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EVALUATION OF GENOTOXICITY INDUCED BY SODIUM BENZOATE IN NORMAL CELL LINE OF GINGIVAL FIBROBLAST (HGF) BY COMET ASSAY METHOD

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Abstract

Background and Objective: Sodium benzoate is an artificial compound known as a food preservative, although it has a few other usages. Sodium benzoate is the first preservative approved by the FDA for foods and it is also a food additive. In this regard, the current study aimed to evaluate the genotoxicity induced by sodium benzoate in the normal cell line of gingival fibroblast (HGF) by the comet method.

Methods and Materials: The statistical population of the current study includes both comet investigation and measurement of the Intracellular glutathione (GSH) as well as the Reactive Oxygen Species (ROS) and the normal cell line of Gingival Fibroblast (HGF) in a way that different concentrations of sodium benzoate were applied on each cell line. Also, all the above steps were separately iterated up to three times for evaluation. The comet assay method was used to determine the genotoxicity of sodium benzoate. Also, to assess the rate of change in oxidative stress parameters induced by the sodium benzoate on the normal cell line of gingival fibroblast (HGF), the rates of GSH and ROS were evaluated.

Findings: The results of the assessment of genotoxic effects of sodium benzoate on the HGF cells, these cells were exposed for 1 h to different concentrations of sodium benzoate (10, 50, 100, and 150 µM). The results were indicative of damage to the DNA in the form of three parameters of tail length, %DNA in the tail, and tail moment, compared to the control group. The ANOVA analysis results were significant for all three parameters. The results obtained from the analysis showed a significant increase in the tail length, DNA percentage in the tail, and tail moment in all concentrations, compared to the control group.

Conclusion: The obtained results indicated that sodium benzoate inflicts significant damage to the DNA compared to the control group. Its genotoxicity also depends on the concentration.

Keywords: Sodium benzoate, genotoxicity, gingival fibroblast, comet method.

INTRODUCTION

Sodium benzoate is a sodium salt of benzoic acid and is widely used in cosmetics. In terms of food chemistry, this substance is used as a preservative to prevent the growth of microorganisms. Animal studies show that sodium benzoate can activate the body's inflammatory pathways directly relative to the amount consumed. It includes the expansion of cancer inflammation (Raposa et al., 2016). Sodium benzoate reduces the release of leptin, an appetite-suppressing hormone. It is effective in appetite suppression with a percentage of 49 to 70% (Ciardi et al., 2012). The sodium benzoate increases the oxidative stress that subsequently damages the healthy cells with the creation of free radicals and increases the risk of chronic

diseases (Yetuk et al., 2014). And also, a small percentage of people may have allergic reactions such as itching and swelling after consuming foods or personal care products that contain sodium benzoate (Moyano et al., 1996).

Sodium benzoate has medicinal benefits. In schizophrenia, a daily dose of 1000mg of sodium benzoate along with the standard medicines can reduce the symptoms by 21% compared to consumption of placebo (Lin et al., 2018). And also, it may slow the progress of MS down. This substance stimulates the production of myelin (the sheath of the nerves) (Rezaei et al., 2016). In patients with severe depression, they showed a 64% improvement of the symptoms as a result of sodium benzoate consumption and their brain MRI showed the improvement of brain structure relevant to the depression (Lai, 2013). Despite its potential benefits, sodium benzoate can have side effects such as nausea, vomiting, and abdominal pain (Misel et al., 2013). In addition, consumption of the medicinal doses of sodium benzoate may empty the body from the carnitine amino acid which is very important for energy production. In such conditions, taking a carnitine supplement might be necessary (Van Hove et al., 1995). That is why sodium benzoate is only used as a prescribed drug in carefully controlled doses and under constant monitoring.

Genotoxicity discusses the effects of mutagenicity of chemicals and radiations, and subsequently, the health of people exposed to mutagens (Neeley et al., 2006). There are two main reasons for concerns about human contact with mutagens. First, the increase in the mutagenicity of human sex cells can lead to the rise in genetic disorders in future generations. Second, the mutation in somatic cells can be involved in different disorders such as cancer in people exposed to this substance (Douche et al., 2010). Thus, the current study has investigated the genotoxicity of sodium benzoate on the cancer-derived and normal cell lines (Sarami, S., et. al., 2020; Alotaibi, N. S., et. al., 2021).

