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**GEABĂ CLAUDIA**



# **PHD THESIS**

**NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AS  
REPURPOSED THERAPIES IN MALIGNANT DISEASES –  
ACTIVITY, SYNERGISM AND PUBLIC HEALTH ISSUES**

## **A B S T R A C T**

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## ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of drugs commonly used to reduce inflammation, pain, and fever. They work by inhibiting the enzyme cyclooxygenase (COX), which plays a crucial role in the production of prostaglandins, chemical compounds that contribute to inflammation and pain.

Recently, the potential of NSAIDs in the treatment of cancer has been studied. Some important points are as follows: (i) chronic inflammation and malignancies – evidence is available that chronic inflammation may contribute to the development and progression of certain types of cancer; NSAIDs, by reducing inflammation, could contribute to lowering the risk of developing cancer or inhibiting its progression; (ii) clinical studies - some studies have shown that the use of NSAIDs, especially celecoxib, a selective COX-2 inhibitor, may have beneficial effects in patients with certain types of cancer, such as colorectal and breast cancer; thus, these drugs have been studied for their potential to improve the efficacy of chemotherapy- and radiotherapy-based therapeutic protocols; (iii) mechanisms of action - in addition to the ability to inhibit prostaglandin synthesis, NSAIDs can affect other biological pathways relevant to the development and progression of malignant cells, including inhibition of angiogenesis, the process by which new blood vessels are formed that feed tumours and the modification apoptosis, i.e. programmed cell death; (iv) side effects and risks – despite the fact that NSAIDs may offer benefits in the context of malignant diseases, their use is not without risks. Among the side effects are those related to damage to the gastrointestinal, cardiovascular and renal systems, it being essential that the use of NSAIDs in the therapeutic approach of malignant diseases is monitored by health professionals and (v) ongoing research - scientific research, continues to determine the more effective treatment regimens that include NSAIDs, as well as identify patients who may benefit most from the use of these.

Nonsteroidal anti-inflammatory drugs may have an important role in cancer treatment, with the potential to reduce inflammation and improve therapy outcomes. However, further studies are needed to fully understand their effects and to establish clear indications for their use in oncology.

The present work is structured in accordance with the rules for drafting the doctoral thesis and includes the following main parts: the general part, the special part, the main conclusions and personal contributions and the bibliography. In the general part, current issues related to nonsteroidal anti-inflammatory drugs (NSAIDs), malignancies as a public health problem, and the roles of NSAIDs in malignancies are discussed

The present theme is interdisciplinary and is part of the research directions of the research group. It is timely and of significant importance locally, regionally, nationally and internationally.

The main objectives of the present work were:

- 1) Evaluation of the effects exerted by NSAIDs in preclinical and clinical models of skin cancer
- 2) Preclinical investigation of the effects exerted by aspirin and its combinations with fisetin on malignant melanoma cells
- 3) Investigating, through preclinical methods, the effects exerted by aspirin and its combinations with genistein on malignant colon cells

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drugs approved by the US Food and Drug Administration (FDA). They are recognized as therapeutic agents that reduce inflammation, pain and fever, comprising around 10% of all drugs prescribed globally each year. These anti-inflammatory drugs are commonly used to treat several health conditions, including fever, migraines, muscle pain, acute and chronic pain, gout, biliary and ureteral colic, and rheumatologic disease. Additionally, they serve as non-opioid agents, particularly in cases of acute trauma.

Extensive background on the pharmacological properties of NSAIDs (including their anti-inflammatory, antipyretic, and analgesic effects) support their efficacy and safety when used according to medical guidelines. However, improper use or repeated administration can lead to a variety of side effects, having a negative impact on patients. These side effects include gastrointestinal, renal, cardiac, hepatic, reproductive, neural, overdose, respiratory, and hematologic toxicities, as well as potential drug interactions. In addition, NSAIDs can impose a significant financial burden on the health system and contribute to environmental degradation, particularly in aquatic ecosystems.

