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# EXPERIMENTAL CARCINOGENESIS WITH 7,12-DIMETHYLBENZ(A)ANTHRAZENE (DMBA) AND ITS INHIBITION WITH ISOTHIOCYANATES.

## Carcinogénesis experimental con 7,12-dimetilbenzantraceno (DMBA) y su inhibición con isitiocianatos.

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**ABSTRACT:**

Introduction: DMBA is a chemical carcinogen that induces carcinomas within a few weeks of its application. We developed an experimental model of carcinogenesis induced by DMBA dissolved in 0,5% paraffin oil (DMBA-PO), verifying the inhibitory effect of the carcinogenicity of phenyl isothiocyanate (PhITC), phenethyl (PhnITC) and benzyl isothiocyanate (BITC).

Material and Methods: For this, 88 hamsters were distributed into three groups: one exposed to DMBA-PO (Group 1, n=12), three subgroups (n=12) exposed to PhITC, PhnITC, BITC and DMBA-PO (Group 2, n=36) and four control subgroups (n=10) that were not exposed to the carcinogen in which PO (paraffin oil) and isothiocyanates were applied (Group 3, n=40).

Results: The experiment had a duration of 20 weeks, at the end of which the inhibitory effect was established by comparing the lesions developed in the groups that received isothiocyanates with the group that was only treated with DMBA-PO. The carcinogenic effect of DMBA-PO is 100% (35 carcinomas) and the inhibitory effect was 0, whereas in the presence of isothiocyanates the carcinogenic effect decreases, with an inhibitory effect of 86% for BITC (5 carcinomas) and 74% for PhITC (9 carcinomas).

Conclusion: The inhibitory effect for PhnITC is 80% in relation to invasive OSCC (1 carcinoma).

**KEYWORDS:**

*Carcinogenesis, Chemoprevention; Isothiocyanates; 9,10-Dimethyl-1,2-benzanthracene (DMBA); oral squamous cell carcinoma (OSCC); Carcinogens; Animal models.*

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**RESUMEN:**

**Introducción:** El DMBA es un carcinógeno químico que induce carcinomas a las pocas semanas de su aplicación. Desarrollamos un modelo experimental de carcinogénesis inducida por DMBA disuelto en aceite de parafina al 0,5% (DMBA-Ap) comprobando el efecto inhibidor de la carcinogénesis de los isotiocianatos fenil (PhITC), fenetil (PhnITC) y bencil isotiocianato (BITC).

**Material y Métodos:** Para ello, se distribuyeron 88 hámsteres en 3 grupos: uno expuesto al DMBA-Ap (Grupo 1, n=12), tres subgrupos (n=12) expuestos a PhITC, PhnITC, BITC y DMBA-Ap (Grupo 2, n=36) y cuatro subgrupos controles (n=10), no expuestos al carcinógeno en el que se aplicaron Ap e isotiocianatos (Grupo 3, n=40).

**Resultados:** El experimento tuvo una duración de 20 semanas, al final de la cual se establece de forma comparativa el efecto inhibidor comparando las lesiones desarrolladas en los grupos que recibieron isotiocianatos con respecto al grupo tratado sólo con DMBA-Ap. El efecto carcinógeno del DMBA-Ap es del 100% (35 carcinomas) y el efecto inhibidor 0, mientras que en presencia de isotiocianatos el efecto carcinógeno disminuye, con un efecto inhibidor del 86% para BITC (5 carcinomas) y del 74% para el PhITC (9 carcinomas).

**Conclusión:** El efecto inhibidor del PhnITC es del 80% en relación con el COCE invasivo (1 carcinoma).

**PALABRAS CLAVE:**

*Carcinogénesis; Quimioprevención; Isotiocianatos; 9,10-Dimetil-1,2-benzantraceno (DMBA); carcinoma oral de células escamosas (COCE); carcinógenos; modelos animales.*

## INTRODUCTION.

The DMBA (7,12-dimethylbenzanthracene) is a polycyclic aromatic hydrocarbon that derives from processes such as the combustion of tobacco (especially cigarettes) or animal fat when grilling meat. Additionally, DMBA is also found in various types of meats and smoked fish.

This hydrocarbon is considered a potent chemical carcinogen as it can induce precancerous lesions and carcinomas within a few weeks of its topical administration.[1-3] Because of this, it is thought to play a key role in the carcinogenesis of oral and lung cancer, as well as in the pathogenesis of digestive cancer.

In 1954, Salley[4] was the first to successfully demonstrate the carcinogenic potential of DMBA through its topical application, with previous dissolution in acetone, in the oral mucosa of hamsters. Nowadays, DMBA is still considered an effective chemical carcinogen, which is why it is still widely used in experimental carcinogenesis studies, causing, through its topical application, the development of squamous cell carcinomas in the oral mucosa of experimental animals, especially rodents. The development of experimental models with animals is common and their aim is to deepen the knowledge of the mechanisms of carcinogenesis and their inhibition with different anticancer agents of natural origin. DMBA-induced oral carcinogenesis in hamsters is one of the most widely accepted models due to the accessibility of this body area for treatment and study, and the similarity of the lesions produced with those observed in humans.[5-9]

In the 1980s, Solt *et al.*,[2] established the types of tumors that DMBA is capable of producing[3,10,11] and perfected the □-glutamyl transpeptidase (GGT) histochemical staining technique of experimentally induced lesions in their earliest stages, developed

by Rutenberg *et al.*,[12] in 1968. Cancer chemoprevention is defined as the use of chemical agents of natural, biological, or synthetic origin with the aim of reducing the risk of cancer.

