Anti toxic effect of broccoli extract on stannous dichloride toxicity[1]

Efeito antitóxico do extrato de brócolis na toxicidade do dicloreto de estanho

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ABSTRACT

PURPOSE: Since Technetium-99m (^{99m}Tc) has favorable physical and chemical characteristics, it is widely used radioisotope in Nuclear Medicine. However, stannous dichloride ($SnCl_2$) has been widely used as a reducing agent in labeling procedure of pharmaceutical with radionuclide, it has been realized that $SnCl_2$ have genotoxic and cytotoxic effects on biological systems. In previous studies, it has been shown that some herbal extract can reduce genotoxic and cytotoxic effects of $SnCl_2$. In the present study, it is aimed to evaluate the effect of the broccoli extract on the survival of *E. coli* ATCC 25922 strain against to toxic effects of $SnCl_2$.

METHODS: Broccoli was extracted with methanol extraction. HPLC and TLC analysis of broccoli extract were performed. Then antitoxicity and dose response assays were performed on bacterial strain.

RESULTS: The broccoli extract had dose dependent protective effect against $SnCl_2$ toxic effect on *E. coli*.

CONCLUSIONS: The consumption of broccoli may alter the stannous dichloride toxicity. Broccoli extract may use as a new protective strategies against the toxic effect of $SnCl_2$ on patients who were taken ^{99m}Tc radiopharmaceuticals.

Key words: Broccoli. Stannous dichloride. Technetium-99m. Escherichia coli. Toxicity.

RESUMO

OBJETIVO: Em face de suas características físico-químicas, o Tecnécio-99m (^{99m}Tc) é um radiofármaco amplamente utilizado na Medicina Nuclear. Todavia, o dicloreto de estanho ($SnCl_2$) tem sido largamente aplicado como um agente redutor no procedimento farmacêutico de marcação com radionuclídeos. Constatou-se que o $SnCl_2$ apresenta efeitos genotóxicos e citotóxicos nos sistemas biológicos. Em estudos prévios, foi demonstrado que alguns extratos de ervas podem reduzir tais efeitos. O estudo atual objetivou avaliar os efeitos do extrato de brócolis na sobrevida da cepa *E. coli* ATCC 25922, exposta ao efeito tóxico do $SnCl_2$.

MÉTODOS: O extrato de brócolis foi obtido mediante extração com metanol. Analises com HPLC e TLC foram efetuadas. Avaliou-se a antitoxicidade e realizou-se um ensaio dose-resposta para uma cepa de bactérias.

RESULTADOS: O extrato de brócolis mostrou um efeito protetor dose dependente para os efeitos tóxicos do $SnCl_2$ sobre a *E. coli*.

CONCLUSÕES: O consumo de brócolis pode alterar a toxicidade do dicloreto de estanho O extrato de brócolis pode ser utilizado como uma nova estratégia para proteção de pacientes contra os efeitos tóxicos do $SnCl_2$, nos quais foi administrado o radiofármaco Tecnécio-99m.

Descritores: Brassica. Compostos Estanho. Tecnécio. Escherichia coli. Toxicidade.

Introduction

Technetium-99m radiopharmaceuticals have favorable physical and chemical characteristics[1,2] and widely used in Nuclear Medicine practice[1,3]. Since stannous dichloride ($SnCl_2$) is the most common reducing agent in ^{99m}Tc radiopharmaceuticals preparation, it has been shown that severe biological effects on central nervous system and oral mucosa in animal studies[1,4]. Also it has been reported that $SnCl_2$ had lethal effect on *Escherichia coli* (E. coli) with cytotoxic and genotoxic effects[5-8]. Genotoxic effect of $SnCl_2$ may be directly on deoxyribonucleic acid (DNA) or indirectly by generating free radicals[9].

In environment oxygen and stannous (II) may carry out some reactions and these reactions, leads to mutations in the DNA and it caused death of the cell by the formation of hydrogen peroxide (H_2O_2), as follows[10-12].