The notable increase in the use of NSAIDs – both as prescription drugs and through self-medication – during the pandemic years, together with their availability as over the counter (OTC) products in many countries, intensified these toxic effects. The incidence and severity of NSAID-related complications have emerged as a significant public health concern, requiring urgent strategies aimed at increasing awareness of the effects and risks associated with NSAID self-medication, along with a focus on patient education.

The concept of drug repurposing, also known as the development of new therapies, based on already approved or investigational drugs, has gained considerable scientific interest in recent years. This approach focuses on identifying new pharmacological targets that differ from the original clinical indications of these drugs. Several benefits support this interest, including the availability of existing clinical and toxicity data, which can save time in studying drug effects, reduce costs, and use

computational methods that improve our understanding of mechanistic insights. Drug repurposing has become particularly common in cancer research.

Non-steroidal anti-inflammatory drugs (NSAIDs) have complex mechanisms of action mainly related to cyclooxygenase (COX). These mechanisms can be divided into two categories:

1. COX-dependent mechanism: This involves the inhibition of prostaglandin synthesis, which is essential in the inflammatory process.
2. COX-independent mechanism: This includes modulation of nuclear receptors, inhibition of key signalling pathways such as MAPK and PI3K/AKT, suppression of transcription factors such as AP-1 and NF- $\kappa$ B, and inhibition of matrix metalloproteinases, among others.

These diverse mechanisms position NSAIDs as strong candidates for repurposing in cancer chemoprevention and treatment. Preclinical and epidemiological studies have shown promising results regarding the effects of NSAIDs as chemopreventive agents in various types of cancer, including colorectal, prostate, gastric, breast and skin cancer.

Strategies to prevent and treat skin cancer are particularly challenging due to the various factors that contribute to its development, including environmental, genetic, and behavioural risk factors, along with the complex and heterogeneous nature of melanoma. Ultraviolet (UV) radiation is a primary factor in promoting skin cancer because it induces DNA damage in skin cells, suppresses the immune system, and causes oxidative stress, inflammation, and changes in microenvironmental signalling.

UV exposure activates the prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) signalling pathway in skin cells, leading to the proliferation and migration of tumour cells, which ultimately contribute to tumour development and progression. PGE<sub>2</sub> synthesis is regulated by the COX-2 enzyme, making it a promising therapeutic target for the prevention and treatment of melanoma.

Recent studies have indicated that COX-2 expression is increased in squamous cell carcinoma (SCC) lesions, lower in basal cell carcinoma (BCC), and significantly overexpressed in melanoma. This overexpression is considered a stage-dependent negative prognostic factor because it plays a role in promoting tumour progression. In addition, COX-2 is involved in various stages of carcinogenesis, including angiogenesis, further establishing it as a crucial biological target for the identification of promising candidates for the prevention and treatment of skin cancer. Consequently, non-steroidal anti-inflammatory drugs (NSAIDs) are emerging as some of the most viable options for drug repurposing in this context.

While non-steroidal anti-inflammatory drugs (NSAIDs) offer multiple therapeutic benefits, in recent years the scientific community has increasingly focused on the wide range of adverse effects associated with their use. The incidence and severity of these adverse effects have increased considerably. It is important to emphasize that short-term administration and therapeutic doses of

NSAIDs are generally well tolerated. However, chronic treatment, high doses, misuse and polypharmacy significantly increase the risk of adverse effects.

Among the most frequently analysed adverse effects related to the use of NSAIDs are gastrointestinal, cardiovascular, renal, hepatic, and hematologic toxicities. In addition, neural, respiratory and reproductive toxicities have also been reported.

The harmful effects of NSAIDs are largely attributed to their mechanism of action, particularly in the case of non-selective NSAIDs that inhibit the COX-1 isoform. This enzyme plays a crucial role in gastric and renal protection, mucus production, platelet aggregation and macrophage differentiation.

