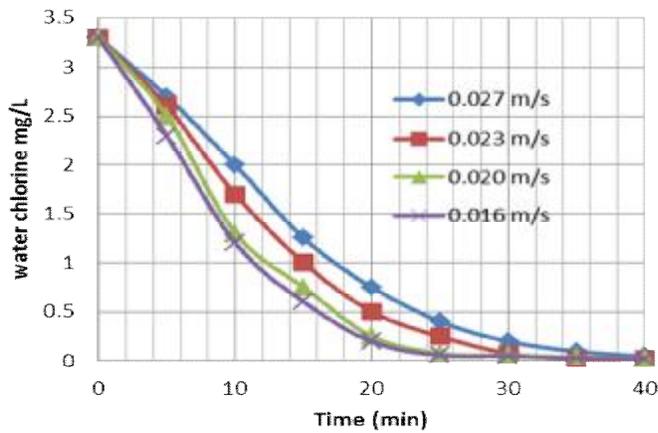
The addition of pH correction chemicals used to be done manually or with a chemical feeder. It is now done on demand with automatic controllers.

A chemical controller monitors the pH of the water with an electronic sensor consisting of a glass pH electrode.

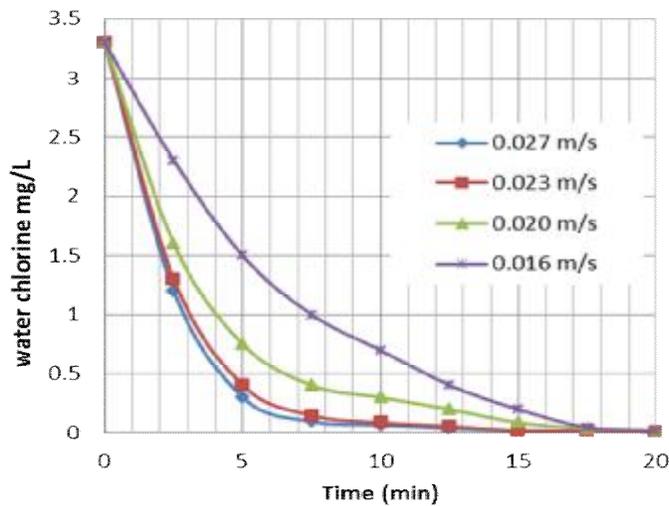
#### CONTENT OF CHLORINE

Figure 9-10 shows the effect of concentration of activated carbon for deferent gas velocities along with time it shows the effect of time and solid concentration of AC more obvious than gas velocity.

Increasing contact time allows greater amounts of contaminant to be removed from the water, contact is improved by increasing the amount of AC in the Bubble column slurry reactor (abbreviated as BCSR) and reducing the flow rate of air through the BCSR.



Figure( 9)Effect of the exposure time on the content of chlorine  
With 5 gm/L of activated carbon.



Figure( 10)Effect of the exposure time on the content of chlorine  
With 20 gm/L of activated carbon.

These Figures 9-10 gives the guidelines of design provide a good starting point for activated carbon concentration 20 gm/L, gas velocity 0.023 m/s and contact period of time more 17 minutes. However, these may not be enough to provide a guarantee in certain situations. Other contaminants, such as organics, in the water may compete for sites on the carbon slowing the reaction kinetics with chloramines.

### CONCLUSIONS

Chlorine Removal: AC catalyzes removal of free chlorine with little consumption or degradation of the carbon during the process. This ability, however, requires tremendous surface area and organics in the water will gradually

adsorb onto the carbon particle, blocking or occupying pores. This leads to gradual loss of dechlorination ability and the need to replace the carbon. Such carbon can be replaced and this is frequently done; however, reprocessed carbon should only be used in waste water applications.

These test results show that changing the pH of a water containing chloramines will speed the reaction kinetics between the chloramine and activated carbon by converting monochloramine to dichloramine. For existing equipment, which may be undersized to treat monochloramine, this would mean better removal with run time after 10 minutes (breakthrough) and gas velocity 0.023 m/s.

Dechlorination occurs very rapidly with AC concentration 20 gm/L than 5 gm/L nearly to half its time required. One advantage of carbon for dechlorination is its low operating cost once installed and virtual "fail safe" operation. A disadvantage, however, is that once the chlorine is removed in the top one inch or so of the media.

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