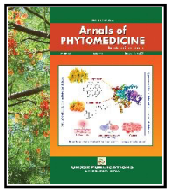
Annals of Phytomedicine 14(1): 34-43, 2025

DOI: http://dx.doi.org/10.54085/ap.2025.14.1.4

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Review Article : Open Access

Indole-3-carbinol (I3C) in cancer therapy: Mechanisms, clinical potential, and future perspectives

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Article Info

Article history
Received 20 January 2025
Revised 6 March 2025
Accepted 7 March 2025
Published Online 30 June 2025

Keywords
Indole-3-carbinol
Cancer
Anticancer mechanisms
Precision medicine
Cruciferous vegetables

Abstract

Indole-3-carbinol (I3C), a bioactive compound derived from cruciferous vegetables such as broccoli, cabbage, and Brussels sprouts, has garnered significant attention for its potential role in cancer prevention and treatment. This review comprehensively examines the mechanisms by which I3C exerts its anticancer effects, including its ability to modulate estrogen metabolism, induce cell cycle arrest, promote apoptosis, and inhibit angiogenesis. Preclinical studies have demonstrated I3C's efficacy in suppressing tumor growth in various cancer models, including breast, prostate, and lung cancers. Additionally, I3C has shown promise in enhancing the effectiveness of conventional chemotherapy agents while mitigating their toxic side effects. Despite these promising findings, clinical evidence remains limited, and further research is needed to establish optimal dosing, bioavailability, and long-term safety profiles. This review also explores the potential of I3C in precision medicine, where its ability to target specific molecular pathways could offer personalised therapeutic strategies. The safety and toxicity of I3C are discussed, highlighting the need for caution in high-dose supplementation, particularly in vulnerable populations such as pregnant women. Future research directions include developing novel delivery systems to enhance I3C's bioavailability and exploring its synergistic effects with other anticancer agents. Overall, I3C represents a promising natural compound for cancer prevention and treatment, warranting further investigation to fully realize its therapeutic potential.

1. Introduction

Indole-3-carbinol (I3C) is recognized as one of the most abundant and biologically active anticancer compounds identified to date from extracts of the Brassicaceae family. This family includes various members of the *Brassica* genus, which are well known for their high levels of I3C across different plant parts. It is commonly isolated from extracts of various cruciferous vegetables, including cabbage, broccoli, Brussels sprouts, cauliflower, radishes, garden cress, arugula, and horseradish (Sunitha *et al.,* 2024). These vegetables are not only staples in human nutrition and diet but are also extensively utilized in the pharmaceutical and cosmetic industries for their numerous beneficial properties (Figure 1). In essence, I3C can be classified as a derivative of benzyl alcohol (Centofanti *et al.,* 2023). Moreover, it is defined as a metabolite derived from indole-3-methylglucosinolate. This transformation occurs when the glucosinolate is hydrolyzed by the plant enzyme myrosinase, which is released from plant cells upon cellular damage or disruption. When the human body digests these cruciferous vegetables, I3C exerts significant bioactive effects, activating various detoxification enzymes (Zheng *et al.,* 2023). Additionally, it may enhance estrogen metabolism and influence multiple hormonal activities, collectively playing a crucial role in the complex interplay of hormones within the human estrogenic framework.

1.1 Chemical structure and properties

Indole-3-carbinol (I3C), which is also recognized by the name 3-(hydroxymethyl) indole, is an important bioactive ligand that is prominently found in various cruciferous vegetables such as broccoli, cauliflower, and Brussels sprouts (Reyes-Hernández *et al.,* 2023). The chemical stability of I3C can be quite sensitive, potentially leading to the loss of its bioactivity, if it is not handled with care and proper techniques. To effectively prevent the risk of oxidation, hydrolysis, and polymerization reactions that could compromise the compound's integrity, I3C should ideally be stored under acidic conditions, at lower temperatures, and kept shielded from light exposure. This careful handling is crucial to maintaining its beneficial properties (Lim *et al.,* 2021). I3C is categorized within the larger class of organic compounds known as indoles and their derivatives, which are identified as organic compounds that contain an indole structure (Li *et al.,* 2022). The indole itself is a bicyclic ring system that is composed of a six-membered benzene ring that is intricately fused to a five-membered nitrogen-containing pyrrole ring, forming a unique chemical framework. Indoles and their derivatives form a distinct family of compounds characterized by the presence of an indole moiety, which is typically derived from carbazole through the substitution of one of the nitrogen atoms with a second aromatic C-nucleoside (Poloznikov *et al.,* 2020). It is also notable that indole, being a non-cyclic compound, is formed as a result of the fusion of a

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benzene ring with a pyrrole ring. The compound plays several significant roles in biology, functioning as a nutraceutical, a metabolite involved in various biological pathways, a mouse metabolite essential for certain research studies, and a key metabolite derived from *Escherichia coli*, contributing to our understanding of microbial metabolism (Baez-Gonzalez *et al.*, 2023).