Drug repurposing, also known as drug repositioning, has emerged as a cost-effective approach to drug development that seeks to identify new therapeutic applications for existing, clinically approved, and marketed drugs. This strategy has become increasingly popular for discovering new treatments for various cancers, including melanoma (MM).

One of the earliest examples of drug repurposing involves aspirin (ASA), a nonsteroidal anti-inflammatory salicylate with a wide range of pharmacological activities, including antipyretic, analgesic, and antiplatelet effects. Recent investigations have focused on the anti-neoplastic activity of ASA, with several studies specifically linking chronic administration of ASA with a reduced risk of developing skin cancer.

Aspirin has demonstrated anti-melanoma effects through multiple mechanisms, such as suppression of colony formation and cell motility, induction of mitochondrial toxicity, and generation of reactive oxygen species (ROS). Beyond its intrinsic anticancer properties, ASA has also been evaluated in combination with various drugs and natural compounds (eg, exemestane, cisplatin, 5-fluorouracil, and genistein) as a means of improving therapeutic outcomes in cancer treatment, with results promising results reported in these studies.

Colorectal cancer (CRC) is a complex pathology influenced by both non-modifiable patient-related factors (such as age, genetic predisposition and inflammatory bowel diseases) and modifiable environmental factors. According to the GLOBOCAN 2022 report, CRC is the first cancer in Romania in terms of incidence, with over 13,500 new cases, and mortality, with over 7,300 deaths, affecting both sexes. It ranks second as the type of cancer most frequently diagnosed in women, after breast cancer.

While CRC is generally characterized by slow progression, late diagnosis contributes to its status as the second leading cause of cancer-related mortality and the third most common cancer globally. Recent studies indicate sex-related differences in CRC incidence rates between men and women. A higher incidence of CRC in men has been reported worldwide, with these differences attributed to factors such as sex dysmorphism (including sex chromosomes, hormones and immune response) as well as environmental risk factors, collectively referred to as the exposome. This topic has been thoroughly discussed in various reviews.

Strong evidence supports the anticancer and chemopreventive potential of aspirin in various types of cancer, including lung, breast, ovarian, stomach, and colorectal cancer. However, in 2022,

recent studies on the use of low-dose aspirin as a prophylactic agent to reduce the incidence of colorectal cancer (CRC) concluded that the evidence is insufficient. These suggested that aspirin had no significant effect on the incidence of CRC. Consequently, the chemopreventive activity of aspirin remains a matter of debate and further research is needed to clarify its role in cancer prevention.

In recent years, researchers have explored the potential synergistic effects of various anticancer drugs and natural compounds, such as 5-fluorouracil (5-FU) and genistein, as chemopreventive alternatives for colorectal cancer (CRC). There has even been interest in combining aspirin with a vegan diet, with promising results. In addition, a recent study examined several hybrid scaffolds based on aspirin and genistein for their chemopreventive potential in colorectal tumour cells, demonstrating a synergistic effect. However, further studies are needed to clarify the protective mechanisms of the aspirin-genistein combination.

The present study had three specific objectives, and several research activities were carried out to achieve them. The main objectives were the following:

1. Evaluation of the effects exerted by NSAIDs in preclinical and clinical models of skin cancer.
2. Preclinical investigation of the effects of aspirin and its combinations with fisetin on malignant melanoma cells.
3. Preclinical investigation of the effects of aspirin and its combinations with genistein on malignant colon cells.

All three objectives were fully achieved, and the dissemination of the obtained results can be found in the author's publications in ISI indexed journals with impact factors.

The main general conclusions that can be drawn are presented below.

