

Original Research Article

Stomach cancer and parabens

ABSTRACT:

Background and Objectives: Parabens is one of the chemicals widely used in preserving foods and pharmaceutical preparations, its action ~~that~~ mimics estrogen in the body when it is linked to its receptors, ~~and as~~ known ~~as~~ estrogen receptors ~~which~~ are present in many systems of the body, such ~~as~~ digestive system and breast, therefore it may have a role in cancer incitation in those systems.

The purpose of this ~~manuscript article~~ is ~~to investigating~~investigate the presence of methyl, ethyl and propyl paraben in stomach cancer in men and women.

Methods: Parabens was extracted from stomach cancer samples in Al Assad University Hospital and then analyzed by HPLC/MS in science faculty/Damascus university.

Results: All samples have the three types of parabens with total mean concentration ~~is of~~ (22.5±0.4 ng/g).

The concentration of methylparaben was the highest (8.2 ± 0.3 ng/g) then propylparaben (7.4 ± 0.4 ng /g) and finally ethyl paraben (6.9 ± 0.2 ng /g).

Conclusion: more research about parabens must be done in this ~~field~~.

Comment [KA1]: The conclusion section of the abstract needs to be improved. The authors should tell us the impact of their findings.

Keywords: methyl paraben, ethyl paraben, propylparaben, stomach, cancer.

1.INTRODUCTION:

There is no doubt that food and drink (especially processed foods) that a person eats have a major role to the occurrence of many diseases, foremost of which is cancer.

According to Global Cancer Statistics 2018:[1]

Stomach cancer is more prevalent in men compared to women, and this percentage increases with age, as it is in general the third most common cause of death from cancer.

In men stomach cancer is the fourth most common type of cancer which forms 7.8% of cancer cases in the world, and is considered to be the forefront of cancer diseases leading to death in men. It is also the fifth most common type of cancer in women, with 4.3%.

Comment [KA2]: Reference?

Stomach cancer is characterized by:

It grows slowly as the symptoms often appear only at a later stage, which contributes to poor prognosis, and therefore is often diagnosed in advanced stages of the disease.

Studies indicate that there is a relationship between the type of foods that a person eats and possibility of developing these types of malignant tumors.

Parabens is one of the chemicals widely used in preserving foods, oils, sweets and chips, as well as it is the most used in pharmaceutical preparations, as it works to prevent reproduction and activity of (bacteria, fungi and yeasts), and has a strong effectiveness within a wide PH range.

70-100% of parabens which is taken up orally are absorbed [2].

Although parabens are rapidly absorbed, metabolized and excreted, only 2% of the amount received via the digestive pathway remains in the cells and is not excreted outside the body, it is believed that the presence of non-metabolized parabens is what causes toxic effects in the body [2],[3].

For paraben which is taken orally:

71- 83% is excreted through the urine.

4% excreted through feces.

While 2% of it remains in the cells.

Therefore, excretion all kinds of parabens, methyl, ethyl, propyl, without metabolism in urine, is evidence that parabens taken from a digestive source are not completely metabolized to B-hydroxybenzoic acid (PHBA).

It is believed that the presence of parabens in the body may lead to a cumulative action that stimulates the abnormal proliferation of cells due to their high ability to penetrate into the fat cells, especially since parabens have little dissolution in water and high in fat, as this degradation increases with increasing length of the side chain of the ester.

So, entering parabens to the body may lead to increase its accumulation in the fatty tissues as a manner similar to those lipophilic pollutants known as cumulative action [4].

Parabens is characterized by an action that mimics estrogen in the body when it is linked to its receptors, and since estrogen receptors are present in many systems of the body, such as the digestive system and breast, therefore it may have a role in cancer incitation in those systems.

Estrogen plays a pivotal role in many vital functions in both genital and non-genital organs, as it is able to make cell modifications when it binds to its own receptors.

Estrogen hormones are found in both women and men. The estrogen receptors (ERs) are divided into two types:(ER- α and ER- β)

ER- β is a type present with high concentrations in non-estrogen-dependent tissues, including the stomach [5].

Thus, estrogen is important in the functions of a variety of systems, and it mediates mostly through its receptors.

The presence of ER- β in the mucous membrane of the digestive system, especially the stomach, enhances its functional role in the proposed proliferation of cancerous cells.

Although there is a lot of controversy and diversity between the results related to the estrogen hormone in the development of stomach cancer, many recent studies indicate that estrogen stimulates the growth of gastric cancer cells [6].

