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EVALUATION OF TOXICITY OF HEAVY METALS FOR *ESCHERICHIA COLI* GROWTH

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ABSTRACT

Iron is essential to virtually all organisms but it can be toxic in excess. High concentration of iron and other trace elements could restricted bacterial growth and modify their metabolic pattern as well. However, this study aimed to find out the influence of iron, chromium, cadmium and synergism or antagonism between these elements on growth of a gram negative bacterium. In the series of experiments, *E. coli* has been cultured in a nutrient broth which supplemented with Fe^{+2}, Fe^{+3}, Cr^{+3}, Cd^{+2} alone or in combination with together, at 37°C for 5 h. Bacterial growth was measured every half an hour using spectrophotometer. Findings obtained from this study indicated that bacterial growth reduced at presence of 1 mM/L concentration of Fe^{+3} and 0.5 mM/L Fe^{+2} in comparison with control. Growth of the bacteria was completely inhibited by 1 mM/L concentration of iron (II). Chromium has also inhibitory effects on growth of the bacteria and cadmium is very toxic. Cr^{+3} and Cd^{+} have antagonism effect with iron on the growth of bacteria. This work suggested that trace elements could interact in their metabolism in bacteria. It has also concluded that toxic effects of trace element could be another view against pathogenic bacteria particularly in complex with antibacterial activity of various antibiotics.

Key words: Iron, chromium, cadmium, *Escherichia coli*, toxicity

INTRODUCTION

Iron is an essential nutrient for living agents due to its noticeable activity in electron transport reactions in biological systems, but its insolubility and reactivity lead to problems of poor availability and toxicity, respectively (Andrews, 1998). Due to insolubility of this element at physiological pH all living agents have involved to use iron transport systems and storage proteins. Bacteria elaborate and secrete high-affinity extracellular ferric chelators (siderophores) and many of them have ferrous iron transporter, to soluble iron prior to transport (Andrews *et al*., 2003, Köster, 2001). They may posses other iron transport systems (Velayudhan, *et al*., 2000, Marlovits *et al*., 2002). Furthermore, it was demonstrated that extracellular iron is not the only source of available iron and many bacteria deposit intracellular reserves of this nutrient within iron storage proteins. These iron stores can then be used to enhance growth when external iron supplies are restricted (Andrews, 1998). However, a relationship between concentration of iron and microbial infection was seen by many investigators using experimental studies on human and laboratory animal. These revealed that pathogens often use low environmental iron levels as a signal for the induction of virulence genes. For example, induction of exotoxins and proteases by many bacteria such as enterohaemorrhagic *E. coli* (Shiga-like toxin I) which affected the bacterial virulence (Litwin and Calderwood, 1993, Calderwood and Mekalanos, 1987). In other hand, high concentration of iron is extremely toxic and may implicate to enhance bactericide effects of antimicrobial agent or noxious substances (Gelvan, 1997, Chamnongpol, 2002).

Cadmium is a heavy metal which widely distributed in the environment. It can induce multiple toxic effects on tissues and also affected immune response to bacterial pathogen (Banjerdkij

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et al., 2004; Simonet *et al*., 1984). Vido and co-workers study's indicated that cadmium increases oxidative stress of *S. cerevisiae* and strains which are deficient in antioxidant defense enzymes have a high sensitivity to cadmium (Vido *et al*., 2001). However, some microorganisms are able to accommodate to growth inhibiting-concentration of cadmium (Tornabene and Edwards, 1972). Also, it may have played an important role in iron metabolism in bacteria.

Chromium is another metal ion which has been established to be biologically significant at all the levels of living organisms. Out of the two stable oxidation states of chromium, -VI and -III, trivalent chromium has been shown to play positive role in controlling carbohydrate and lipid metabolism (Juturu and Komorowski, 2003; Ryan *et al*., 2003). Recently, bacterial growth inhibition and decrease of pathogencity induced by chromium suggested by Yamini *et al*., 2004.

However, various trace elements such as chromium and cadmium may interact with iron metabolism in living organisms (Stohs *et al*., 1995). In other hand, toxic effects of essential and trace element is another view against pathogenic microorganisms particularly in complex with antibacterial activity of various antibiotics as suggested by Sultana *et al*., 2005. Therefore, this work aimed to evaluate toxic effects of iron (Ferric and Ferro), chromium and cadmium on *E. coli* growth. It has also focused on synergism or antagonism effects of these elements on the bacterial growth.