#### First objective

NSAIDs (nonsteroidal anti-inflammatory drugs) are well-established anti-inflammatory, antipyretic, and analgesic agents known for their diverse pharmacological properties, including potential anticancer effects, and are generally considered safe when used in therapeutic doses. However, there is concern about the increasing incidence of adverse effects related to NSAID use. Contributing factors include their wide availability (being over-the-counter drugs in many countries), polypharmacy (leading to an increased risk of drug-drug interactions), misuse, and overdose. Consequently, effective strategies are needed to prevent or minimize these avoidable events, which place a significant burden on healthcare systems. Educating the public about NSAID side effects, combined with more prudent prescribing

practices and targeted guidance from both health care professionals, could help reduce the incidence of NSAID-related adverse reactions.

Regarding the antitumor effects of NSAIDs, most data in the current literature provide only parts of a puzzling mechanism that remains largely unexplored. Future research directions could investigate this further. The results of epidemiologic studies, randomized trials, meta-analyses, and cohort studies examining the potential of NSAIDs to reduce skin cancer risk remain controversial. These studies have not definitively established a direct link between NSAID use and a lower risk of skin cancer due to the many variables involved, including those related to the therapeutic agent (whether used alone or concomitantly, dose, duration, etc.), factors (such as sex, age, pre-existing conditions), study design, biochemical and biological parameters assessed, and statistical methods used.

## Second objective

Aspirin (acetylsalicylic acid) and genistein are two substances that have gained attention in oncology research due to their potential to influence cellular processes involved in the development and progression of cancer. Aspirin is well known for its anti-inflammatory and antithrombotic properties, while genistein, an isoflavone derived from soy, is recognized for its antioxidant and anticancer effects. In the case of skin cancer, recent studies suggest that these two substances may have beneficial effects, either by preventing the onset of cancer or by limiting its progression. Aspirin has been studied extensively for its potential in preventing and treating various types of cancer, including skin cancer. The main mechanisms by which aspirin may affect skin cancer include: (i) inhibition of Cox-2, (ii) effects on tumor cells, (iii) chemoprevention. Genistein, a natural compound in the isoflavone family, is known for its antioxidant, anti-inflammatory and anti-cancer effects. The new findings of the current study showed that the combined treatment of aspirin (ASA) and fisetin (FIS) could be a promising complementary alternative for malignant melanoma (MM) therapy. This combination demonstrated significant cytotoxicity, induced apoptotic-like features, and suppressed cell motility in A375 melanoma cells carrying the BRAFV600E mutation. In addition, it showed anti-angiogenic activity in the chick embryo chorioallantoic membrane (CAM) assay. This research serves as an initial preclinical evaluation of the ASA + FIS combination for potential applications in MM therapy, paving the way for further investigations in this area.

Both aspirin and the natural compound have shown considerable potential in the prevention and treatment of skin cancer, and their combination may represent a promising future strategy. Their anti-inflammatory, anti-proliferative and anti-angiogenic effects may contribute to limiting the development and progression of skin tumours. However, further studies are needed to evaluate the efficacy and safety of this combination in clinical settings, as well as to better understand the mechanisms involved.



### Third objective

Findings from in vitro and in ovo studies suggest that the combination of low-dose ASA (2.5 mM) and GEN (10-75  $\mu$ M) exhibits a significant dose-dependent cytotoxic effect on human colorectal cancer cells (HCT-116). This effect is significantly greater than that seen with either treatment alone. Key indicators of this cytotoxicity include:

- Decreased cell viability: The combination treatment resulted in a marked reduction in the percentage of viable cells.
- Morphological changes: Notable changes in cell morphology were observed, including reorganization of the actin cytoskeleton and impairment of nuclear integrity.
- Low confluence: The cell culture showed low cell confluence, indicating inhibited growth
- Apoptotic features: The typical features of apoptosis were present in the treated cells.

In addition, the combined treatment showed an antimigratory effect, characterized by suppression of cancer cell migration and downregulation of mRNA expressions for matrix metalloproteinases MMP-2 and MMP-9, which are involved in tumour invasion and metastasis. In addition, the combination demonstrated antiangiogenic effects in the chicken chorioallantoic membrane (CAM) model, indicating its potential to inhibit the formation of new blood vessels that supply tumours.