Reaction I: $\text{Stannous (II)} + O_2 \longrightarrow \text{Stannous (III)} + O_2^-$

Reaction II: $\text{Stannous (III)} + O_2^- + 2H^+ \longrightarrow \text{Stannous (IV)} + H_2O_2$

Reaction III: $O_2 + \text{Stannous (II)} + 2H^+ \longrightarrow H_2O_2 + \text{Stannous (IV)}$

In recent studies, some authors have also described that genotoxic and cytotoxic effects of $SnCl_2$ can be altered by herbal extract, chemicals and pharmaceutics on *E. coli*[5,6,8-13].

Lima *et al*.[6] demonstrated that cauliflower from *Brassicacea* family was abolished the lethal effect of $SnCl_2$ on the *E. coli* strains.

In our study we used broccoli extract which is widely consumed in Turkey[14]. Broccoli (*Brassica oleracea italica*) from the *Brassicaceae* family is a nutrient source of bioactive components including glucosinolates, flavonoids, minerals and antioxidants[15-17]. In the current study we aimed to evaluate the effect of the broccoli extract on the survival of E. coli ATCC 25922 strains against to toxic effect of $SnCl_2$.

Methods

Broccoli was extracted with methanol extraction. HPLC and TLC analysis of broccoli extract were performed. Then antitoxicity and dose response assays were performed on bacterial strain.

Extraction of broccoli

A similar procedure to that for the extraction of broccoli has been applied as described previously[19]. Broccoli was purchased from local market, dried at room temperature and powdered. The samples (250 mg) were extracted with 5 mL of methanol/water (60:40, v/v) using ultrasonic bath (Ceia P104) for 60 min. at room temperature. The slurry mixture was centrifuged at 2500 rpm for 15 min. The supernatant was collected and 5 mL of methanol/water (60:40, v/v) was added in the remaining parts in the tube. All procedure was repeated 3 times. All the supernatants collected and kept at -20 ^{0}C until the experiments.

High performance liquid chromatography (HPLC)

The procedure used for HPLC for broccoli extract was similar to that reported in other studies[19]. A low pressure gradient HPLC system (LC-10ATvp quaternary pump, SPD-10A/V UV detector, RF-10AXL Fluorescence detector, RAD501 single channel analyzer, a syringe injector equipped with a 1 mL loop and 7-µm VP 250/21 Nucleosil 100-7 C18 column (Macherey - Nagel)) was used for the analytical experiments.

HPLC of Broccoli Extract: Fluorescence was monitored with a model RF-10AXL Shimadzu Fluorescence Detector. The samples were analyzed at a flow rate of 1 mL/min and injection volume was 20 µL. The excitation was monitored at 290 nm and emission at 360 nm. The mobile phase was consisted of two solvents which were water–formic acid (0.33%) (A) and methanol (B). The elution gradient profile used was as 10–17% B in 30 min., 17–40% B in 10 min., 40–100% B in 1 min., 100% B in 5 min. Retention time (R_t) values were given in Figure 1.

Thin layer chromatography (TLC)

Five µL of broccoli extract was applied on ITLC-SG (Merck-5554) plates. As mobile phase, 60% methanol in water was used. Then UV lamp was used to determine location of components in extract. Relative front (R_f) values of the components were calculated.

Antitoxicity assay

Bacterial strain

The *E. coli* ATCC 25922 strain was provided from Adnan Menderes University Science and Technology Research and Development Center (ADUBILTEM Aydin, Turkey) Epidemiology Laboratory and used in all experiments. The bacteria concentration of ~10^8 cfu/mL was monitored spectrophotometrically as optical density of 0.1 at A625 nm (Thermolabsystems Multiscan Spectrum).

Bacterial viability assay

For determining the effect of $SnCl_2$ and extract on bacteria viability, bacteria were resuspended in PBS at 10^8 CFU/

ml and incubated with i. $SnCl_2$ ii. Extract + $SnCl_2$ and iii. Extract (as control group) with the 1, 3, 10, 30, 100 µg concentrations for both $SnCl_2$ and extract for 3h at 35ºC. After the incubation bacteria was washed twice in PBS and resuspended. Samples were diluted appropriately and plated on Mueller Hinton Agar (Merck). Following 24h incubation at 35ºC, the total number of bacteria for each dilution was counted and colony-forming units per milliliter (cfu/mL) were determined. The survival fraction (N/N_0) was calculated as previously described[6].