The genetic measurements are used for identification of the sex cells mutagens, somatic cells mutagens, and potential carcinogens as well as evaluation of different types of genetic mutations. Many techniques including the Ames test, various in vitro and in vivo tests such as intracellular ROS test, alkaline wash, sister chromatid displacement, micronucleus test, USD test, 8-hydroxy guanosine (8-OHdG) measurement, and Comet method have been developed for evaluation of the ability of chemicals to damage DNA that may cause cancer. These tests are aimed to determine the effects of a substance on the cell's genome or the formation of cancer (Williams, 1989). The Comet assay or the single cell gel electrophoresis (SCGE) is a sensitive and fast technique for the determination and analysis of the damage to the DNA in single cells. This technique is one of the techniques used in the cancer research field to assess the genetic damage and efficacy of the drugs used in chemotherapy protocols. The images obtained from this technique (Comet) are similar to a comet that consists of a separate head and a tail. The head contains healthy DNA when the tail contains damaged DNA (single or double-stranded breaks) or broken pieces of DNA.

In this method, the rate of DNA migration from the core is used for the assessment of the extent of the damage. The measured parameters include tail length, tail moment, and percentage of DNA in the tail. Tail length indicates the length of the tail and in fact, the distance the DNA moves from the core body. The tail moment is tail length multiplied by the percent of the DNA in the tail. The number of intact or broken pieces is also determined by the amount of DNA in the tail (Ticeet et al., 2000). In the current study, the authentic Comet assay has been used for the evaluation of the damage to DNA.

Modi et al., in a study (2015) extensively used sodium benzoate, cinnamon metabolite, and a food preservative in the anti-inflammatory activities and the immune system. The results indicated that sodium benzoate can increase the mRNA and CNTF protein expression in mouse primary astrocytes and human oligodendrocytes and primary astrocytes. Saatci et al. in a study

(2016) showed that the use of sodium benzoate can inflict damage to the DNA and increase the formation of micronucleus. It is recommended to pregnant women avoid consuming foods containing sodium benzoate as a preservative (Saatci et al., 2016). Zengin et al., (2016) investigated the genotoxic effects of sodium and potassium benzoate on the cultured peripheral lymphocytes. The results indicated that sodium benzoate significantly increases the damage to the DNA, however, potassium benzoate did not significantly increase the damage to DNA. Loutsidou et al. (2012) evaluated the toxicity of two food preservatives, sodium nitrate and sodium benzoate on Tetrahymena pyriformis protease based on the changes in DNA content, and the results indicated that stimulation of the mitosis process is associated with stimulation of protein activity.

Yetuk et al. (2014) showed in a study that oxidative stress leads to the creation of free radicals which can damage healthy cells and increase the risk of chronic diseases. Regarding the extensive use of sodium benzoate in foods, a more careful investigation of its genotoxicity in the in vitro and in vivo models is necessary. Therefore, in the current study, the genotoxicity induced by the sodium benzoate in the normal cell line of gingival fibroblast (HGF) with the Comet assay was investigated.

Methods and Materials:

1- Cell Culture and Maintenance:

Ready-made deionized water was received and one liter of it was poured into the autoclavable glass container and it was sterilized 15 atmospheres and 120 °C.

To prepare the PBS (Phosphate Buffered Saline) solution, 2.16 grams of Na_2HPO_4, 0.2 grams of KH_2PO_4, 8 grams of NaCl, and 0.2 grams of KCl were dissolved in deionized water and it reached a volume of one liter. Then, the pH of the solution was adjusted to 7.3-7.6 by the use of concentrated NaOH and HCl and it was autoclaved in an autoclavable glass container for 20 minutes at the pressure of 15 atmospheres and temperature of 120 °C. The obtained solution was kept in the refrigerator (Azarova et al., 2007).

Then, the pre-ready frozen sterile trypsin solution was purchased and divided into 5 ml Eppendorf and stored at -20 °C. To prepare trypan blue solution, 0.4 grams of trypan blue color were dissolved in 100 ml of physiological serum and stored in the screw-capped tubes (Azarova et al., 2007).