This term was initially introduced by Sporn *et al.*,[14] and today it represents an emerging field in cancer prevention, which is focused on the search for agents that, with the least possible toxicity, can inhibit the development of cancer in people at risk, thus falling within the scope of primary preventio.[14]

Regarding dietary habits, various epidemiological studies report a positive correlation between the consumption of fruits and vegetables and the reduction in the risk of developing cancer.[15,16]

Stoner GD, since the early 1990s, became involved in cancer chemoprevention research and was one of the first to study the anticarcinogenic effect of isothiocyanates on animal models, making use of nitrosamines as chemical carcinogens.[17-19] Nowadays, several studies have shown the anticarcinogenic effects of isothiocyanates on various types of cancer,[20] such as oral squamous cell carcinoma,[21] breast,[22,24] prostate,[25] lung,[16] pancreas,[26] or bladder,[27] cancer, both in animal models and in human studies.

The following are the most frequently studied isothiocyanates: phenyl-isothiocyanate (PhITC), phenethyl-isothiocyanate (PhnITC), and benzyl-isothiocyanate (BITC). Of these, PhnITC[21,28] and BITC2[2-24,26] are the most relevant, due to their high inhibition capacity in experimental animal models and minimal toxicity to normal cells; the latter is an essential requirement for any future chemopreventive agent.[28-30]

The most recent studies also highlight their therapeutic potential through various molecular mechanisms, among which the production of ROS and the arrest of the cell cycle in the $G^2$/M phase stand out as forms of apoptosis induction of cancer cells, in such a way that the production of ROS in large quantities induces death by apoptosis in cancer cells upon reaching ROS levels that exceed their toxicity threshold.[21,25,26]

In addition, in some studies it has also been possible to observe anti-migratory and anti-invasive actions that would suggest a possible anti-metastatic potential in some types of cancer.[22]

## MATERIALS AND METHODS.

Animals, chemical reagents, treatment, histochemical labeling with □-glutamyl transpeptidase (GGT), and histological study.

### Materials

Eighty-eight male Golden Syrian hamsters were used, each were 4-6 weeks old and weighed 60-80 grams at the beginning of the experiment. The animals came from the Harlan® Experimental Animal Breeding Facility (Barcelona) and were kept in the animal facility of the School of Medicine, specially conditioned for animal experimentation. Animals were given feed and water *ad libitum*. Sacrifice by $CO_2$ inhalation was used as the euthanasia procedure.

All the experiments were approved by the Ethics Committee for Animal Experimentation of Universidad de Sevilla (07-11-2005). The project met the requirements for animal experimentation set forth by the regulations in force in Spain and the European Union.

To carry out the experiment, the following chemical products were used: 7,12-dimethyl-1,2-bezanthracene (DMBA), isothiocyanates (phenyl, phenethyl and benzyl isothiocyanates), and paraffin oil, from Sigma-Aldrich Co. St. Louis, USA GMNA (□-L- glutamyl-4-methoxy-2-napthy-lamide), Fast Blue BB Salt and Trizma base for histochemical staining technique (GGT) from Vega Biotechnologies Inc. Tucson, USA.

### Methods

The eighty-eight animals were divided into 3 large groups that would be exposed to different treatments for 20 weeks. Group 1, 12 animals: DMBA dissolved in paraffin oil (PO) at 0.5%. Group 2: three subgroups of 12 animals (n=36), DMBA-PO and isothiocyanates. The control group (Group 3, n=40): four subgroups of 10 animals, in which only the topical application of PO and each of the


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isothiocyanates was carried out, without being exposed to the carcinogen. All isothiocyanates were administered at the inhibitory concentration of 10 µmol/ml and dissolved in paraffin oil, previously calculated using the GGT technique. The topical application of the chemical products was carried out in both cheek pouches of the hamsters, following the methodology suggested by other authors.[1-6]

The experiment had a duration of 20 weeks and was divided into 2 stages. The first stage lasted from the 1st to the 7th week (inclusive), and the objective was to determine carcinogenesis initiation zones using the GGT technique.

The second stage began at the start of the 8th week and lasted until the end of the 20th week, when a conventional histological study was carried out. Of the 88 animals, 40 were studied in the first stage and 48, in the second.

The experimental strategy is described below and is presented in Table 1.

Group 1, DMBA-PO (n=12). During the first two weeks, both buccal pouches of the twelve animals were brushed daily with PO. Subsequently, from the third to the seventh week inclusive (5 weeks), DMBA-PO at 0.5% was applied on Mondays and Fridays. Only PO was applied on Tuesdays, Wednesdays, and Thursdays. After the seventh week, five animals were sacrificed. From this point on and until the 20th week was completed, PO was continued to be applied on the remaining animals.

Group 2, DMBA-PO/isothiocyanates (n=36). Isothiocyanates PhITC, PhnITC and BITC were applied daily in the three subgroups of 12 animals until the end of the second week. Subsequently, for a period of 5 weeks, the application of DMBA-PO at 0.5% (Monday and Friday) was alternated with that of the different isothiocyanates (Tuesday, Wednesday, and Thursday). After seven weeks, five animals from each subgroup were sacrificed. The remaining animals continued being treated exclusively with isothiocyanates on a daily basis until the end of the twentieth week.