1.2 Natural sources, dietary intake, and their importance for health

Indole-3-carbinol (I3C) is a fascinating compound formed during the breakdown of glucobrassicin, a naturally occurring substance in cruciferous vegetables. This bioactive compound is predominantly found in various cruciferous vegetables, which are well known for their numerous health benefits (Bradlow *et al.,* 1996). These nutritious vegetables, including popular choices such as broccoli, cauliflower, cabbage, and Brussels sprouts, are widely recognized for their positive impact on health. Among cruciferous vegetables, Brussels sprouts, along with broccoli and cauliflower, stand out for their high concentrations of glucobrassicin (Michnovicz and Bradlow, 1991). This unique characteristic makes them excellent dietary sources of I3C. Regular consumption of these vegetables provides significant amounts of essential nutrients and beneficial compounds, including I3C, which contributes to overall well-being and health (Aggarwal and Ichikawa, 2005).The transformation of glucobrassicin into indole-3-carbinol (I3C) in the human body follows multiple pathways, influenced by factors such as gastric acidity, the type of vegetable consumed, its preparation method (raw, cooked, or processed), and the composition of gut microbiota (Weng *et al.,* 2008). When cruciferous vegetables containing glucobrassicin are chopped, chewed, or otherwise processed, the enzyme myrosinase is activated, leading to the breakdown of glucobrassicin (Figure 2). This enzymatic reaction results in the formation of I3C along with other metabolites, including oxazolidine-2-thione and related compounds.

If, I3C is consumed in supplement form or large quantities from cruciferous vegetables, the body's pH levels remain largely unaffected, preventing significant alterations in gastric conditions following high-dose intake (Leong *et al.,* 2001). Under these circumstances, I3C may bypass the conventional metabolic pathway and enter the bloodstream directly, where it undergoes further conversion into biologically active derivatives. These metabolites are believed to exert significant physiological effects, contributing to various health benefits (Cover *et al.,* 1999).

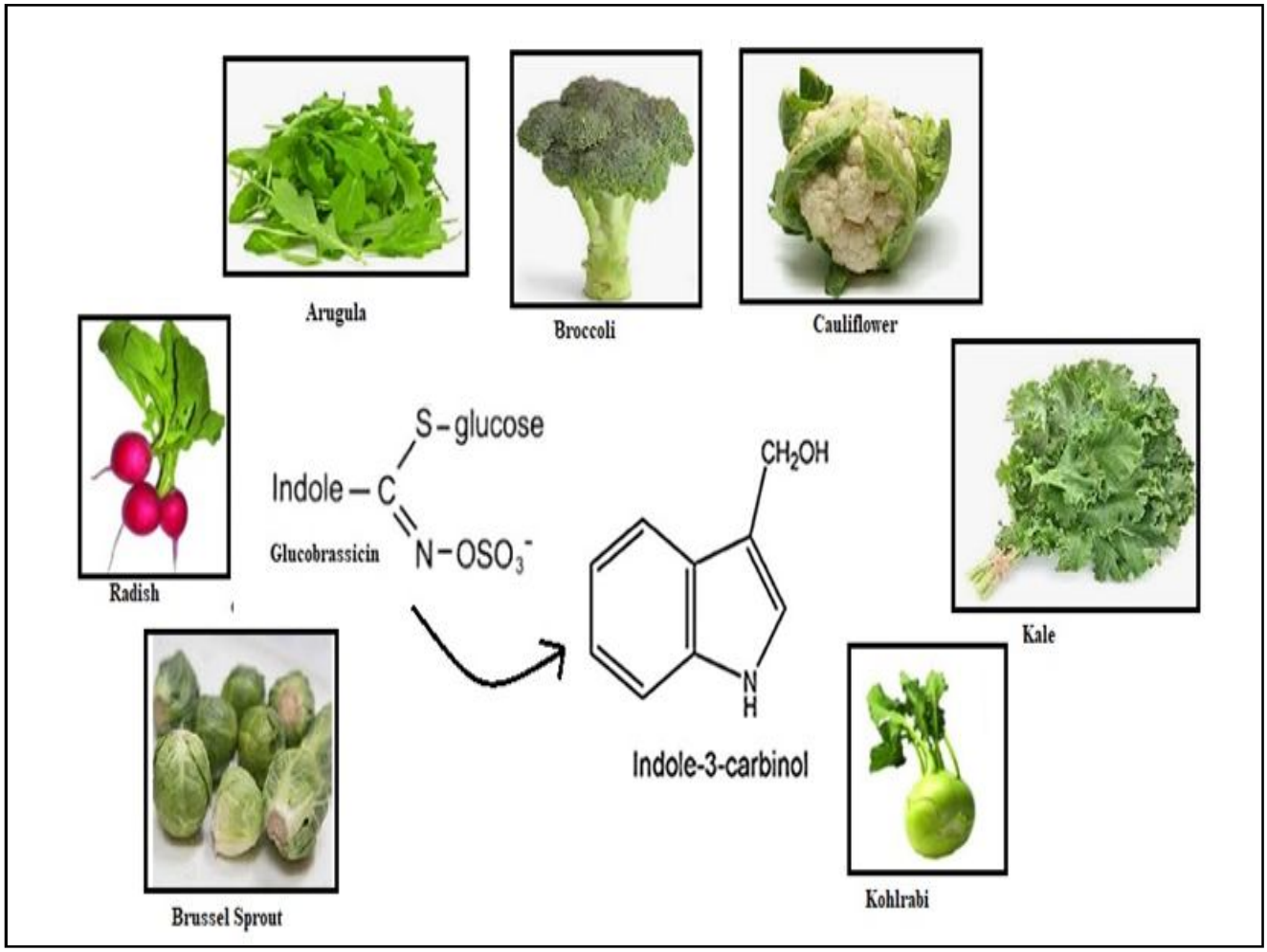

Figure 1: Members of genus *Brassica* which contain glucobrassicin that produce indole-3-carbinol.