To prepare a complete culture medium, 45ml of the RPMI-1640 medium was poured into a 50ml sterile falcon tube and then, 500 microliters of antibiotic (Penicillin/streptomycin) and 500 ml of sterile Fetal Bovine Serum were added to it, and stirring was done. The complete culture medium can be stored in the refrigerator for one month, however, it is better to use it one week after preparation. Besides, all steps of culture medium preparation and the work with the cells should be executed in a completely sterile medium and under the exhaust hood (Azarova et al., 2007).

2- Preparation of the Solutions Needed for Comet Assay:

In addition to the PBS solution, the lysis solution is also needed. To prepare this solution, needed amounts of NaCl, Tris HCl, EDTA, and NaOH powders are dissolved in deionized water. Triton X-100 is a neutral substance and does not change the pH. Triton should be also added after adjusting the pH to 10-10.5. Since the lysis solution can be stored for two weeks without Triton X-100, after dissolving the mentioned substances, the deionized water reached a volume of 990ml. To obtain better quality, half an hour before performing the test, Triton was added to the lysis solution in a precise volume and then it was used (Azarova et al., 2007).

To prepare the buffer tank from the stock solutions, 10 N NaOH and 200mM of EDTA were used. To prepare the 10 N NaOH stock solution, 200g of NaOH was weighed and dissolved in 500ml of deionized water. NaOH strongly absorbs air moisture. Therefore, the lid of the container must be completely closed after each use. to prepare the 200mM EDTA stock solution, 14.8g of EDTA powder is mixed in the

deionized water and it was completely stirred to be dissolved. Then, the solution's pH was adjusted to 10-10.5 and the solution reached a volume of 200ml to be stored in the refrigerator.

3- Cell Culture:

The HGF cell line has been used in the current study. This cell was received from the Pasteur Institute of Iran (IPI) in a flask containing the culture medium. All steps of the cell culture were performed in an aseptic medium and under a laminar hood.

When the HGF cell line fills the bottom of the flask as a single layer, it indicates that it has consumed the nutrients of the culture medium. To prevent cells death during this period, the cell content of a flask should be transferred to several new flasks and their old culture medium should be changed, a process named subculture. After disposing of the old culture medium, and rinsing the cells with PBS, to remove the cells from the flask bottom surface, the trypsin-EDTA solution was used. After removal of the cells, the trypsin effect is neutralized by the new culture medium and after centrifuging, the supernatant was disposed of. The new culture medium was added to the obtained cell deposit, and after uniformity of the resulting cell suspension, it was divided based on the number of available flasks and some fresh culture medium was added to it and placed in a CO2 incubator (Neeley et al., 2006).

To count the cells, the use of hemocytometer method is an accurate and effective method for cell counting by the use of which the amount of the cells in a milliliter of suspension can be calculated (Dhar et al., 2002). To perform the Comet assay, the number of alive cells should be above 90% (Morgan, 2003).

4- Plate Preparation and Proximity of the Cell and the Sample:

For the cells to face proper concentration of sodium benzoate, first, the needed number of cells should be prepared. After preparing the cell suspension containing 25×10^4 cells, 1ml of the cell suspension is poured into each well of the 24-well plate. Different concentrations of sodium benzoate were calculated and added to the wells in a specified volume. The total volume of each well was selected as 2ml. The concentration of the prepared sodium benzoate was 300mM. The molecular weight of sodium benzoate is 144g. The molar mass of sodium benzoate was obtained as follows.

Table 1: Preparation of different concentrations of sodium benzoate

Cell suspension volume	The volume of the prepared sample	The final concentration of the sample
1000mL	1000mL from 20mM concentration	10mM
1000mL	1000mL from 100mM concentration	50mM
1000mL	1000mL from 200mM concentration	100mM
1000mL	1000mL from 300mM concentration	150mM

The prepared plate was placed inside a CO_2 incubator for 1 hour, after writing the specifications down, and after that, each well was separately trypsinized and a concentration of 25×10^4 cells in each milliliter of the culture medium was prepared and used for the Comet assay.

5- Comet Assay:

The main purpose of preparing the slide is to obtain a uniform and stable gel until the end of

the process and a visible Comet with the least background parasites. The slides were prepared one day before the experiment. 500mg of NMA was weighed and dissolved in 50ml of PBS solution. The resultant solution is placed inside the microwave for 1 minute to become clear. For the preparation of the slides, cleaned slides were soaked in the 1% warm and freshly prepared solution, and then, they were removed horizontally and slowly to be put on a flat surface at room temperature. After drying the slides, the surface beneath each slide was cleaned and free of any agars. These slides are stored away from moisture and at room temperature. A high concentration of agars restricts the DNA's power and migration rate. Thus, in this stage, high precision is needed for the preparation of the slide with a uniform gel and proper concentration. The slides can be stored for weeks. They are marked after drying. In the current study, 3 slides are prepared for each sample (Oren et al., 2010).