Dose response of extract

To determine the protective effect of extract; amount of extract was increased 100 µg and 300 µg while $SnCl_2$ concentrations remain stable at 30 µg. Bacterial assay were performed as described above.

Statistical analysis

All experiments were repeated three times and analysis of variance (ANOVA) was used to compare the mean responses among experimental and control groups. Probability values $p<0.05$ were considered as significant.

Results

HPLC and TLC studies of broccoli extract were performed. The relevant chromatogram was given in Figure 1. According to HPLC chromatogram, R_t values of broccoli extract were 3.00, 11.50, 14.50 respectively.

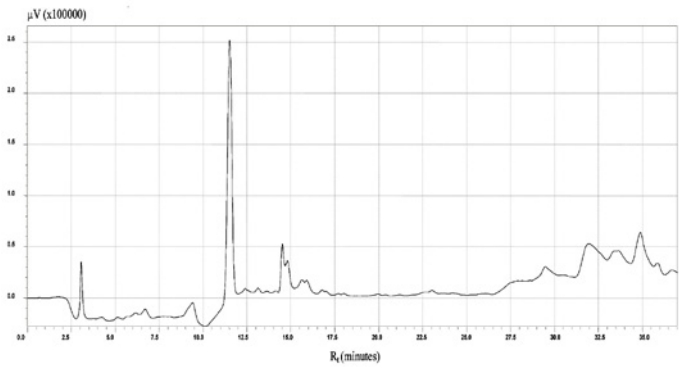

FIGURE 1 - HPLC chromatogram of the broccoli extract.

We have concluded that both of the Rt values were similar (as seen in Figure 1). Table 1 was showed R_f values of broccoli extract by TLC methods.

TABLE 1 - R_f values of broccoli extract by TLC methods.

	Broccoli extract		
Solvent	60% methanol in water		
R_f values	0.34	0.70	0.98

Figure 2 represents survival fractions of bacteria in the presence of broccoli extract. In the control group (Extract), there was no difference in survival fraction values for all concentrations. Survival fraction of *E. coli* was decreased after 3 µg/mL $SnCl_2$ with future dose-dependent decrements at 10 µg/mL as seen in Figure 2.

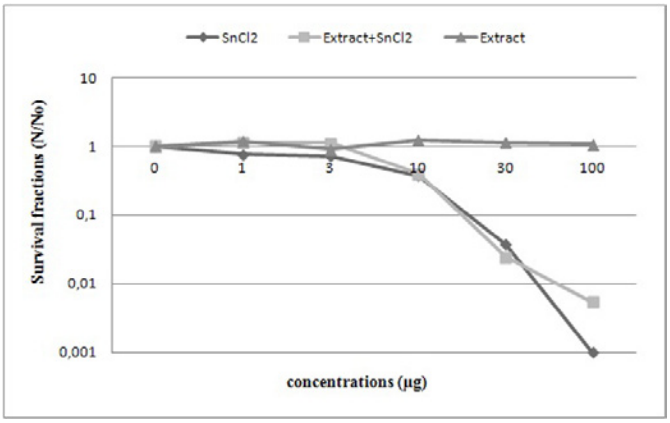

FIGURE 2 - Survival fractions of bacteria in the groups of i. $SnCl_2$, ii. Extract + $SnCl_2$, iii. Extract.

When we compare $SnCl_2$ group with Extract+$SnCL_2$ group, similarly survival fraction was decreased after 3 µg/mL $SnCl_2$. In Extract + $SnCl_2$ group, *E. coli* survival fraction is 0.005 at 100 µg. It was seen, at 100 µg/mL, survival fraction of *E. coli* in Extract + $SnCl_2$ group was more than $SnCl_2$ group. These data suggest that, $SnCl_2$ had toxic effect on *E. coli*. However, in Extract+ $SnCl_2$ group survival fraction was expected increasing after 100 µg/mL.