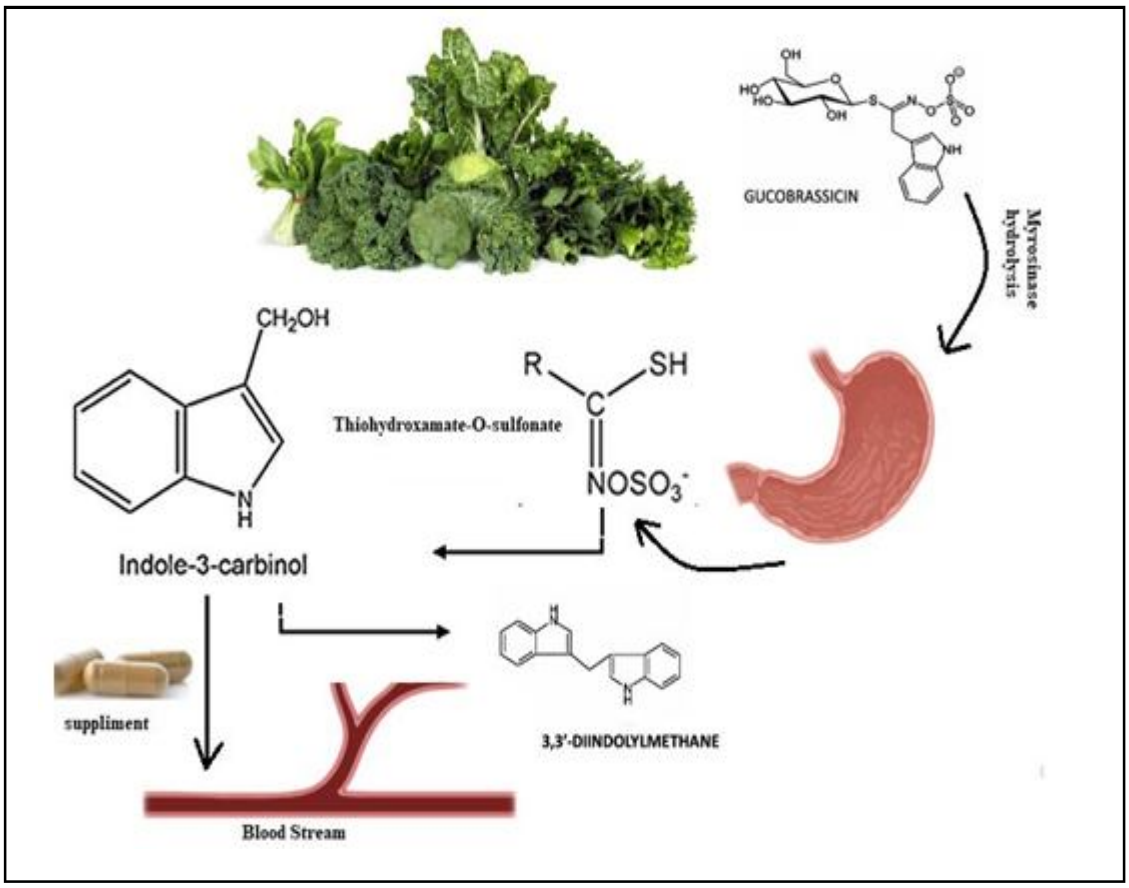

Figure 2: The pathways illustrating the conversion of glucobrassicin to diindolylmethane in the digestive tract, as well as the direct absorption of the supplement into the bloodstream without pH alteration.

2. Cancer: A global health challenge

Cancer is an ancient disease, but its recognition as a global health challenge in the modern era is relatively recent. Cancer research and treatment advancements accelerated following the discovery of X-rays' effects on tumours over a century ago; however, a definitive cure remains elusive. Cancer progression, metastasis, and patient tolerance to advanced treatments continue to present major challenges (Rahman and Sarkar, 2005; Banu, 2019). Over the years, various lifestyle-based and dietary agents have demonstrated the potential to prevent or treat cancers without the severe side effects associated with conventional therapies (Chinni *et al.,* 2001; Banu, 2024; Saidaiah *et al.,* 2024). These natural compounds enter cells gradually, are not typically included in drug cocktails, and can help protect normal cells from the toxic effects of radiation and chemotherapy. Among these agents, indole-3-carbinol (I3C) has emerged as a promising candidate for cancer prevention and treatment. Extensive research has shown that I3C, alone or in combination with other compounds and its dimeric derivatives, exhibits antiproliferative, pro-apoptotic, antiangiogenic, antimetastatic, and radiosensitizing properties (Bonnesen *et al.,* 2001). This analysis extensively discusses research data derived from various sources, including *in vitro* cell line studies, comprehensive *in vivo* animal models, retrospective studies, early-phase clinical trials, and industry-sponsored clinical investigations. Additionally, molecular mechanistic studies that provide insights into the bioavailability and pharmacodynamics of indole-3-carbinol (I3C) are thoroughly examined (Nachshon-Kedmi *et al.,* 2003).The scope of this review extends beyond the general aspects of I3C, encompassing critical topics such as bioavailability, pharmaco-dynamics, and clinically relevant data regarding its anticancer effects. These studies have been conducted across diverse geographical regions, including North America, Europe, and Asia, offering a broad international perspective (Kim *et al.,* 1997). Furthermore, future directions for the application of I3C both as a standalone agent and in combination with other cancer therapeutics are explored. Particular emphasis is placed on strategies to enhance its bioavailability and apoptosis-inducing properties, ultimately improving its efficacy in oncology (Meng *et al.,* 2000).

