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Xanthohumol and Breast Cancer

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Xanthohumol and Breast Cancer

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Xanthohumol and Breast Cancer

Narrative Review

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Review article

Xanthohumol and Breast Cancer

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Abstract:

Background: Breast cancer remains one of the most prevalent cancers among women, posing significant challenges in treatment. Xanthohumol (XN) has demonstrated notable chemopreventive and chemotherapeutic properties against breast cancer cells.

Method: This literature review analyzes research from the Medline database via PubMed using the search term "Xanthohumol AND breast cancer." Studies published between 1999 and 2024 were screened, yielding 45 relevant articles, with 35 selected based on their focus on the anticancer effects of XN. The review covers *in vitro*, *in vivo*, and *ex vivo* studies describing the impact of XN on cancer cell proliferation, viability, apoptosis, invasion ability, and chemoresistance. Additionally, studies on XN derivatives were reviewed to assess their potential in overcoming current therapeutic limitations of XN.

Results: XN exhibits potent antiproliferative and cytotoxic effects by inducing apoptosis, modulating key signaling pathways, and inhibiting DNA synthesis in breast cancer cells. It enhances chemotherapy and radiotherapy efficacy by downregulating multidrug resistance-associated proteins. Several XN derivatives have shown improved potency. *In vivo* studies demonstrate the ability of XN in suppressing tumor growth and metastasis via multiple mechanisms, including Notch signaling inhibition and immune modulation.

Discussion: Despite its promising antitumoral effects, clinical application of XN is hindered by poor solubility, rapid metabolism, and low oral bioavailability. Structural modifications have enhanced stability and efficacy, but optimized delivery methods are needed. Further studies are required to evaluate potential off-target effects, long-term safety, and the role of XN metabolites in its anticancer activity.

Conclusion: XN presents strong anticancer potential against breast cancer by inhibiting proliferation, inducing apoptosis, and overcoming chemoresistance, making it a promising candidate for future therapeutic strategies.

Keywords: Xanthohumol (XN), breast cancer, anticarcinogenic effects, cancer prevention, cancer therapy, Xanthohumol (XN) derivatives

1. Introduction

1.1. Hops and Xanthohumol

Hops (*Humulus lupulus* L., family *Cannabaceae*), or more accurately, the female plant's flowers (cones), are currently widely used in the brewing industry to provide a characteristic flavour and bitterness to beer. However, hop has been used since 200 A.D., with therapeutic treatments beginning as early as the 9th century [1]. When it was discovered in 79 A.D., it was popular as a vegetable, but it was later utilized as a dye and a culinary flavor ingredient [2,3]. It was also used in the production of coarse textiles and paper. Hop plants were traditionally used as a sedative for treating sleeplessness and restlessness, as well as for relieving ear discomfort, toothache, and loss of appetite [1]. Currently, hop products are also often used as dietary supplements because of their hypnotic and anxiolytic characteristics, and they are utilized in the treatment of postmenopausal symptoms in women. Furthermore, they are broadly applied in the cosmetic and pharmaceutical industries, owing to their antibacterial and antiviral properties [4,5].

Hop versatility lies in the fact that it possesses a wide range of physiologically active compounds [6,7]. Among hundreds of compounds present in hops, the most significant chemical is the chalcone xanthohumol (XN) (corresponding up to 1% of dried hop cones).

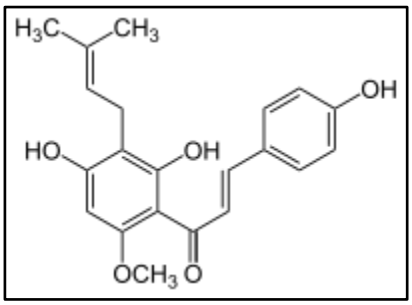

Fig. (1). Chemical structure of XN.