**Subgroup 2a (n=12):** PhITC (10µmol/ml)-DMBA-PO/PhITC (10µmol/ml) – PhITC (10µmol/ml)

**Subgroup 2b (n=12):** PhnITC (10µmol/ml)-DMBA-PO/PhnITC (10µmol/ml) – PhnITC (10µmol/ml)

**Subgroup 2c (n=12):** BITC (10µmol/ml) – DMBA-PO/BITC (10µmol/ml) – BITC (10µmol/m)

Group 3, control (n=40). Divided into four subgroups of 10 hamsters, the animals in the control groups were treated daily for twenty weeks with PO (subgroup 3a) and isothiocyanates at the inhibitory concentration: PhITC (subgroup 3b), PhnITC (subgroup 3c), and BITC (subgroup 3d). From each subgroup, five animals were sacrificed after seven weeks of treatment and the rest continued treatment until the end of the twenty weeks.

**Subgroup 3a (n=10): PO**
**Subgroup 3b (n=10): PhITC (10 µmol/ml)**
**Subgroup 3c (n=10): PhnITC (10 µmol/ml)**
**Subgroup 3d (n=10): BITC (10 µmol/ml)**

All the animals that were euthanized after seven weeks of treatment (n=40) had their buccal pouches removed. Subsequently, a complete assembly of the oral epithelium was carried out, followed by a histochemical study with the aim of determining GGT activity, according to the Rutenburg method modified by Solt *et al.*[1]

GGT-positive lesions were counted and morphometrically studied (Figure 1) with a Kontron image analyzer (Kontron Imaging System, Carl Zeiss). In order to measure the surface of the buccal pouches, a 10x10 cm scale with a projection at 1.2X magnification was used, each field corresponding to 72.25 $mm^2$.

As positive controls of this technique, 5 µm cryostat sections of hamster kidney were used, considering that cells from the renal tubules of hamsters produce GGT. The positivity in these sections indicates that the technique was correctly performed. In each group, the following data was determined: the whole of the analyzed surface, the number of GGT-positive lesions, and the maximum diameter found.

In the remaining animals that completed the treatment up until the end of the twenty weeks (n=48), a morphological, macro, and microscopic study was performed with hematoxylin and eosin staining for typing, quantification, and determination of the size of the tumors originating in the oral epithelium, esophagus, and stomach. For the classification of benign and malignant tumors, the following parameters were used: histological appearance (flat or papillary), differentiation, presence of nuclear atypia and mitotic activity, preservation or lack of the epithelial-connective tissue interface, and invasion of the stroma as flaps or frank invasion by isolated cells.

Three distinct patterns of squamous cell carcinoma were observed: carcinoma *in situ* or intra-epithelial carcinoma, the exophytic variant, very well differentiated or verrucous carcinoma, and conventional squamous cell carcinoma.[35] To determine the carcinogenic/inhibitory effect, the carcinogenic effect in animals belonging to the DMBA-PO group was considered to be 100% and the inhibitory effect was defined as 0. Thus, the inhibitory effect of the isothiocyanates was established by the percentage difference over 100, considering values greater than 50% as inhibitory effect.

## RESULTS.

After seven weeks, at a macroscopic level, neither group showed apparent neoplastic lesions. Microscopically, all control groups were GGT-negative, and GGT-positive lesions were only observed in groups that received the carcinogen during treatment. The highest proportion was observed in the group that was treated exclusively with DMBA-PO (4.68 lesions/cm$^2$) and, at a lower rate, in the groups that received isothiocyanates along with the carcinogen. The lowest proportion was recorded in the group that received PhnITC (1.3 lesions/cm$^2$)(Table 2).

After seven weeks of treatment, it was observed that isothiocyanates do have an inhi-bitory effect in the initiation stage of carcinoge-nesis with DMBA greater than 50%. PhnITC was reported to be the isothiocyanate that develops higher carcinogenesis inhibition, followed by PhITC with slightly less inhibition, and finally BITC, which would produce negligible inhibition.

After twenty weeks, the morphological study (Table 3) at a macroscopic level revealed lesions in the oropharyngeal bags, esophagus, and stomach. Control subgroups did not present lesions. From a microscopic point of view, the histological study

**Table 1.** Results of the determination of GGT activity in each group at 7 weeks of treatment.

| Weeks | 1-2 | 3-7 (5 weeks) | 8-20 |
|---|---|---|---|
| DMBA-PO (12) | Daily PO | DMBA-PO (M and F); Ap (T, W, and Th) | Daily PO |
| DMBA-PO / PhITC (12) | Daily PhITC | DMBA-PO (M and F) and PhITC (T, W, and Th) | Daily PhITC |
| DMBA-PO / PhnITC (12) | Daily PhnITC | DMBA-PO (M and F) y PhnITC (T, W, and Th) | Daily PhnITC |
| DMBA-PO / BITC (12) | Daily BIPC | DMBA-PO (M and F) y BIPC (T, W, and Th) | Daily BIPC |
| PO (10) | PO, daily | PO, daily | PO, daily |
| PhITC (10) | PhITC, daily | PhITC, daily | PhITC, daily |
| PhnITC (10) | PhnITC, daily | PhnITC, daily | PhnITC, daily |
| BITC (10) | BITC, daily | BITC, daily | BITC, daily |

**DMBA:** Dimethylbenzanthracene, **PO:** Paraffin oil, **PhITC:** Phenyl isothiocyanate, **PhnITC:** Phenethyl isothiocyanate, **BITC:** Benzyl isothiocyanate, **M:** Monday, **T:** Tuesday, **W:** Wednesday, **Th:** Thursday, **F:** Friday. In parentheses: number of animals.