2.1 Epidemiology and impact

Worldwide, cancer is the second leading cause of death among various human diseases. Estimates from a dedicated cancer research agency indicate that approximately 12.7 million new cancer cases and 7.6 million cancer-related deaths occurred in 2008 alone. Projections for 2030 suggest that the number of new cancer cases could rise to around 22.2 million, with an expected 13.2 million deaths (Stresser *et al.,* 1995). This alarming increase is primarily driven by population growth and ageing demographics. Cancer is a complex disease characterized by multiple genetic factors and diverse biological pathways (Meng*et al.,* 2000). It typically arises from significant alterations in tumour suppressor genes and proto-oncogenes, which are crucial for cell regulation and growth (Auborn *et al.,* 2003).

Furthermore, changes in the human genome can result from various factors, including exposure to endogenous hormones, ionizing and ultraviolet radiation, and chemical agents encountered in the environment, including dietary components. Additionally, lifestyle factors, such as high-fat diets and other unhealthy habits, can further contribute to cancer risk and progression (Sarkar and Li, 2004).

The initial evidence highlighting the significant role of diet in cancer prevention emerged from several epidemiologic studies (Michnovicz and Bradlow, 1990). These studies revealed a notable inverse association between diets rich in fruits and vegetables and the risk of developing various diseases, including certain types of cancer (Chen *et al.,* 2001). The findings strongly suggest that numerous health benefits may be attributed to specific dietary bioactive compounds.

Among these bioactives, indole-3 derivatives, particularly those found in the Brassicaceae family of plants, have garnered significant attention. Within this plant family, indole-3-carbinol stands out as a particularly intriguing compound, believed to contribute to a measurable reduction in disease risk (Jin *et al.,* 1999). This review provides an in-depth exploration of the bioactivity, cancer prevention potential, and therapeutic capabilities of indole-3-carbinol. Additionally, it examines the reasons why indole-3-carbinol is not widely recommended as a standard treatment option (Reed *et al.,* 2006).

2.2 Cancer etiology and risk factors

Cancer is a highly complex and multifactorial disease, arising from the progressive accumulation of genetic and epigenetic alterations in critical cellular regulatory pathways (Kumar *et al.,* 2016). These alterations do not act independently; rather, they interact dynamically, often in unpredictable ways. Key oncogenic events, including prolonged exposure to carcinogenic chemicals or ionizing radiation, activation of proto-oncogenes, and the concurrent inactivation of tumour suppressor genes, frequently cooperate to drive malignant transformation (Johnson *et al.,* 2018). This intricate interplay of genetic defects unfolds over a multistage process known as tumorigenesis. Carcinogenesis is initiated by various exogenous and endogenous agents that interact with DNA, inducing mutations that can profoundly alter cellular homeostasis and promote neoplastic progression. However, not all individuals exposed to established carcinogens ultimately develop cancer, highlighting the role of additional modifying factors in tumorigenesis (Rawla *et al.,* 2019). Cancer development is influenced by a complex interplay of intrinsic and extrinsic factors. Genetic predisposition plays a crucial role, with individual susceptibility varying widely. Age is another significant determinant, as the risk of cancer increases with advancing years. Additionally, gender and race-associated disparities contribute to differences in cancer incidence and progression (Lundell, 2010).

Environmental exposures, including air pollutants, occupational hazards, and dietary patterns, further modulate cancer risk. Lifestyle factors such as tobacco use, alcohol consumption, physical inactivity, and obesity are well-established contributors to carcinogenesis. Moreover, psychosocial factors, including chronic stress and psychological distress, have been implicated in modulating cancer susceptibility (Petrakis, 1977). Endocrine and metabolic dysregulation, along with subtle molecular changes at the epigenetic level over time, further contribute to cancer pathogenesis. Understanding these multifactorial interactions is essential for developing effective preventive and therapeutic strategies against cancer. The majority of human cancer cases are strongly associated with exposure to various physical, chemical, and environmental carcinogens, highlighting the significant role of lifestyle factors in both cancer development and prevention (Sorgun *et al.,* 2018). While conventional cancer therapies, including chemotherapy and radiation, have been extensively employed, they often lack specificity for distinct cancer types and may exhibit carcinogenic properties that can pose additional risks to patients undergoing treatment. As a result, contemporary cancer research is increasingly focused on the exploration of non-toxic, naturally occurring compounds as potential alternatives to conventional therapies (López Lázaro, 2018).

These bioactive compounds often exhibit multi-targeting capabilities, influencing multiple signaling pathways simultaneously, and their pleiotropic effects provide broader therapeutic benefits beyond their anticancer activity. Among the various naturally derived anticancer agents, indole-3-carbinol (I3C) has attracted substantial scientific interest due to its promising chemopreventive and therapeutic potential. Predominantly found in cruciferous vegetables such as broccoli, cabbage, and Brussels sprouts, I3C has demonstrated potent antiproliferative, pro-apoptotic, and anti-metastatic properties in various preclinical and clinical studies. Its emerging role as a natural cancer-preventive agent warrants further investigation to optimize its bioavailability, elucidate its precise molecular mechanisms, and explore its potential for integration into standard cancer treatment regimens (Licznerska and Baer-Dubowska, 2016).