1.2. Chemical structure of Xanthohumol (XN)

The molecular structure of XN was discovered in 1957 (Fig. (1)), but its favorable pharmacological properties were not recognized until the 1990s [9]. Numerous

works carried out in recent years have demonstrated its anti-inflammatory, antibacterial, antiviral, antifungal, antiplasmodial, and anticancer properties [10-12]. Because XN possesses multiple beneficial properties in addition to its natural and relatively non-toxic nature, it could be a very promising active substance for further *in vivo* and clinical investigations as well as possible applications in a range of therapies.

However, the main challenge for the clinical application of XN is its poor delivery to the target site due to its high hydrophobicity, low stability, high photosensitivity, short half-life, and poor oral bioavailability [5].
Indeed, the observation that both XN and its metabolites are primarily excreted in feces within 24 h of administration is an important factor limiting the clinical application of this compound [25]. Therefore, it is necessary to create innovative formulations that overcome all of the restrictions associated with the low XN oral bioavailability and delivery to the target site while maintaining the biotransformation steps.

Because of its molecular instability, due to a high sensitivity to light, pH and temperature, most XN is readily converted into metabolites. In acidic environments like the gastric lumen or through the process of brewing beer, or on alkaline environments, isomerization of XN into Isoxanthohumol (IXN) occurs leading to the formation of this metabolite. The same reaction can happen through heat exposure and enzymatic reduction by the gut microbiota or cytochrome P450 enzymes in the liver [65, 66]. IXN can in fact be the molecule available in very high concentrations and thus be the one exerting effects in the human cells. IXN is a more stable molecule than XN but nevertheless it is still frequently converted to 8-prenylnaringenin (8-PN) and less frequently to to 6-prenylnaringenin (6-PN) by the gut microbiota and liver enzymes [65]. So, although one might conclude that a given effect is caused by XN, further investigation evaluating whether the reported effects are not in fact caused by its metabolites is however required. For instance, the estrogen-inactive XN possesses the potential of being converted to the estrogenic IXN and 8-PN [2,8]. So, elucidation of the activity of XN metabolites is incredibly important.

1.3. Breast cancer and Xanthohumol

The global incidence of breast cancer increased rapidly during the 1980s and 1990s, largely due to enhanced detection methods [13]. After the death rate peaked in the late 1980s, it sharply decreased as the way in which breast cancer was managed changed [24]. Both mass mammography screening and better treatment have contributed to breast cancer mortality reduction. However, in recent years, the

decline in mortality has slowed to 1.3%, which could result from the gradual increase in the incidence of breast cancer associated with a stable prevalence of screening [22]. Indeed, since the beginning of the XXI century, the burden of breast cancer is rising, and this trend continues today [17,20,21]. The explanations might encompass increased body mass index (BMI) and decreased birth rate [16]. In 2020, female breast cancer became for the first time the most commonly diagnosed cancer globally, with an estimated 2.26 million new cases reported [18]. Correspondingly, the latest global statistics showed that breast cancer was one of the leading causes of cancer-related mortality among women, ranking fifth in overall cancer deaths [14].

Although breast cancer treatment seems to be ever more effective with higher rates of success, there are still several cases where chemotherapy resistance or rapid disease progression inevitably leads to unsuccessful treatment and reduction of the quality of life of these patient. The most recent prediction suggests that by 2040, the global burden of breast cancer is expected to increase to over 3 million new cases annually [19].

In this context, further research on new potential therapeutic agents for breast cancer is warranted. XN appears to be a very promising chemopreventive and chemotherapeutic drug for breast cancer. First, XN possesses several beneficial effects on human health, including chemopreventive and chemotherapeutic effects [25]. Secondly, the natural origin and low cost of production are two very important characteristics that should be considered and motivate further research on its effects. It can also be said that it is rather safer option than current treatments available in healthcare, supported by the presence of Hops (the plant) in the human diet for several generations.