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**Table 2.** Results of the determination of GGT activity in each group at 7 weeks of treatment.

| | DMBA-PO | DMBA PO/PhITC | DMBA PO/PhnITC | DMBA-PO/BITC | PO | PhITC | PhnITC | BITC |
|---|---|---|---|---|---|---|---|---|
| Surface ($cm^2$) | 46.7 | 55.9 | 32.6 | 51.3 | 56.3 | 57.9 | 54.1 | 55.3 |
| No. of GGT lesions | 219 | 87 | 43 | 104 | 0 | 0 | 0 | 0 |
| No. of lesions/$cm^2$ | 4.68 | 1.55 | 1.31 | 2.28 | 0 | 0 | 0 | 0 |
| Maximum diameter (mm) | 10.5 | 7.1 | 4.8 | 11.3 | 0 | 0 | 0 | 0 |
| Carcinogenic effect (%) | 100 | 33 | 28 | 48.7 | 0 | 0 | 0 | 0 |
| Inhibitory effect (%) | --- | 67 | 72 | 51.3 | 0 | 0 | 0 | 0 |

**DMBA:** Dimethylbenzanthracene, **PO:** Paraffin oil, **PhITC:** Phenyl isothiocyanate, **PhnITC:** Phenethyl isothiocyanate, **BITC:** Benzyl isothiocyanate.

**Table 3.** Histological study of DMBA-PO/Isothiocyanates carcinogenesis after 20 weeks of treatment.

| | | DMBA-PO | DMBA-PO/ PhITC | DMBA-PO / PhnITC | DMBA-PO/ BITC |
|---|---|---|---|---|---|
| Squamous papilloma | Oral epithelium | 1 (0.4 mm) | 4 (0.5-1 mm) | 2 (0.9 y 1 mm) | 1 (0.6 mm) |
| | Esophagus | 9 (0.2-1.2 mm) | 11 (0.7-1 mm) | 9 (0.5-1.2 mm) | 5 (0.5-1 mm) |
| | Stomach | 14 (0.3-1.2 mm) | 2 (0,2 y 0.3 mm) | 12 (0.2-1 mm) | 7 (0.7-1,5mm) |
| Total papillomas | | 24 | 17 | 23 | 13 |
| Carcinoma *in situ* | Oral epithelium | 20 (0.1-0.4 mm) | 3 (0.2-0.3 mm) | 9 (0.2-0.5 mm) | 2 (0.3 y 0.5 mm) |
| | Esophagus | 0 | 1 (0.4 mm) | 4 (0.2-0.3 mm) | 0 |
| | Stomach | 1 (0.6 mm) | 0 | 0 | 0 |
| Verrucous carcinoma | Oral epithelium | 1 (1.7 mm) | 1 (0.6 -1 mm) | 2 (0.4-1 mm) | 1 (6 mm) |
| | Esophagus | 3 (0.8-1 mm) | 2 (1-2 mm) | 3 (0.7-1.5 mm) | 0 |
| | Stomach | 5 (0.8-2 mm) | 0 | 1 (2 mm) | 0 |
| Conventional SCC | Oral epithelium | 1 (0.5 mm) | 1 (3 mm) | 1 (2mm) | 0 |
| | Esophagus | 2 (2 y 4 mm) | 1 (1.2mm) | 0 | 2 (2 y 3 mm) |
| | Stomach | 2 (1.5 y 9 mm) | 0 | 0 | 0 |
| Total carcinomas | | 35 | 9 | 20 | 5 |

**PhITC:** Phenyl isothiocyanate, **PhnITC:** Phenethyl isothiocyanate, **BITC:** Benzyl isothiocyanate. **SCC:** Squamous cell carcinoma. **In parentheses:** tumor size, expressed in millimeters.

**Table 4.** Neoplastic effect of DMBA and inhibitor effect of isothiocyanates in relation to the total number of tumors.

| | | DMBA-PO | DMBA-PO /PhITC | DMBA-PO /PhnITC | DMBA-PO /BITC |
|---|---|---|---|---|---|
| Squamous papilloma | Proliferative effect | 100 % (24) | 71% (17) | 96% (23) | 54% (13) |
| | Inhibitory effect | 0 | 29% | 4% | 46% |
| Carcinoma *in situ* (intraepithelial) | Carcinogenic effect | 100% (21) | 19% (4) | 62% (13) | 10% (2) |
| | Inhibitory effect | 0 | 81% | 38% | 90% |
| Verrucous carcinomas | Carcinogenic effect | 100% (9) | 33% (3) | 67% (6) | 11 % (1) |
| | Inhibitory effect | 0 | 67% | 33% | 89% |
| Conventional squamous cell carcinoma | Carcinogenic effect | 100% (5) | 40% (2) | 20% (1) | 40% (2) |
| | Inhibitory effect | 0 | 60% | 80% | 60% |

DMBA: Dimethylbenzanthracene, PO: Paraffin oil, PhITC: Phenyl isothiocyanate, PhnITC: Phenethyl isothiocyanate, BITC: Benzyl isothiocyanate. In parentheses: number of tumors.

revealed the presence of benign tumors (squamous papillomas) and three different patterns of squamous cell carcinoma: carcinoma in situ or intraepithelial carcinoma, verrucous carcinoma, and conventional squamous cell carcinoma. All variants were observed in the oral epithelium, esophagus, and stomach.