3. Understanding cancer development

The continuous possibility of cancer development arises from the persistent accumulation of intrinsic mutations, coupled with the selective advantage conferred upon certain cellular populations. This dynamic process is tightly regulated by a specialized subset of cells within multicellular organisms known as stem cells. From the earliest stages of embryonic development, stem cells originate from fertilized egg cells, subsequently proliferating and differentiating into various specialized somatic cells that constitute the body (Ebben *et al.,* 2010). However, stem cells do not disappear upon differentiation; rather, they maintain a critical role by giving rise to subsequent generations of stem cells, ensuring tissue homeostasis and regeneration (Ebben *et al.,* 2010).

Stem cells possess two defining properties: self-renewal, which enables them to undergo numerous rounds of division while retaining an undifferentiated state, and the ability to differentiate into specialized somatic cells, which perform essential physiological functions (Malin *et al.,* 2006). These characteristics are fundamental for proper development and tissue maintenance throughout an organism's lifespan. In adults, stem cells remain crucial for the regeneration and repair of various tissue-specific cell types, contributing to the maintenance of organs such as the intestine, pancreas, bone marrow, and brain (Cheng *et al.,* 2012).In the context of cancer, the significance of stem cells lies in their ability to generate diverse cellular lineages within adult tissues. While most cancer cells exhibit disrupted differentiation pathways, leading to uncontrolled proliferation, tumours originating from cells with stem cell-like properties remain relatively rare compared to other malignancies (Papaccio *et al.,* 2017). The histological distinctions between tumours and normal tissues are a cornerstone of cancer diagnostics, as pathologists rely on cellular morphology to identify malignancy

(Soltanian and Matin, 2011). However, this diagnostic approach has limitations, particularly in identifying cancers that do not conform to conventional classification systems, thereby posing challenges in achieving accurate and timely diagnoses (Ward and Dirks, 2007).

3.1 Carcinogenesis and tumour formation

The body's intricate response to indole compounds and their various derivatives is governed by a complex interplay of physiological and molecular factors (Wogan *et al.,* 2004). These factors include the presence of specific hormones within the biological system, the distinct characteristics of various organs, and the diverse cell types involved in these processes (Kontomanolis *et al.,* 2020). Moreover, these responses reflect a dynamic influence on gene expression, highlighting the interconnected nature of biological pathways. The modulatory effects of pro- and anti-estrogens further underscore the complexity of these physiological responses, demonstrating that they arise from an intricate network of interdependent molecular events (Cosper *et al.,* 2021).The interaction of estrogens with diverse receptor subpopulations throughout the body plays a critical role in hormonal signaling, profoundly affecting overall health. The broad spectrum of effects exerted by these bioactive compounds on hormone metabolism is a key factor underlying their potential as cancer-preventive agents (Bizzarri and Cucina, 2014). This is particularly evident in hormone-sensitive organs such as the mammary gland, a frequent site of tumorigenesis in women. Furthermore, hormone-dependent modulation plays a pivotal role in catalyzing hypertestosterone and estrone catechols, leading to the formation of hormonally inactive hydroxy-estrones. These inactive metabolites may contribute to chemoprevention by mitigating the effects of hormone-driven carcinogenesis. Given these mechanisms, indole-3-carbinol and its structurally related molecules have been extensively evaluated in preclinical models, demonstrating a range of biological benefits. These findings have been further validated in well-structured human trials, providing substantial evidence for their efficacy (Wang *et al.,* 2017). The accumulated research significantly enhances our understanding of the molecular basis of cancer prevention and opens new avenues for therapeutic exploration in this field (Schedin and Elias, 2004).

3.2 Key molecular pathways in cancer

Therapeutic strategies for cancer treatment often involve the precise surgical removal of malignant tissue, followed by adjuvant chemotherapy or the administration of chemopreventive agents (Dep Prete *et al.,* 2011). Chemoprevention, which utilizes naturally occurring, non-toxic dietary compounds, represents a promising avenue in cancer treatment and prevention research (Prickett and Samuels, 2012). The supplementation of these bioactive compounds in the diet is a scientifically supported approach to reducing cancer risk. Historically, various foods have been valued not only for their nutritional benefits but also for their prophylactic and therapeutic effects against numerous diseases, including cancer. Among these, sulforaphane and indole-3-carbinol, found in cruciferous vegetables, have garnered significant attention for their anticancer properties. These compounds selectively target mechanisms involved in cancer development and progression, making them potential candidates for effective cancer therapies (Mantovani, 2010).The natural prevention of cancer by these dietary compounds is believed to involve multiple molecular pathways. Bioactive agents have been extensively recognized for their role in regulating apoptosis, cell cycle progression, and cellular proliferation, key processes that influence the transformation of premalignant cells (Gonzalgo and Isaacs, 2003). The anticancer activity exhibited by these compounds in preclinical models highlights their potential therapeutic impact. Their ability to inhibit the dysregulation of critical cellular processes through modulation of molecular pathways is a pivotal factor in their protective effects. Consequently, contemporary anticancer strategies increasingly focus on targeting deregulated apoptotic and cell survival signaling pathways to suppress cancer progression. Dysregulation of these pathways has been directly implicated in tumorigenesis, further underscoring the significance of dietary chemopreventive agents in cancer management (Gonzalgo and Isaacs, 2003).