To prepare the cell suspension, 0.1g of LMA powder was weighed and it reached a volume of 10ml mixed in PBS solution. The resultant solution is the LMA with a 1% concentration which was placed inside the microwave for 1 minute until it became clear.

The cell suspension in each falcon is pipetted and 1ml of 1% concentration LMA solution with a temperature of 37 ◦C is added to each falcon, and then, 100mL of the falcons' contents are added to pre-prepared and numbered slides.

In the slide preparation stage, the positive control was applied for the determination of the concentration inducing the genotoxicity. In this stage, except for the positive control slide, the other slides were stored in a dark and cool environment. 400mL of the 200mM H_2O_2 solution was added to two positive control slides by a sampler, and it was stored in a refrigerator for 20 minutes. After taking it out of the refrigerator, the positive control slides were rinsed three times with deionized water, each time for 15 minutes (Morgan, 2003).

All slides were placed in the cold and freshly prepared lysis solution for 40 minutes and then were transferred to the refrigerator. After the lysis stage, to remove the salt sedation, detergent, and additional materials from the slide's surface, they were rinsed with cold deionized water three times, each time for 15 minutes.

The slides should be placed inside an alkaline electrophoresis buffer before performing the electrophoresis so that the single-strand DNA is created and the alkali-labile sites (ALS) are expressed in the Single-Stranded Breaks (SSB). In the current study, the slides were stored in the buffer tank for 40 minutes in the refrigerator.

To neutralize the slides (due to the alkalinity of the electrophoresis medium), after performing the electrophoresis, the neutralizing buffer was used. This buffer contains 0.4M Tris with a pH of 7.5. In this stage, the slides were placed inside the neutralizing buffer for 10 minutes until the alkaline pH induced by the buffer tank is neutralized.

6- Measurement of the Intracellular Glutathione Levels (GSH):

1.5mL of TCA and EDTA (10%) were added to the falcon tune containing the normal cell line of gingival fibroblast (HGF) which were treated with different concentrations of Sodium Benzoate in the form of pre-treatment for protein deposition. In the next stage, the samples were centrifuged for 15 minutes in the XG 3500. Then, 1mL of the supernatant is removed and 2.5mL of Tris buffer with the pH=8.9 was added to it, and in the next stage, 0.5mL of DTNB (40%) was added to it and incubated for 15 minutes until the reaction was completed. The tube was then shaken well until a uniform yellow color could be seen. Finally, absorption was measured by the spectrophotometry device at 412 nm (Azari et al., 219).

7- Measurement of the amount of Reactive Oxygen Species (ROS):

To evaluate the amount of the reactive oxygen species (ROS), the normal cell line of gingival

fibroblast (HGF), and sodium benzoate, the treatment was following process:

- The DCFHH-DA stock solution was prepared in the DMSO in a 10mM form to be stored at a temperature of 4 centigrade.
- The lysis solution is used like the one used in the comet assay.
- The incubated cells with the intended drugs are rinsed with PBS twice, based on the time protocol.
- The working solution DCFH-DA with the final concentration of 100mM is prepared from the primary stock solution.
- The working solution DCFH-DA with the final concentration of 100mM is added to the cells and incubated for 1 hour at a temperature of 37 centigrade.
- The DCFH-DA is removed from the medium and then the cells are rinsed two more times.
- The cold lysis solution is added to the cells and after 1 minute, the contents of the wells are collected and centrifuged in 2800g for 5 minutes.
- In the following, 200 microliters of the supernatant are added to each well in the 96-well plate.
- Fluorescence is measured by microplate reader as Emission: 530nm and Excitation: 485nm.
- In all stages, the cells should be in a dark place and aluminum foil should be used.
- In this test also, negative control of cells incubated with culture medium alone and positive control of 0.1mM H_2O_2, were considered (Ghassemi-Barghi et al., 2016).