The squamous papillomas were made up of vascular connective axes covered by a squamous epithelium with hyperkeratosis and without the presence of atypia or mitotic activity (Figure 2).

Carcinomas in situ showed a proliferation of neoplastic cells with nuclear atypia and mitosis, which did not exceed the epithelial-connective tissue interface (Figure 3). Verrucous carcinomas showed an exophytic growth, whose epithelium had a papillary appearance and invaded the connective tissue as epithelial flaps. The proliferating cells showed low atypia and mitoses, and no invasion of the stroma by isolated neoplastic cells was observed (Figure 4).

Conventional squamous cell carcinoma showed a proliferation of squamous epithelium that, in the form of nests, cords, and isolated cells, invaded the underlying stroma. The proliferating cells presented atypia and mitotic activity, as well as foci of abnormal keratinization in the form of corneal pearls (Figure 5).

Representation of results in percentages of the neoplastic and inhibitory effect, in relation to the total number of carcinomas, is shown in Table 4. The inhibitory effect of BITC and PhITC was 86% (five carcinomas) and 74% (nine carcinomas), respectively. It can be seen that, in animals belonging to the DMBA-PO/PhnITC group, there is no inhibitory effect for in situ and verrucous carcinomas (19 carcinomas), with a value of less than 50%, and only a small number of conventional squamous cell carcinomas are produced, via frank invasion (one carcinoma).

## DISCUSSION.

Chemopreventive agents must be substances with the least possible toxicity, well tolerated and with minimal side effects since their administration may be required for long periods of time.


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Therefore, much of the current research in chemoprevention focuses on the study of agents of natural origin; a field in which experimental carcinogenesis induced in animal models plays a fundamental role.

In this sense, substances such as tea and curcumin,[7] propolis,[8] derivatives of isoflavone,[9] olive oil[32-34] or isothiocyanates,[17-26,28,31] have already been studied. In this research, it is proposed an experimental carcinogenesis model induced by the chemical carcinogen,[7] 12-dimethylbenzanthracene (DMBA) dissolved in paraffin oil (PO) at 0.5% in the oral mucosa of hamsters, as well as its inhibition, caused by isothiocyanates present in cruciferous vegetables, such as broccoli, cabbage, cauliflower, or Brussels sprouts. Isothiocyanates are seen as the future chemopreventive agents, which makes them rank among the most studied natural products today. With this purpose in mind, one of the most widespread experimental carcinogenesis models in the scientific community was replicated in this study: the topical application of DMBA on the oral mucosa of hamsters, which is considered an excellent model for experimentation with chemopreventive agents due to the convenience for its study and the similarity of the lesions produced in humans.[5-8] To determine that isothiocyanates have a significant inhibitory effect on DMBA carcinogenesis, they were administered before, during, and after exposure to the carcinogen.

DMBA produces oral squamous cell carcinomas (OSCC) within weeks of topical administration in the buccal pouches. The aim was to study the effect of isothiocyanates on DMBA-induced oral carcinogenesis in rodents. However, due to the method of application of the compounds in the buccal pouches, in the necropsy carried out at the conclusion of the study after twenty weeks, lesions were observed not only in the oral epithelium, but also in the esophagus and stomach, because concentrations of the carcinogen may have also reached them by dripping or swallowing when it was applied to the oral mucosa.

In addition, except for the carcinoma in situ, no relevant differences were found between the different groups with respect to the number of carcinomas developed in the oral epithelium. For this reason, the total number of papillomas and carcinomas were taken into account in the analysis of the results, considering the carcinogenic and inhibitory effect in each group without making organ-specific conjectures.

At seven weeks, differences were observed in each of the control and treated groups with respect to the surface area ($cm^2$) of the analyzed tissue. Specifically, a smaller surface in the group exposed to DMBA-PO and DMBA-PO/PhnITC was reported, which could be due to the fact that inflammatory phenomena with proliferation of a higher fibrous component that originated tissue retractions were produced in these groups, (Table 2). Results show that the inhibitory effect of initiated lesions is greater than 50% in all groups of animals treated with isothiocyanates.

At twenty weeks, it was observed that none of the isothiocyanates showed an apparent inhibitory effect on the development of squamous papillomas, and in some cases a greater number of these lesions were observed in the groups treated with DMBA/isothiocyanates than in the group that was only treated with the carcinogen. Considering the proliferative and benign nature of the papilloma, the results were deemed satisfactory since they correlate with a lower development of carcinomas in all groups, including conventional squamous cell carcinomas. Therefore, it was deduced that the neoplastic effect of DMBA is restricted in the presence of isothiocyanates, limiting it to a proliferative, less aggressive effect, and thereby limiting most of the lesions to a benign state.