MATERIALS AND METHODS

Preparation of stock solutions

a) Fe^{+2} and Fe^{+3}: 556 mg of $FeSo_4$, $7H_2O$ and 541 mg of $FeCl_3$, $6H_2O$ were solved in 50 mL distilled water and then adjusted to 100 mL to make 20 mM/L of Fe (II) and (III), respectively.

b) Cd^{+2}: 402.8 mg of $CdCl_{2,}$ H_2O were solved in 50 mL distill water and then adjusted to 100 mL to make 20 mM/L of cadmium chloride.

c) Cr^{+3}: 533 mg of $CrCl_{3,}$ $6H_2O$ were solved in 50 mL distill water and then adjusted to 100 mL to make 20 mM/L of chromium chloride.

Preparation of bacterial culture medium

Nutrient agar and nutrient broth (Merk, Germany) were prepared as manufacture recommendation. All culture medium and stock solution of trace elements were sterile with autoclave at 121ºC, 15 pound/in^2 pressure for 15 min. This work was carried out at pH=~7.

Bacteria and culture medium

E. coli (Microbial collection, microbiology laboratory, Isfahan University) was used and cultivated on N.A using streak plate method. The plate was incubated 24h at 37ºC and then stored at 4ºC.

Three colonies of *E. coli* was added to 100 mL N.B and cultivated for 14h at 37ºC before performing main experiments. 1, 5 and 10 mL of iron or chromium stock solution was added to 199, 195 and 190 mL of N.B in order to have 0.1, 0.5 and 1 mM concentrations of these elements. 0.1, 0.5 and 1 mL of cadmium stock solution was added to 199.9, 199.5 and 199 mL of N.B in order to have 0.01, 0.05 and 0.1 mM/L concentrations of cadmium and mixed well. 5 mL of each solution was taken out and used as blank. Then, three mL of the 14h bacterial culture was added to each flask, mixed well and incubated at 37ºC on a shaker. Bacterial growth was measured every half an hour using spectrophotometer (Bausch) at 520 Nanometer (nm).

To examine the synergism or antagonism effects of these elements various concentrations of these elements were used together. For example, 10 mL of iron stock solution and 0.1 mL of cadmium stock solution was added to 189.9 mL N.B to examine a combination of 1 mM/L of iron (II, III) and 0.01 mM/L of cadmium on bacterial growth as described above. Bacterial growth was measured after 3.5h incubation at 37ºC on a shaker using spectrophotometer (Bausch; Spectronic 70) at 520 nm.

Any experiment repeats at least three times and the bacteria were cultured in N.B culture medium without adding any trace elements solution which used as control. Data were analyzed using T-test and T-paired by SPSS software.

RESULTS

Results obtained from cultivation of *E. coli* at presence of various concentrations of iron are shown in Fig. 1. At presence of 0.1 mM/L and

0.5 mM/L concentration of Fe (II and III) bacteria grew approximately as well as control while the bacterial growth decreased to 31.3 % when 1 mM/L concentration of Fe (III) was used (OD 0.5mM/lFe^{+3} = 0.25 Vs ODcontrol=0.8) and they did not grow at presence of 1 mM/L concentration of Fe (II) (OD=0.09) (P<0.001).

In other part of the study bacterial growth was measured after treatment with cadmium chloride and combination of cadmium with iron. The findings demonstrated that bacteria grew as well as control at presence of 0.01 mM/L concentration of cadmium (OD=0.8) but bacterial growth reduced extremely at presence of 0.05 mM/L of this element (OD=0.1). Bacteria did not enter to growth phase at presence of 0.1 mM/L of Cd^{+2} (OD=0.06) (P<0.001) (Fig. 2). Findings obtained from interaction between cadmium and iron showed that inhibitory effect of cadmium on bacterial growth was partially supported by iron (III). For example, bacterial growth was reduced from 12.5% to 6 % at presence of 0.1 mM/L of Fe^{3+} and 0.05 mM/L of Cd^{2+} in comparison with 0.05 mM/L of cadmium alone (OD control=0.8, OD 0.05 mM/L Cd^{2+}=0.1 and OD 0.05 mM/L of Cd^{2+} and 0.1mM/L Fe^{3+}=0.05). In other hand, effects of cadmium in combination with iron (II) on *E. coli* growth were also measured. Results obtained here showed that bacterial growth was increased from 12.5% to 20% at presence of 0.1 mM/L of Fe^{2+} and 0.05 mM/L of Cd^{2+} compared with 0.05 mM/L of cadmium alone (OD control=0.8, OD 0.05mM.lCd^{2+}=0.1 and OD 0.05 mM/L of Cd^{2+} and 0.1 mM/L Fe^{2+}=0.16). It has also demonstrated that bacterial growth was increased to 97% in comparison with control when 1 mM/L of Fe^{2+} and 0.01 mM/L of Cd^{2+} was used (OD control and 0.01 mM/L Cd^{2+}=0.8, and OD 0.01 mM/L of Cd^{2+} and 1 mM/L Fe^{2+}=0.78) (Fig. 2).