The results have been reported based on the (SEM±Mean) obtained from three iterations of the experiment, and the statistical analyses have been done by the PRISM III. The statistical tests including the one-way ANOVA test were done by Post-test: Tukey and the chart are also drawn with the same graphics program. The significance level is defined as $p<0.05$ (Ghassemi-Barghi et al., 2016).

Findings:

Using the Comet assay, first, the concentration inducing proper genotoxicity of sodium benzoate in the normal cell line of gingival fibroblast (HGF) was determined. Then, different concentrations of sodium benzoate were tested for the determination of the protection rate. The negative control in all experiments was the cells incubated with the complete culture medium without any additional materials. The positive control was cells incubated with the intended concentration of sodium benzoate which, in the DNA protection studies, the comparison of the treatment was done with it.

1- Investigation of the Sodium Benzoate Genotoxic Effects on the Normal Cell Line of Gingival Fibroblast (HGF):

To evaluate the genotoxic effects of sodium benzoate, the HGF cells were exposed to different concentrations of sodium benzoate (10, 50, 100, and 150 µM). The charts (2, 3, and 4) are indicative of the damage to the DNA in the form of three parameters of tail length, DNA percentage in the tail, and tail moment compared to the control group. The one-way ANOVA analysis results were significant for all three concentrations. According to the results obtained from the analysis, all concentrations showed a significant increase in the tail length, percentage of DNA in the tail, and tail moment compared to the control group. (*), (**), (***), and (****) are indicative of the significant difference with the control group ($p<0.05$, $p<0.01$, $p<0.001$, and $p<0.0001$).

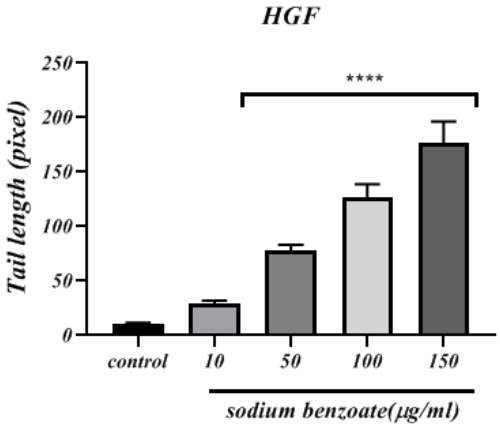

Chart 2: Comparison of the tail length obtained from the Comet assay test of different sodium benzoate concentrations in the HGF cell culture medium

In this chart, the tail length of different concentrations of sodium benzoate compared to the control group is shown negative. The results in the form of Mean ± SD obtained from three iterations are represented. (****) is indicative of the significant difference with the control group ($p<0.0001$). By control group, we mean the cells that are incubated for 24 hours in the complete culture medium without any additional materials. The tail length parameter in all three concentrations of sodium benzoate (50, 100, and 150 µM) in the cell line of HGF have been significantly increased which is indicative of the damage to the DNA.

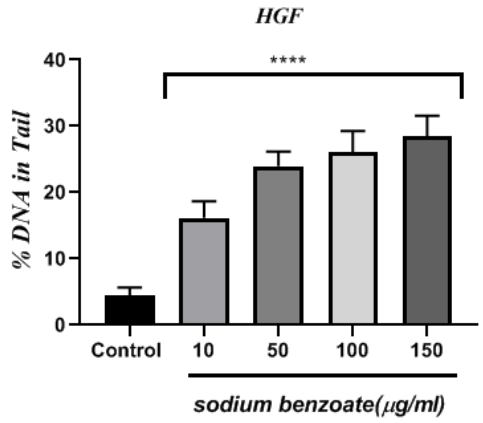

Chart 3: Comparison of the percentage of DNA in tail obtained from the Comet assay test of different sodium benzoate concentrations in the HGF cell culture medium

In this chart, the rate of DNA migration in different concentrations of sodium benzoate is shown as compared to the control group. The results are reported in the form of Mean ± SD obtained from three iterations. (****) is indicative of the significant difference with the control group ($p<0.0001$). By control group, we mean the cells that are incubated for 24 hours in the complete culture medium without any additional materials. The percentage of DNA in the tail parameter in all four concentrations of sodium benzoate (10, 50, 100, and 150 µM) in the cell line of HGF has been significantly increased which is indicative of the damage to the DNA.