4. Indole-3-carbinol (I3C) and cancer prevention

Cancer, as a group of diseases, results from the excessive growth of cells, wherein the body loses the ability to maintain homeostasis. The abrogation of cancer initiation may serve as a novel approach to treating or preventing cancer development. 3,3'-Diindolylmethane DIM, a major product of indole-3-carbinol I3C, plays a crucial role in the cancer preventive and therapeutic effects of I3C (Nachshon-Kedmi *et al.,* 2003). Indole-3-carbinol, present in cruciferous vegetables, exhibits cancer-preventive activity in various animal models of chemical carcinogenesis. It is also noted for its ability to induce cell cycle arrest followed by apoptosis in tumour cells. Indole-3-carbinol influences cyclin and cyclin-dependent kinase protein levels in tumour cells, although, dose-response and time-course relationships have yet to be fully elucidated. It causes both cyclin-dependent kinase inhibitor 1A CDKN1A-mediated G1 cell cycle arrest and downregulation of cyclin B1 and cyclin A, leading to apoptosis in luminal-like human breast cancer cells (Megna *et al.,* 2016).Indole-3-carbinol inhibition is associated with the induction of CDKN1A and BCL2-associated X, as well as proteasomal degradation of cyclin B1. It directly inhibits cyclin A and cyclin B1-cyclin-dependent kinase 1 complexes, resulting in the nuclear localization of cyclin B1 mediated by cyclin-dependent kinase inhibitor 1B protein. This inhibition leads to G2/M cell cycle arrest. These findings highlight the significance of timing and dosage in administering potential cancer-preventive agents. Indole-3-carbinol may not only mediate cyclin B1 proteasomal degradation but also influence cyclin-dependent kinase inhibition (Wong *et al.,* 1997).

4.1. Mechanisms of action

Several potential anticancer actions have been thoroughly described for I3C. Many of these notable effects have been attributed specifically to diindolylmethane DIM, the principal *in vivo* product of I3C (Michnovicz *et al.,* 1997). I3C notably increases the rate of estradiol 2-hydroxylation while simultaneously reducing 16 alpha-hydroxylation. This significant shift in hydroxylase activity favours the enhanced synthesis of the weaker estrogen, estrone, while concurrently decreasing the production of the more potent estrogen, estradiol. The net effect of these biochemical changes results in lower intracellular concentrations of biologically active estrogen. Consequently, there is reduced exposure of estrogen target tissues to estrogen, potentially leading to a decreased risk of certain estrogen-dependent cancers (Michnovicz *et al.,* 1997).

CYP1A2 plays a crucial role in the metabolic process of estradiol 2-hydroxylation (Sepkovic *et al.,* 1994). This metabolic pathway is

essential for converting estradiol into its 2-hydroxylated form, which may influence its biological activity and overall effects in the body. The observation that I3C enhances estradiol 2-hydroxylation strongly suggests that CYP1A2 is induced by this compound. Supporting this, a study by Sepkovic *et al*. (1994) confirmed that the 2-hydroxylation of estrogens, specifically when tested on primary cultured human hepatocytes, shows a marked increase in the presence of I3C (Sepkovic *et al.,* 1994). However, this phenomenon's apparent contradiction could stem from varying I3C concentrations. Cytochrome P4501A2 induction appears to occur at lower concentrations of I3C, while at higher concentrations, this induction may not be as effective. Furthermore, the elevated mRNA levels of additional enzymes involved in estradiol 2-hydroxylation, including CYP1A1 and CYP1B1, due to I3C exposure, provide further compelling evidence supporting these findings and enhancing the understanding of this complex biochemical interaction (Sepkovic *et al.,* 1994).