Also, survival fraction values of all groups were the same up to 3 µg. Survival fraction value of Extract + $SnCl_2$ group (0.005) was higher than group of $SnCl_2$ (0.00) at 100 µg concentration ($p<0.05$). These data thought that extract protect *E. coli* against toxic effect of $SnCl_2$ at high concentrations. So, we decided to do a dose response study for to determine extract protective effect with high concentrations (Figure 3). For this aim, 30 µg $SnCl_2$ + 100 µg extract and 30 µg $SnCl_2$+ 300 µg extract group were prepared addition to 1-1, 3-3, 10-10 and 30-30 µg concentrations were used in bacteria viability assay. As shown in Figure 3, viable bacteria in 30 µg $SnCl_2$ + 300 µg extract group was 10.82 fold ($p=0.001$) high compared to 30 µg $SnCl_2$ + 30 µg extract group.

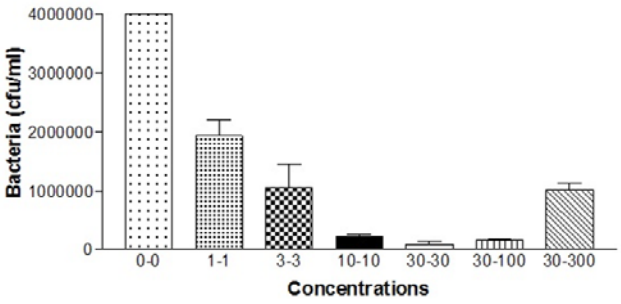

FIGURE 3 - Counts of bacteria at different concentrations of $SnCl_2$+broccoli.

Discussion

Our HPLC results were confirmed with Lin *et al*.[20] LC-MS study results with phenolic components of colloid greens, kale and Chinese broccoli. The results of HPLC and TLC analysis were compatible.

Although its toxic effects were well documented, $SnCl_2$ has been used as ^{99m}Tc reducing agent in majority of ^{99m}Tc radiopharmaceutical kits. In previous studies it was reported that genotoxic effect mediated by the production of reactive oxygen species on pro- and eukaryotic microbial test systems[1,7]. So $SnCl_2$ may have harmful effect on *E. coli*.

In recent studies, it was reported; the genotoxic and cytotoxic effects of $SnCl_2$ can be altered by herbal extract, chemicals and pharmaceutics on *E. coli*[5,8,10-13]. One of these studies was performed by using cauliflower which is in the same family with broccoli. In this study, *E. coli* AB1157 strain was treated with $SnCl_2$ in the presence of cauliflower and it abolished the lethal effect of $SnCl_2$ on the *E. coli* strains[6].

In another study, it was evaluated the influence of the *Cymbopogon citratus*, *Maytenus ilicifoli*a and *Baccharis genistelloides* crude extracts on the survival of *E. coli* AB1157 (wild type) strain. *Maytenus ilicifolia* extract had high protection effect on *E. coli* survival compared to other extracts and this protection was highly related with antioxidant and/or oxidant properties of the extracts[11]. Similarly, the *Ganoderma lucidum* (reishi) extract had protective effect on *E. coli* against the oxidative effect of $SnCl_2$, and the chemical compounds in reishi extract had redox/chelating activity[5]. Although, it has been shown that some other herbal extracts have been eliminated the toxic effects of $SnCl_2$[5,8,9,11], action of mechanisms are not well known yet.

Broccoli has nutritional antioxidants, such as vitamins C and E, but also a great quantity of non-nutritional antioxidants, such as flavonoids, flavones, and other polyphenolic compounds[16,21]. In our study broccoli extract had dose dependent protective effect against $SnCl_2$ toxic effect on *E. coli*. We suggest that consumption of broccoli may alter the $SnCl_2$ toxicity.

Conclusions

The consumption of broccoli may alter the stannous dichloride toxicity. Broccoli extract may use as a new protective strategies against the toxic effect of $SnCl_2$ on patients who were taken ^{99m}Tc radiopharmaceuticals.

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