Results regarding the development of total carcinomas reveal that only BITC and PhITC show an inhibitory effect. However, when analyzing the findings by lesion subtype, it is possible to see that the group treated with PhnITC showed a significant inhibitory effect only in regard to the development of conventional squamous cell carcinomas, in an even greater proportion than

the BITC and PhITC groups. Nevertheless, this group also presents a greater number of in situ and verrucous carcinomas. On the other hand, the groups treated with BITC and PhITC had a lower number of *in situ* and verrucous carcinomas but had a higher proportion of conventional squamous cell carcinomas (Table 3).

Thus, although PhnITC did not prevent the development of malignant lesions, it did show a greater ability to control tumor progression and the development of squamous cell carcinoma with frank invasion compared to the other isothiocyanates.

Therefore, taking these results into account, it is possible to conclude that, to a greater or lesser extent, all isothiocyanates have an inhibitory effect on carcinogenesis, either by acting on its initiation or by modulating the later phases of tumor differentiation and progression, giving rise to better differentiated and less aggressive tumors. Although the limitations of this study do not allow it to go beyond the deductions made, they are equally important since they coincide with the results shown by the most recent reviews on PhnITC and BITC. In both cases, advances in the knowledge of their anticancerogenic functions lead them to be considered not only as chemopreventive agents but also as chemotherapeutic agents.

Therefore, they would not only be capable of inhibiting the carcinogenesis stages of initiation and progression, but could also selectively act upon cancer cells, inducing death by apoptosis.[20-22,26] In the case of PhnITC, Solt *et al.*,[28] observed that treatment with phenethyl isothiocyanate produces, as noted in this study, a reduction in the number of GGT lesions in the oral epithelium and the formation of malignant tumors, although the carcinogen used in that case was a nitrosamine.

Recently, Yeh *et al.*,[21] concluded that PhnITC inhibits growth and induces apoptosis in oral squamous cancer cells (OSCC) with mutations in p53. Therefore, the detection of this mutation in people with OSCC may be of clinical interest both at a chemopreventive level for people at risk, and also for its use at a therapeutic level. Regarding BITC, Kim *et al.*,[22] reported that the growth[23] of stem cells in breast cancer, both *in vitro* and *in vivo*, is inhibited in the presence of BITC. This complements a previous study in which results showed how oral administration of BITC in mice with breast cancer inhibits cell proliferation through the suppression of angiogenesis and the induction of apoptosis in tumor cells, also observing a decrease in the development of lung metastases, thus clearly outlining the usefulness of BITC as a preventive agent in breast cancer metastasis.

In a recent systematic review, which offers a comprehensive analysis of the anticancerogenic mechanisms of PhnITC Gupta *et al.*,[31] expose its use in combined therapies with conventional anticancerogenic agents, as a way of enhancing their effects and of combating the resistance to chemotherapeutic drugs that cancer cells have the capacity to develop. In this sense, PhnITC would be a promising agent, although more in-depth studies regarding toxicity profiles and interaction with these drugs would be necessary, considering the already established effect of isothiocyanates on drug metabolism and detoxification enzymes.

Although the systematic analysis of current epidemiological studies concludes that there is an inverse relationship between the consumption of isothiocyanates in the form of cruciferous vegetables and the overall incidence of cancer, better designed studies are required to avoid the confounding factors responsible for some of the differences observed between the study populations, such as: time of consumption of the vegetables by each subject, variations in the amount of isothiocyanates found in the different vegetables in relation to geographical locations, form of vegetable consumption, or errors and lack of correlation between the questionnaires with which each study evaluates consumption.

Therefore, considering the correlation of the results obtained in this study with those of other


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authors and based on current evidence, it can be stated that isothiocyanates have significant anti-cancerogenic potential.

## CONCLUSION.

In this experimental model of DMBA-induced carcinogenesis and its inhibition with isothiocyanates at a concentration of 10 µm/ml, it is evident that:

Phenethyl, phenyl and benzyl isothiocyanates cause a reduction in the number of GGT-induced lesions with an inhibitory effect of 72%, 67%, and 51%, respectively.

The inhibitory effect on carcinogenesis in relation to the total number of carcinomas was 86% and 74% for BITC and PhITC, respectively. PhnITC showed the greatest inhibitory effect against the development of conventional squamous cell carcinomas with frank invasion, with an inhibition of 80%.

All the isothiocyanates studied modulate to a greater or lesser extent the experimental DMBA-induced carcinogenesis, preventing the development of carcinomas or giving rise to well-differentiated and less aggressive tumors.

## Conflict of interests:

None of the authors has conflicts of interest to declare.

## Ethics approval:

Study approved by the Ethics Committee for Animal Experimentation of Universidad de Sevilla (07-11-2005).

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## Authors' contributions:

All authors contributed to the study execution of this manuscript.

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## REFERENCES.