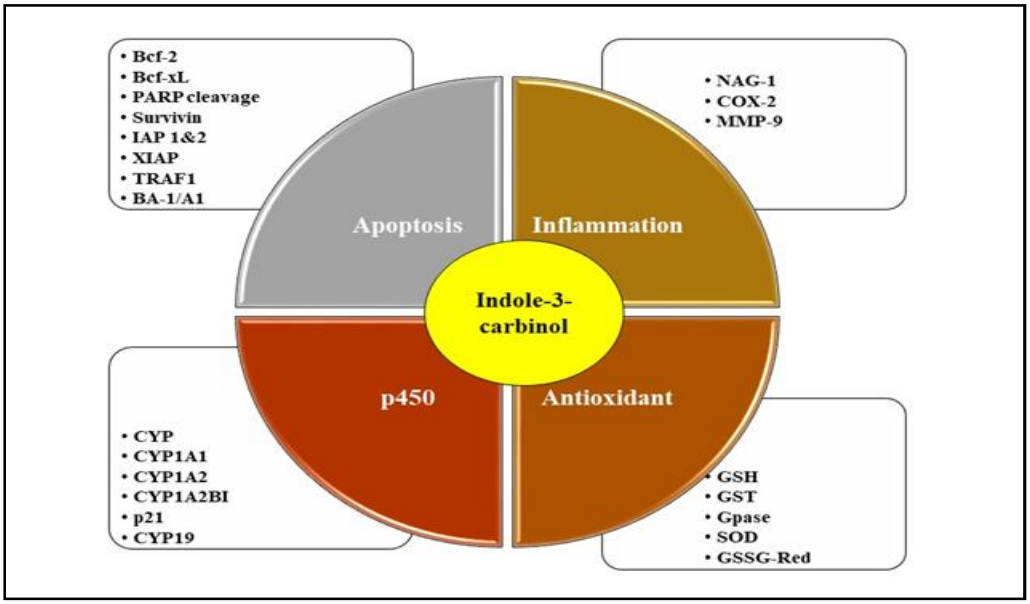

Figure 3: Different therapeutic targets of I3C and its analogues. The superscript indicates the reference number. The abbreviations used are: NF-κB, nuclear factor-κB; IκB, inhibitory subunit of NF-κB; IKK, IêBákinase, IAP, inhibitor-of-apoptosis protein; XIAP, X-chromosome-linked IAP; COX, cyclooxygenase; MMP, matrix metalloproteinase; TRAF, TNF receptor-associated factor, UDPGT; UDP-glucuronosyl transferase, GSH; Glutathione, GST; glutathione S-transferase, GSSG-Red; glutathione reductase, glutathione peroxidase, GPase; CYP; cytochrome P450, FMO;flavin-containing monooxygenase, PKB; phosphatidylinositol 3'-kinase and protein kinase B (also called AKT), CDK; cyclin-dependent kinase,MUC; NAG; Non-steroidal anti-inflammatory drug-activated gene-1,ATF; activating transcription factor; Nrf2; Nuclear factor-E2-related factor 2, PARP; poly (ADP ribose) polymerase,ER; estrogen receptor, BRCA-1; breast cancer susceptibility gene 1, EROD; ethoxyresorufin O-deethylase, DR; TRAIL death receptor, JNK; c-Jun NH2-terminal kinase, DTD; DT-diaphorase = NAD(P)H-quinone reductase; superstar refers to activation or phosphorylation.

5. Safety and toxicity of indole-3-carbinol

Indole-3-carbinol (I3C) is a naturally occurring compound found in cruciferous vegetables that has potent anticancer properties. However, no two individuals are the same, and the efficacy of chemoprotective agents differs among individuals (Centofanti *et al.,* 2023). The tendency is to emphasize the essential novelty of these compounds without paying nearly as much attention to their safety and potential adverse effects. One of the major components of I3C metabolism is 3,3'-diindolylmethane (DIM), which is often used as a potential dietary supplement for its health-promoting potential in humans. Consumption of high cruciferous vegetable intakes would likely supply considerable quantities of I3C; however, high volumes may be intolerable, and potential liver sequelae could occur. Studies indicate that, in general, dietary consumption of cruciferous vegetables or I3C supplementation and/or DIM does not produce any significant adverse effects (Chen *et al.,* 2001). In this regard, although, I3C is generally regarded as nontoxic, reproductive data remain sparsely investigated, and information that can be obtained is based on animal models. As the prevalence of reproductive disorders increases, it is important to understand sexual differentiation and the development of the reproductive system (Michnovicz and Bradlow, 1991). The data suggest that I3C is an endocrine disruptor and can interfere with sexual development. Given the numerous benefits of I3C, if a woman is unlikely to consume the suggested daily servings of cruciferous vegetables, then consuming I3C as a supplement would be an alternative. However, consuming supplements when unnecessary could lead to hypervitaminosis and unintended health problems. I3C should be used cautiously by women who are pregnant, as infections and developmental disorders have previously been described with insufficient information on their toxicity (Michnovicz *et al.,* 1994).

5.1 Exploring the bioavailability and metabolism of compounds

Oral supplements of I3C have recently become widely available in the market, attracting significant attention from health enthusiasts and researchers alike (Julliard *et al.,* 2017). However, due to the variable degradation and disappearance of I3C, as found in both experimental and analytic studies of various I3C preparations, pharmacokinetic studies of I3C can be very challenging to design and execute properly. Research on such product development remains quite meager compared to the tremendous amount of studies demonstrating I3C's role in enzyme induction and its anticancer effects (Julliard *et al.,* 2017). Naturally occurring I3C, found in vegetables like broccoli, has consistently been reported to exert a notable suppressing effect on chemical carcinogen-induced cancers in experimental animals, leading to increased interest in its potential health benefits. However, it is still unknown whether biologically detectable levels of I3C would be absorbed following ingestion of relatively small quantities of food that naturally contain I3C. Some glucosinolates related to I3C have been reported to exhibit no activity against the diethylstilbestrol-stimulated neo-ACL-T1 focus in the rat mammary gland. Consequently, whether I3C is the only active agent responsible for the observed effects or if it must first be absorbed and subsequently converted into its other metabolites to exert its full effectiveness remains a pertinent question. This issue awaits further experimental animal studies involving various forms and concentrations of I3C to provide a clearer understanding of its mechanisms (Sunitha *et al.,* 2024).

5.2 Adverse effects and interactions

Several small clinical trials have tested I3C in combination with chemotherapy drugs. One trial investigated the effects of radiation therapy, chemotherapy, and I3C in recurrent respiratory papillomatosis, a rare childhood airway tumour caused by human papillomavirus. In these trials, researchers aimed to increase patients' levels of cytochrome P450, a protective body protein, using I3C before administering a chemotherapy agent (Centofanti *et al.,* 2023). Other trials have explored the potential of I3C to reduce chemotherapy-induced toxicity. However, many of these studies are significantly underpowered to conclusively answer their research questions. Animal studies suggest that I3C can enhance the activity of AHL lactonase, an enzyme that mitigates bacterial-induced damage to host tissues when exposed to chemotherapy drugs. In preclinical models, this effect appears to reduce the gastrointestinal and haematological toxicities associated with high doses of 5-fluorouracil, without compromising the drug's efficacy. Further research is needed to confirm this potential role of I3C in humans.