Studies have been conducted to investigate the roles that estrogen and its receptors play in stomach cancer, especially since the incidence of stomach cancer is much higher in men than in women. Epidemiological studies indicate the ratio of gastric cancer prevalence in male to female over the world is 2:1.

Qualitative studies have shown a documented presence of (ER- α) and (ER- β) at the cellular and tissue levels of stomach cancer. These results confirm that these cancerous tissues have E2 binding sites, thus, they have the same biochemical properties as in breast cancer but its effect on disease progression remains unclear [7], [8].

In this paper, we investigated the presence of parabens in stomach cancer samples in men and women.

2. MATERIALS AND METHODS:

The samples of stomach cancer were collected immediately after surgery in Al Assad University Hospital -Damascus and stored in (-80° C), then after parabens extraction from the samples, they had been analyzed by HPLC/MS in science faculty lab -Damascus university, Damascus, Syria

1.2. Reagents and solutions used in analysis:[4],[9]

Ethanol, acetone and methanol (PRS Panreac, Spain)

Standard chemicals: methylparaben, ethyl paraben and propyl paraben (Roth, Germany). Sampling analysis: HPLC / MS (SHIMADZU LC MS-2020)

Mobile phase: ammonium acetate (mM 15, PH = 4.5) in pump A, acetonitrile in pump B, (t=0min A90%, t=15 A40%, t=16 A90%, t=25 next injection), column type C18, 150mm, wavelength 254 nm.

Table1. Parabens standers: HPLC retention times

Stander: 1 μ g/ml	HPLC Retention time(min)
Methyl paraben	8.3
Ethyl paraben	10
Propel paraben	11.7

2.2. Parabens extraction from studied samples:

Sample (0.5 g) is cut well by sterile razor blade and homogenize with (6.25 ml Ethanol + 6.25 ml acetone). This mixture is left all night with periodic shaking.

The next day, the mixture is placed in a bench centrifuge at 2500 rpm for 10 min at room temperature, to separate the phases. The floating layer containing parabens is taken and placed in a clean tube while the remaining tumor mass is re-extracted by adding (1.5 ethanol+ 1.5 ml acetone) and placed in a bench centrifuge at 2500 rpm for 10 min at room temperature. The floating layer which containing parabens is placed in the previous clean tube. The two upper layers dried to get a spot of dry oily extract containing parabens.

The dry oily residue was mixed with 6 ml of 70% aqueous methanol and incubate all night at (-20°C). At the next day we separate the phases by putting the mix in a bench centrifuge at 3200 rpm for 20 min at

Comment [KA3]: Ethical clearance? The authors failed to provide ethical clearance approving the study.

(4°C), the floating layer which contain paraben is placed in a clean tube. The bottom phase of the oily dry residue is re-extracted again by adding 1 mL of 70% aqueous methanol and put in a bench centrifuge at 3200 rpm for 20 min at (4 °C), the separated floating layer which containing parabens was added to the pervious floating layer and the two layers were dried in the open air.

The dry samples which containing parabens are taken, dissolved in methanol and analyzed by HPLC / MS.

3.RESULTS AND DISCUSSION:

Samples were divided into 6 groups, each group had 2 samples and one blank extraction.

The clinical story of patients as gender, age and tumor degree is shown in table 1.

- Men ages were between (55-65) years old.
- women ages were between (56-60) years old.

Table2. The clinical story of stomach cancer patients

Sample	Male/Female	Age	Tumor degree
1	F	59	III
2	F	56	III
3	M	55	III
4	M	65	III
5	M	60	III
6	M	58	III
7	F	59	III
8	M	63	III
9	F	60	III
10	M	61	III
11	M	57	III
12	F	58	III

Table 3 show the distribution of stomach cancer which is 58% in men and 42% in women.

Table 3. Stomach cancer distribution between men and women

sample	male	female
Total of samples=12	7	5
percentage of samples	58%	42%

Table 3 show the concentration of parabens ng/g in tumor and blank extraction in each group and the mean of parabens concentration is shown in table 4. the Confidence limits of the mean concentration is shown in table 5.