Bacterial growth was also measured after treatment with chromium alone or in combination with iron. Results obtained from supplementation of the bacterial culture medium with Cr^{3+} and in combination with Fe^{2+} or Fe^{3+} are shown in Fig. 3. These finding revealed that Cr^{3+} has partially inhibitory effects on growth of the bacteria. Bacterial growth was reduced to 77% and 38% using 0.1 and 0.5 mM/L concentration of chromium, respectively compared with control (OD 0.1mM/L=0.62, OD 0.5 mM/L=0.3 Vs. OD control=0.8) (P<0.001). Results obtained from cultivation of *E. coli* in presence of combination of chromium with iron show that the inhibitory effects of chromium on bacterial growth was supported by iron while toxic effects of iron removed by chromium. For example, growth of the bacteria was reduced approximately from 38% to 8% using 0.1 mM/L Fe^{3+} or Fe^{2+} and 0.5 mM/L Cr^{3+} (OD 0.5mM/L cr=0.3 OD control=0.8O, OD 0.1 mM/L Fe^{3+} or Fe^{2+} and 0.5 mM/L Cr^{3+}= 0.06) (P<0.001). Bacterial growth was increased to 74.3% compared with control when *E. coli* was cultured at presence of combination of 1 mM/L Fe^{2+} and 0.1 mM/L Cr^{3+}(OD 1mM/L Fe^{2+}= 0.09, OD control=0.80, OD 1mM/L Fe^{2+} and 0.1 mM/L Cr^{3+}=0.6) (P<0.001) (Fig. 3).

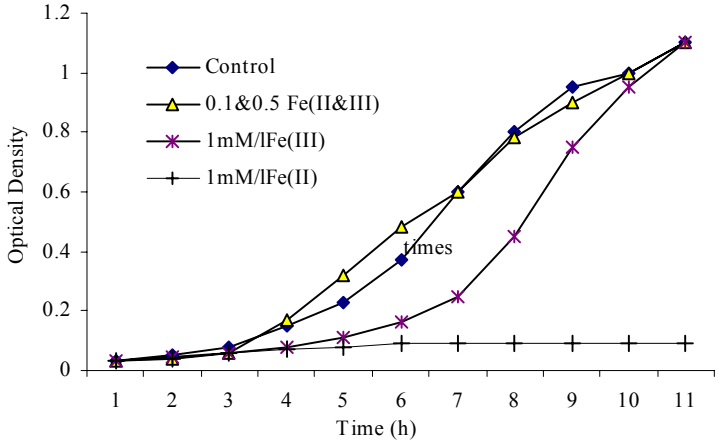
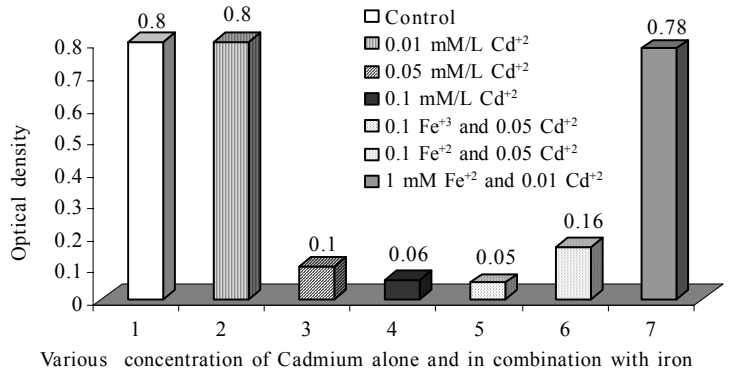
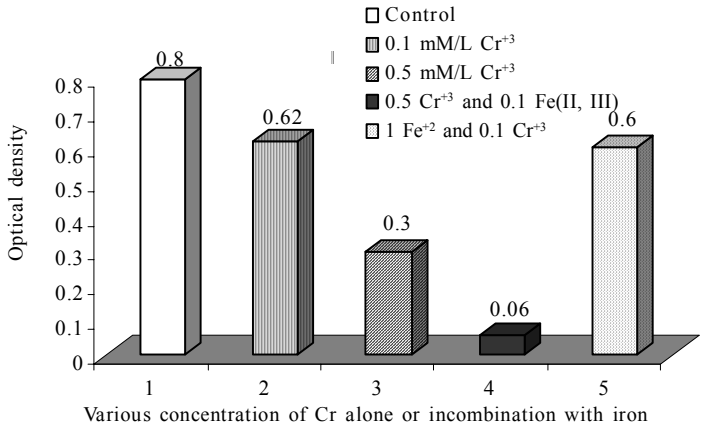


Fig. 1: Study of toxic effects of iron (II) and (III) on *E. coli* growth using 0.1, 0.5 and 1 mM/L of Ferro soleplate and ferric chloride