Most of the adverse effects observed in patients were not severe in nature, yet they occurred most frequently at a typical dose of around 200 mg per day. Among the reported adverse effects, individuals experienced symptoms such as dizziness, which can be quite uncomfortable, uncontrolled tremors affecting the whole body, feelings of both euphoria and dysphoria that could lead to significant mood changes, persistent headaches, ataxia which affects coordination, and instances of vomiting that can be distressing. More serious effects have been reported, including conditions such as angiogenic edema, which may lead to swelling, foot drop that can impair mobility, and sudden severe swelling of the jaw that may be alarming (Connor *et al.,* 2011). These mentioned effects are just a sampling of the various treatment-related adverse effects associated with the use of I3C, which is indole-3-carbinol. To prevent the occurrence of adenomas, individuals were subjected to a treatment regimen where they received daily doses of 30 to 60 mg of I3C for a period of 12 months. During this treatment duration, all participants experienced one or more of the aforementioned adverse effects while consuming I3C. Although, it is worth noting that none of the effects experienced were classified as severe, it was also observed that there was no apparent advantage or benefit to taking a larger dose of the compound. Researchers have been diligently attempting to initiate a Phase II study on the effects of I3C. However, it was concluded that the compound would pose significant challenges in being taken as recommended by the medical community. Interestingly, in the year 2015, various toxicity reviews regarding I3C observed notable inconsistencies concerning its previously misapplied use: the administration of lower doses, specifically 100 mg a day or even less, was found to be permissible and remarkably, it did not lead to any of the adverse effects that had been previously documented (Licznerska and Baer-Dubowska, 2016; Adetuyi *et al.,* 2021; Jagtap *et al.,* 2022).

6. Future directions in I3C research

Research on indole-3-carbinol (I3C) has demonstrated promising anticancer potential; however, several limitations persist in both preclinical and clinical studies. To fully elucidate the therapeutic mechanisms and benefits of I3C, critical challenges related to its pharmacokinetics, bioavailability, and systemic effects must be addressed. As a modulator of cytochrome enzymes with cytotoxic activity against cancer cells, I3C and its metabolite diindolylmethane (DIM) are predominantly obtained from cruciferous vegetables such as Brussels sprouts and cabbage, as well as commercial supplements (Michnovicz and Bradlow, 1990). With the growing recognition of I3C's potential health benefits, research efforts must prioritize the development of safe and efficient delivery systems. Prodrug formulations that prevent rapid metabolic conjugation, such as glucuronidation and sulfation, could enhance its bioavailability and therapeutic efficacy.

Discrepancies between *in vitro* and *in vivo* findings highlight the complexity of I3C's pharmacological effects, which are influenced by administration route, dosage, and experimental design. Notably, variations in outcomes have been observed based on whether I3C was administered prophylactically or therapeutically, delivered *via* dietary intake or injection, and whether androgenic modulation was considered. Additionally, oral administration does not necessarily correlate with systemic availability, further complicating its application in cancer prevention (Broadbent and Broadbent, 1998). Beyond oncology, the broader physiological effects of I3C in non-malignant cells remain insufficiently explored. Moreover, the efficiency of I3C detoxification appears to be age-dependent, suggesting that intracellular concentrations of free I3C cannot be reliably inferred from extracellular measurements. Addressing these knowledge gaps will be critical for optimizing I3C's clinical application while ensuring its safety and efficacy across diverse patient populations.

7. Conclusion

Cancer is a major global health burden with a strong dietary influence, making it an ideal model for identifying functional dietary constituents that can modify carcinogenic processes. Phytochemicals, particularly those derived from fruits and vegetables, are increasingly recognized

for their potent cancer-preventive and therapeutic properties. Nutrigenomics, which explores the interplay between nutrition and gene expression, highlights the impact of diet on physiological phenotype.Among the most studied dietary constituents are members of the Brassica family, which are rich in glucosinolates and their bioactive hydrolysis products. This review focuses on indole-3-carbinol (I3C), a key compound in this family, known to inhibit carcinogenesis at multiple stages. I3C exhibits well-characterized antioxidant, anti-inflammatory, detoxification enzyme-inducing, and hormone-modulating properties in various laboratory models. The promising epidemiological evidence supporting cancer prevention underscores the need for further translational research to bridge the gap between laboratory findings and clinical outcomes.A comprehensive, lifestyle-based approach to cancer prevention remains crucial, as it can serve as a major modulatory factor in reducing the risk of cancer progression. The growing body of mechanistic data on I3C's ability to regulate genes and transcription factors provides a strong foundation for advancing its application in clinical settings.

Acknowledgements

Authors are thankful and acknowledge researchers of the original research works whose publications are cited in the present review.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Johra Khan (2025). Indole-3-carbinol (13C) in cancer therapy: Mechanisms, clinical potential, and future perspectives. Ann. Phytomed., 14(1):34-43. http://dx.doi.org/10.54085/ap.2025.14.1.4.**