Table 4. Concentration of parabens ng/g from (tumor and blank extraction)

sample	MP tumor	EP tumor	PP tumor	MP blank	EP blank	PP blank	MP tumor minus blank	EP tumor minus blank	PP tumor minus blank
1	7.8	7.4	7.6	0	1.1	0.7	7.8	6.3	6.9
2	9.2	8.1	7.5	0	1.1	0.7	9.2	7.0	6.8
3	8.5	7.4	7.7	0.2	0.4	0.3	8.3	7.0	7.4
4	7.7	7.5	8.8	0.2	0.4	0.3	7.5	7.1	8.5
5	8.1	7.0	6.0	0.5	0.3	0.2	7.6	6.7	5.8
6	7.4	7.5	9.6	0.5	0.3	0.2	6.9	7.2	9.4
7	7.1	7.3	6.1	1	0.6	0.5	6.1	6.7	5.6
8	10.1	6.7	6.9	1	0.6	0.5	9.1	6.1	6.4
9	8.9	8	7.9	0.4	0	1.2	8.5	8.0	6.7
10	9.2	6.3	8.1	0.4	0	1.2	8.8	6.3	6.9
11	8.7	9.5	8.9	0.3	0.9	0	8.4	8.6	8.9
12	10.9	6.5	9.1	0.3	0.9	0	10.6	5.6	9.1
MEAN	8.6	7.4	7.8	0.4	0.6	0.5	8.2	6.9	7.4
SEM	0.3	0.2	0.3	0.1	0.1	0.1	0.3	0.2	0.4
SD	1.1	0.9	1.1	0.3	0.4	0.4	1.2	0.8	1.3

Table 5. mean concentration of parabens ng/g in stomach cancer

Mean of parabens	MP	EP	PP	Mean of total parabens
Stomach cancer	8.2	6.9	7.4	22.5
SEM	0.3	0.2	0.4	0.4

Table 6 Confidence limits of mean concentration of parabens ng/g in stomach cancer

Tumor minus blank	Mean	Confidence Limit
MP	8.2	7.5-8.9(95%)
EP	6.9	6.5-7.3(95%)

PP	7.4	6.5-8.2(95%)
Total parabens	22.5	21.6-23.4(95%)

Table 7. show the highest concentration of methyl, ethyl and propyl paraben in the samples of stomach cancer.

Table 7. The high concentration of parabens ng/g in stomach cancer

Parabens highest concentration	Stomach cancer
MP	10.6
EP	8.6
PP	9.4

4.CONCLUSIONS:

- ❖ All studied samples have the three types of parabens. The total mean concentration is (22.5±0.4 ng/g).
- ❖ The average old of men was 60 and women was 58 year.
- ❖ All tumors were stage III.
- ❖ The incidence rate of stomach cancer in men is higher than women.
- ❖ The concentration of methylparaben was the highest in the studied samples (8.2 ± 0.3 ng/g) followed by propylparaben (7.4 ± 0.4 ng /g) and finally ethyl paraben (6.9 ± 0.2 ng /g).
- ❖ More research about parabens must be done in this field.

Comment [KA4]: List of abbreviated words should be included

Comment [KA5]: What research needed? The authors should be specific.

Comment [KA6]: The authors should conclude the study by summarizing their findings in one sentence.

REFERENCES:

1-National Cancer Institute (NCI).2018.

2-Brand W, Boon PE, Hessel EVS, Meesters JAJ, Weda M and Schuur AG. The Netherlands Food and Consumer Product Safety Authority (NVWA). National Institute for Public Health and the Environment. Exposure to and toxicity of methyl- ethyl and propylparaben. RIVM Report .2017:1-109.

3-Scientific Committee on Consumer Safety (SCCS), Opinion on parabens.2010.

4-Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ and Pope GS. Concentrations of parabens in human breast tumours. J Appl Toxicol.2004; 24(1):5-13.

5-Millas I, and Liquidato BM. Estrogen receptors alpha and beta in non-target organs for hormone action. Braz J Morphol Sci. 2009;26 (3-4): 193-197.

6-Hogan AM, Collins D, Baird AW, Winter DC. Estrogen and gastrointestinal malignancy. *Mol Cell Endocrinol.*2009;307(1-2):19-24.

7-Saif Ur Rahman M and Cao J. Estrogen receptors in gastric cancer: Advances and perspective. *World J Gastroenterol.*2016;22(8): 2475–2482.

8-Xu CY, Guo JL, Jiang ZN, Xie SD, Shen JG, Shen JY and Wang LB. Prognostic role of estrogen receptor alpha and estrogen receptor beta in gastric cancer. *Annals of Surgical Oncology.* 2010;17(9):2503-9.

9- Barr L, Metaxas G, Harbach CAJ, Savoy LA and Darbre PD. Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum. *J Appl Toxicol.*2012; 32(3):219-32.

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