Columns are: 1. Control; 2. 0.01 mM/L concentration of Cd^{+2}; 3. 0.05 mM/L concentration of Cd^{+2}; 4. 0.1 mM/L concentration of Cd^{+2}; 5. 0.1 mM/L concentration of Fe^{+3} and 0.05 Cd^{+2}; 6. 0.1 mM/L of Fe^{+2} and 0.05 mM/L Cd^{+2} ; 7. 1 mM/L Fe^{+2} and 0.01 mM/L of Cd^{+2} (P<.001)

Fig. 2: Effects of Cd^{+2} and interaction between cadmium with various concentrations of Fe^{+2} and Fe^{+3}, on *E. coli* growth after 3.5 h incubation at 37ºC. Each data point represents the mean of three independent experiments



Columns are: 1. Control; 2. 0.1 mM/L concentration of Cr^{+3}; 3. 0.5 mM/L concentration of Cr^{+3}; 4. 0.5 mM/L concentration of Cr^{+3} + 0.1 mM/L Fe^{+2} and Fe^{+3}; 5. 1 mM/L of Fe^{+2} and 0.1 mM/L of Cr^{+3}. (P<0.001)

Fig. 3: Effects of Cr^{+3} and interaction between chromium with various concentrations of Fe^{+2}, $Fe^{+3,}$ on *E. coli* growth rate after 3.5 h incubation at 37ºC. Each data point represents the mean of three independent experiments

DISCUSSION

Till date very little progress has been made in combating the toxic potential of the microbe through chemical route (Yamini *et al*., 2004). The present work tries to understand the effect of iron, chromium, cadmium and combination of them on *E. coli* growth.

Results obtained here demonstrated that iron has inhibitory effects on bacterial growth at high concentration and Fe^{3+} is also a toxic substance. These findings are in agreement with results obtained from other studies which indicated that Fe (III) is a toxic substance that appears to act on an extracytoplasmic target of gram negative bacteria (Chamnongpol *et al*., 2002, Harrison *et al*., 1992). Another study also showed that increasing concentrations of Fe^{3+} and some other trace elements significantly decreased the surface hydrophobicity of *E. coli* to uroepithelial cells (Saralaya *et al*., 2004). But, the results are not in line with others which have largely been considered that Fe (III) is a non- cytotoxic substance (Braun, 1997, Bruins *et al*., 2000).

Findings obtained from the effects of cadmium on *E. coli* growth suggested that cadmium able to interfere in bacterial metabolism. Our results are

in agreement with results obtained from many studies which indicated that cadmium was very toxic for living agents (Simonet *et al.*, 1984, Laddaga and Silver. 1985, Prozialeck *et al*., 1995). It has also demonstrated that inhibitory effect of cadmium on bacterial growth was partially supported by iron (III). In other hand, effects of cadmium in combination with iron (II) on *E. coli* growth were also showed that the bacterial growth was increased. It has also demonstrated that bacterial growth was extremely increased at presence of toxic amount of iron (II) (1 mm/L) in combination with cadmium (Fig. 2). These results indicating that cadmium is able to extremely remove the inhibitory effects of high concentration of iron (II). However, this work revealed that iron (II) and cadmium have antagonism but it has synergism effects with iron (III) on growth of bacteria. This finding is in agreement with results obtained from Stern and coworkers study's which indicated that the minimal inhibitory concentration (MIC) of cadmium for four *Campylobacter jejuni* strains reduced significantly in the presence of iron (II). Moreover, the numbers of colonies were greater when culture medium supplemented with Fe^{2+} (Stern *et al.*, 1988).

Chromium is a unique transition metal ion, which has been established to be biologically significant at all the levels of living organisms. Thus, in another part of this work, effect of chromium on the bacterial growth was measured. Results obtained showed that Cr^{3+} had partially inhibitory effects on growth of the bacteria. It has also revealed that cultivation of *E. coli* in presence of combination of chromium with the inhibitory effects of chromium on bacterial growth was supported by iron while toxic effects of iron removed by chromium.

It seems that chromium salts act as an iron chelating agent which could precipitate it and thus removed the excess amounts of this elements. This explanation is in agreement with results obtained from a study; demonstrated that chromium could chelating iron in the culture medium and confirm the capacity of a staphylococcus to resist the inhibiting action of transferring (Valenti *et al*., 1980).

In conclusion, this work demonstrated that high level of iron play an important role to inhibit growth of the bacteria and Fe (III) had also inhibitory effect on *E. coli* as a gram negative bacteria. It has also revealed that other trace elements such as chromium and cadmium was toxic and could interact with iron metabolism in bacteria. The toxic effects of the trace elements could support or remove in combination with other elements. Finally, data obtained providing basic data about toxic effects of trace elements against pathogenic bacteria and open another view to apply further studies on mechanism of inhibition or synergy with antibiotics.

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