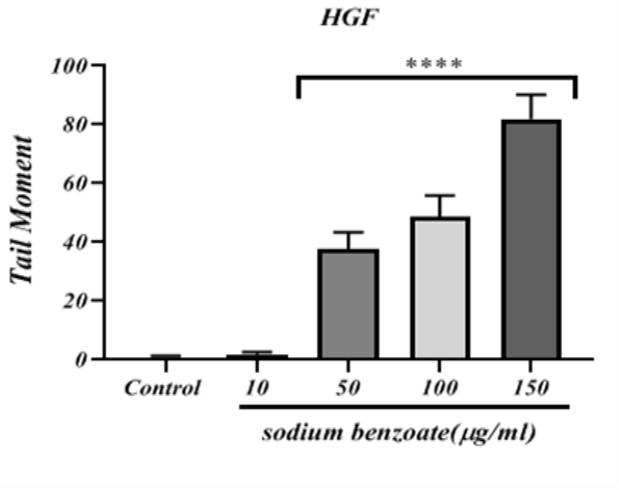

Chart 4: Comparison of the tail moment obtained from the Comet assay test of different sodium benzoate concentrations in the HGF cell culture medium

In this chart, the rate of the tail moment of different concentrations of sodium benzoate is shown as compared to the control group. The results are reported in the form of Mean ± SD obtained from three iterations. (****) is indicative of the significant difference with the control group ($p<0.0001$). By control group, we mean the cells that are incubated for 24 hours in the complete culture medium without any

additional materials. The tail moment parameter in all three concentrations of sodium benzoate (50, 100, and 150 µM) in the cell line of HGF have been significantly increased which is indicative of the damage to the DNA.

2- Measurement of the Sodium Benzoate Induced Intracellular Reduced Glutathione in the HGF Cell Line:

To measure the effects of sodium benzoate on the reduced glutathione, the HGF cells were exposed to different concentrations of sodium benzoate (50, 100, and 150 µM) for 1 hour. Chart (5) shows the mean values of reduced glutathione obtained from fluorimetry at 480-520 nm in different concentrations of sodium benzoate compared to the control group. By control group, we mean the cells that are incubated for 1 hour only in the complete culture medium. The one-way ANOVA analysis results were significant for all three concentrations (50, 100, and 150 µM). according to the results obtained from the analysis, in these three concentrations, a significant reduction in the reduced glutathione was observed, compared to the control group. The results are shown in the form of Mean ± SEM obtained from three iterations. (****) is indicative of a significant difference with the control group ($p<0.0001$).

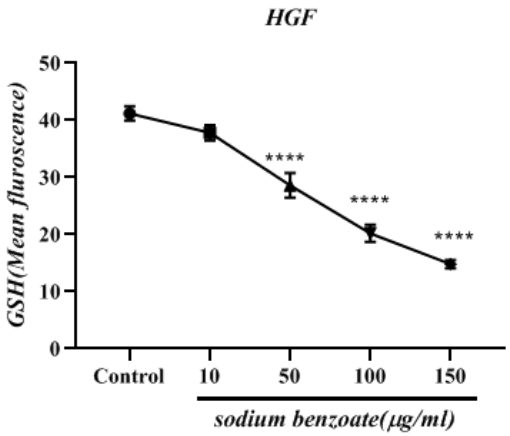

Chart 5: determination of the effects of different concentrations of sodium benzoate on the amount of intracellular reductive glutathione obtained from the comet assay in HGF cells culture medium

3- Measurement of the Amount of Sodium Benzoate Induced Reactive Oxygen Species Obtained from the Comet Assay in HGF Cells culture medium:

To measure the effects of sodium benzoate on the number of reactive oxygen species (ROS), the HGF cells were exposed to different concentrations of sodium benzoate (10, 50, 100, and 150 µM) for 1 hour. Chart (6) shows the mean values of ROS obtained from fluorimetry at 480-520 nm in different concentrations of sodium benzoate compared to the control group. By control group, we mean the cells that are incubated for 1 hour only in the complete culture medium. The one-way ANOVA analysis results were significant for all three concentrations (50, 100, and 150 µM). according to the results obtained from the analysis, in these three concentrations, a significant increase in the reactive oxygen species (ROS) was observed, compared to the control group. The results are shown in the form of Mean ± SEM obtained from three iterations. (****) is indicative of a significant difference with the control group ($p<0.0001$).