1. Solt DB, Shklar G. Rapid induction of gamma-glutamyl transpeptidase-rich intraepithelial clones in 7,12-dimethylbenz(a)anthracene-treated hamster buccal pouch. Cancer Res. 1982;42(1):285-91. PMID: 6119154.
2. Solt DB, Polverini PJ, Ray S, Fei Y, Biswas DK. Early neoplastic commitment of hamster buccal pouch epithelium exposed biweekly to 7,12-dimethylbenz[a] anthracene. Carcinogenesis. 1988;9(12):2173-7. doi: 10.1093/carcin/9.12.2173. PMID: 3142694.
3. Nagabhushan M, Ng YK, Elias R, Polverini PJ, Solt DB. Acute inhibition of DNA synthesis in hamster buccal pouch epithelium exposed to indirect acting carcinogens. Cancer Lett. 1990;53(2-3):163-73. doi: 10.1016/0304-3835(90)90210-o. PMID: 2119880.
4. Salley JJ. Experimental carcinogenesis in the cheek pouch of the Syrian hamster. J Dent Res. 1954;33(2):253-62. doi: 10.1177/00220345540330021201. PMID: 13152263.
5. García FJG, Ortega VV, Sánchez NA, Jornet PL. Estudio comparativo de la aplicación del hidrocarburo aromático policíclico 7,12-dimetil-1,2-benzatraceno (DMBA) sobre la mucosa oral del hámster y del cobaya. Rev Esp Patol. 2009;42:287-95.
6. Nagini S, Kowshik J. The Hamster Buccal Pouch Model of Oral Carcinogenesis. Methods Mol Biol. 2016;1422:341-50. doi: 10.1007/978-1-4939-3603-8_29. PMID: 27246045.
7. Ribeiro DR, Alves ÂV, dos Santos EP, Padilha FF, Gomes MZ, Rabelo AS, Cardoso JC, Massarioli AP, de Alencar SM, de Albuquerque-Júnior RL. Inhibition of DMBA-induced Oral Squamous Cells Carcinoma Growth by Brazilian Red Propolis in Rodent Model. Basic Clin Pharmacol Toxicol. 2015;117(2):85-95. doi: 10.1111/bcpt.12374. PMID: 25556639.
8. Ribeiro DR, Alves ÂV, dos Santos EP, Padilha FF, Gomes MZ, Rabelo AS, Cardoso JC, Massarioli AP, de Alencar SM, de Albuquerque-Júnior RL. Inhibition of DMBA-induced Oral Squamous Cells Carcinoma Growth by Brazilian Red Propolis in Rodent Model. Basic Clin Pharmacol Toxicol. 2015;117(2):85-95. doi: 10.1111/bcpt.12374. PMID: 25556639.
9. Kim BR, Seo JY, Sung MK, Park JH, Suh HJ, Liu KH, Kim JS. Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis by glyceollins. Mol Nutr Food Res. 2015 May;59(5):907-17. doi: 10.1002/mnfr.201400726. Epub 2015 Mar 16. PMID: 25641514.
10. Odajima T, Solt DB, Solt LC. Persistence of gamma-glutamyl transpeptidase-positive foci during hamster buccal pouch carcinogenesis. Cancer Res. 1984;44(5):2062-7. PMID: 6143614.
11. Calderon-Solt L, Solt DB. Gamma-glutamyl transpeptidase in precancerous lesions and carcinomas of oral, pharyngeal, and laryngeal mucosa. Cancer. 1985;56(1):138-43. doi: 10.1002/1097-0142(19850701)56:1<138:aid-cncr2820560122>3.0.co;2-4. PMID: 2408724.
12. Rutenburg AM, Kim H, Fischbein JW, Hanker JS, Wasserkrug HL, Seligman AM. Histochemical and ultrastructural demonstration of gamma-glutamyl transpeptidase activity. J Histochem Cytochem. 1969;17(8):517-26. doi: 10.1177/17.8.517. PMID: 5816239.
13. Sporn MB, Suh N. Chemoprevention: an essential approach to controlling cancer. Nat Rev Cancer. 2002;2(7):537-43. doi: 10.1038/nrc844. PMID: 12094240.
14. Serrano D, Lazzeroni M, Bonanni B. Cancer chemoprevention: Much has been done, but there is still much to do. State of the art and possible new approaches. Mol Oncol. 2015;9(5):1008-17. doi: 10.1016/j.molonc.2014.12.006. PMID: 25556583; PMCID: PMC5528739.
15. Pavia M, Pileggi C, Nobile CG, Angelillo IF. Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. Am J Clin Nutr. 2006;83(5):1126-34. doi: 10.1093/ajcn/83.5.1126. PMID: 16685056.
16. Wu QJ, Xie L, Zheng W, Vogtmann E, Li HL, Yang G, Ji BT, Gao YT, Shu XO, Xiang YB. Cruciferous vegetables consumption and the risk of female lung cancer: a prospective study and a meta-analysis. Ann Oncol. 2013;24(7):1918-1924. doi: 10.1093/annonc/mdt119. PMID: 23553059; PMCID: PMC3690909.
17. Stoner GD, Morrissey DT, Heur YH, Daniel EM, Galati AJ, Wagner SA. Inhibitory effects of phenethyl isothiocyanate on N-nitrosobenzylmethylamine carcinogenesis in the rat esophagus. Cancer Res. 1991 Apr 15;51(8):2063-8. PMID: 2009525.
18. Stoner GD, Morse MA. Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. Cancer Lett. Mar 19;114(1-2):113-9. doi: 10.1016/s0304-3835(97)04639-9. PMID: 9103268.
19. Stoner GD, Kresty LA, Carlton PS, Siglin JC, Morse MA. Isothiocyanates and freeze-dried strawberries as inhibitors of esophageal cancer. Toxicol Sci. 1999;52(2 Suppl):95-100. doi: 10.1093/toxsci/52.2.95. PMID: 10630596.
20. Gupta P, Kim B, Kim SH, Srivastava SK. Molecular targets of isothiocyanates in cancer: recent advances. Mol Nutr Food Res. 2014;58(8):1685-707. doi: 10.1002/mnfr.201300684. PMID: 24510468; PMCID: PMC4122603.
21. Yeh YT, Yeh H, Su SH, Lin JS, Lee KJ, Shyu HW, Chen ZF, Huang SY, Su SJ. Phenethyl isothiocyanate induces DNA damage-associated G2/M arrest and subsequent apoptosis in oral cancer cells with varying p53 mutations. Free Radic Biol Med. 2014;74:1-13. doi: 10.1016/j.freeradbiomed.2014.06.008. PMID: 24952138.