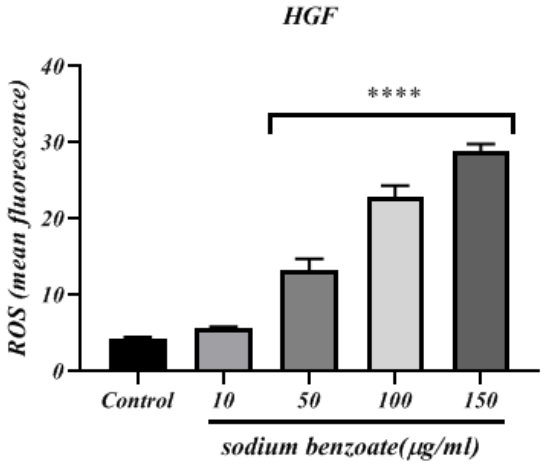

Chart 6: determination of the amount of Reactive Oxygen Species (ROS) induced by sodium benzoate obtained from the Comet assay in HGF cells culture medium

Discussion:

Sodium benzoate is used in cosmetics and food chemistry. It is also useful in medicine. This substance is used as a therapeutic agent in patients born with acute hyperemia due to urea cycle disorder. In addition, it can be used as an inhibitor of the enzyme diamino oxidase. Therefore, the current study aimed to investigate the genotoxicity of sodium benzoate in the normal cell line of the gingival fibroblast (HGF) by the use of Comet assay.

In the current study, the result of the assessment of sodium benzoate genotoxic effects on the HGF cells which were exposed to different concentrations of sodium benzoate (10, 50, 100, and 150 µM) for one hour indicated that the DNA is damaged in three parameters of tail length, percentage of DNA in the tail, and tail moment compared to the control group. The one-way ANOVA analysis results were significant for all three parameters. According to the results obtained from the analysis, all concentrations showed a significant increase in the tail length, percentage of DNA in the tail, and tail moment as compared to the control group.

In the assessment of the amount of Reactive Oxygen Species (ROS) compared to the control group, the one-way ANOVA analysis results were significant for all three concentrations (50, 100, and 150 µM). According to the results obtained from the analysis, in these three concentrations, a significant increase in the amount of ROS was observed, compared to the control group.

In the assessment of the reduced glutathione, compared to the control group, the one-way ANOVA analysis results were significant for all three concentrations of 50, 100, and 150 µM. According to the results obtained from the analysis, in these three concentrations, a significant decrease in the amount of reduced glutathione was observed, compared to the control group.

The results obtained indicated that sodium benzoate causes significant damage to the DNA compared to the control group. Also, the genotoxicity of sodium benzoate depends on the concentration. according to the results, the sodium benzoate in three concentrations of 50, 100, and 150 µM led to a significant increase in the ROS amount of normal cell line of gingival fibroblast (HGF) compared to the control group. Also, in the concentrations of 50, 100, and 150 µM, it led to a significant reduction in reduced glutathione levels in the normal cell line of gingival fibroblast (HGF) compared to the control group.

In the previous studies, sodium benzoate has caused severe toxicity in zebrafish larvae and this toxicity is due to the creation of genetic mutations and the change in enzymatic pathways (Tsay et al., 2007). The results of the current study are in line with the results of Tsay et al.'s study and sodium benzoate leads to the creation of toxicity in the normal cell line of gingival fibroblast cells. In a study on five patients with neonatal nonketotic hyperglycemia treated with sodium benzoate to balance plasma glycine levels, it was revealed that this treatment led to a decrease in seizures and a dramatic increase in wakefulness. High doses of sodium benzoate led to the improvement in life quality of the patients (Van Hove et al., 1995). The results of this study are not in line with those of the current study.

Conclusion:

Sodium benzoate is toxic for normal cell lines of gingival fibroblast (HGF). The results indicated that sodium benzoate leads to severe damage to DNA, compared to the control group. The genotoxicity of sodium benzoate depends on its concentration and higher concentrations than the control group lead to the increased reactive oxygen species (ROS) in the normal cell line of gingival fibroblast (HGF). Also, it leads to a reduction in reduced glutathione level in the liver's cancer cell line (HepG2) and normal cell line of gingival fibroblast (HGF), compared to the control group.

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