**22.** Kim EJ, Hong JE, Eom SJ, Lee JY, Park JH. Oral administration of benzyl-isothiocyanate inhibits solid tumor growth and lung metastasis of 4T1 murine mammary carcinoma cells in BALB/c mice. Breast Cancer Res Treat. 2011;130(1):61-71. doi: 10.1007/s10549-010-1299-8. PMID: 21170677.

**23.** Kim SH, Sehrawat A, Singh SV. Dietary chemopreventative benzyl isothiocyanate inhibits breast cancer stem cells in vitro and in vivo. Cancer Prev Res (Phila). 2013;6(8):782-90. doi: 10.1158/1940-6207.CAPR-13-0100. PMID: 23661606; PMCID: PMC3737245.

**24.** Rao CV. Benzyl isothiocyanate: double trouble for breast cancer cells. Cancer Prev Res (Phila). 2013;6(8):760-3. doi: 10.1158/1940-6207.CAPR-13-0242. PMID: 23842793.

**25.** Chiao JW, Wu H, Ramaswamy G, Conaway CC, Chung FL, Wang L, Liu D. Ingestion of an isothiocyanate metabolite from cruciferous vegetables inhibits growth of human prostate cancer cell xenografts by apoptosis and cell cycle arrest. Carcinogenesis. 2004;25(8):1403-8. doi: 10.1093/carcin/bgh136.PMID: 15016658.

**26.** Sahu RP, Zhang R, Batra S, Shi Y, Srivastava SK. Benzyl isothiocyanate-mediated generation of reactive oxygen species causes cell cycle arrest and induces apoptosis via activation of MAPK in human pancreatic cancer cells. Carcinogenesis. 2009;30(10):1744-53. doi: 10.1093/carcin/bgp157.. PMID: 19549704; PMCID: PMC2757546.

**27.** Abbaoui B, Lucas CR, Riedl KM, Clinton SK, Mortazavi A. Cruciferous Vegetables, Isothiocyanates, and Bladder Cancer Prevention. Mol Nutr Food Res. 2018;62(18):e1800079. doi: 10.1002/mnfr.201800079. PMID: 30079608; PMCID: PMC6196731.

**28.** Solt DB, Chang Kw, Helenowski I, Rademaker AW. Phenethyl isothiocyanate inhibits nitrosamine carcinogenesis in a model for study of oral cancer chemoprevention. Cancer Lett. 2003; 202(2):147-52. doi: 10.1016/j.canlet.2003.08.021. PMID: 14643444.

**29.** Chikara S, Nagaprashantha LD, Singhal J, Horne D, Awasthi S, Singhal SS. Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. Cancer Lett. 2018;413:122-134. doi: 10.1016/j.canlet.2017.11.002. PMID: 29113871.

**30.** Gründemann C, Huber R. Chemoprevention with isothiocyanates - From bench to bedside. Cancer Lett. 2018;414:26-33. doi: 10.1016/j.canlet.2017.10.033. PMID: 29111351.

**31.** Gupta P, Wright SE, Kim SH, Srivastava SK. Phenethyl isothiocyanate: a comprehensive review of anti-cancer mechanisms. Biochim Biophys Acta. 2014;1846(2):405-24. doi: 10.1016/j.bbcan.2014.08.003. PMID: 25152445; PMCID: PMC4260992.

**32.** Moral R, Solanas M, García G, Grau L, Vela E, Escrich R, Escrich E. High corn oil and extra virgin olive oil diets have different effects on the expression of differentiation-related genes in experimental mammary tumors. Oncol Rep. 2008;20:429-5.

**33.** Soto-Castillo JJ, Ortega-Medina I. Carcinogenesis experimental con 7,12-dimetilbenzantraceno (DMBA) y su inhibición con aceite de olive virgin extra y dieta con aceitunas maduras (variedad Picual). Rev Esp Pat. 2017; 50:82-88.

**34.** Soto-Castillo JJ, Ortega-Medina I. Chapter: Experimental Carcinogenesis with 7,12-Dimethylbenz(a)Anthrazene (DMBA) and Its Inhibition with Extra Virgin Olive Oil and a Diet of Mature Olives (Picual Variety). In Olive Oil – New Perpectives and Applications. IntechOpen. March 4th 2021.

**35.** Head and Neck. Oral Cavity. AJCC Cancer Staging Manual, 8th Edition. New York. Springer 2017. (Edition S, Edge S, Byrd D. AJCC cancer staging manual. AJCC cancer staging manual. 2017. ISBN: 978-3-319-40617-6 https://link.springer.com/book/9783319406176


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