2. Methods

This work is a review based on literature research of studies available on the Medline database. The PubMed search engine was used, applying the following query: Xanthohumol AND "breast cancer". All articles from 1999 to 2024 were considered, resulting in a total of 45 papers, which were further assessed based on the title, abstract and full texts. A total of 10 articles were excluded (6 based on the title, 2 on the abstract and 2 based on the analysis of the full). The articles included specifically mention original anticancer effects of XN or XN derivatives on breast cancer cells or breast cancer i*n vivo*. The result was a total of 37 original articles considered for the elaboration of this review, including *in vitro*, *in vivo* and *ex vivo* studies.

3. Results

3.1. In vitro effects of XN on breast cancer cells

3.1.1 Effects of XN on breast cancer cell proliferation and viability

XN shows pleiotropic effects in breast cancer cells, affecting several signaling pathways, and thus influencing cellular function on multiple levels. One of the effects of XN in breast cancer cells is an inhibition of cancer cell proliferation and viability (Table 1).

The antiproliferative effects of XN are achieved by arresting cell cycle progression, by inhibiting DNA or protein synthesis, and by influencing cellular signaling pathways controlling growth. On the other hand, the cytotoxic effects of XN are associated with apoptosis or necrosis in the breast cancer cell lines.

Theoretically, these are two different effects of XN, but in reality, it is arguable that the mechanisms responsible for each are interdependent. Moreover, some of the assays used were differently interpreted as showing antiproliferative or cytotoxic effects in different studies, and so we were unable to make an unequivocal distinction.

3.1.1.1. Antiproliferative effects of XN

The antiproliferative effect of XN on breast cancer cells is particularly interesting, as it supports consideration of XN as a treatment option for breast cancer in the future [26-29,31-34,42,46,49,50-52] (Table 1). While analyzing the potential antiproliferative effects of XN, it is reported that proliferation may be reduced by tighter packing of DNA molecules [26]. XN was also shown to increase the expression levels of proteins such as p21 and p53 that are responsible for inducing cell cycle arrest and thus reduce proliferation [27]. Similar results may be achieved by reducing expression levels of NFKB 2/1 protein, which has been linked to an increase in apoptosis and delay in cell growth [45]. XN can also decrease levels of the survivin and FAK that have been shown to be important stimuli for cell survival and proliferation [27].

Kim *et al*. reported that XN caused a reduction in expression levels of the Bcl-2 protein, which plays an important role as an apoptotic inhibitor, in the MDA-MB-231 breast cancer cell line [28]. XN can also reduce proliferation by reducing DNA synthesis [29].

Sun *et al.* showed that XN treatment reduced proliferation by regulating the Notch1 pathway, as its inhibition causes a reduction in cellular proliferation, a fact

that is also supported by the concurrent reduction of downstream protein such as Hes1 and Hey1, which are both involved in cycle arrest suppression and promote cell proliferation. Also supporting the antiproliferative effect of XN is the increase in p21 and decrease in CDK4 and cyclin D1, increasing antiproliferative stimuli and decreasing cell cycle progression-inducing stimuli, respectively. Moreover, similarly to aforementioned studies, XN decreased survivin and c-myc, both known for their role as proliferation inductors [31].

Another particularly interesting effect of XN is its ability to interfere with E2-induced cell proliferation and thus to reduce ERα-positive tumour growth. The way through which it is achieved seems to be by binding to PHB2 and in doing so disrupting the BIG3-PHB2 interaction [32].

Interestingly, Blanquer-Rosselló et al. described that XN had a biphasic effect on proliferation, reducing it at high concentrations while actually increasing proliferation at low concentrations. A similar outcome was observed in regard to ROS production (a decrease at lower concentrations and increase at higher concentrations), and the explanation for this biphasic effect was that at low concentrations there was a concurrent reduction of catalase, SOD and glutathione reductase and at higher concentrations glutathione reductase and protein carbonylation increased. Moreover, an increase in SIRT1 and OXPHOS complexes was also observed at low concentrations only [33].

In another study, the antiproliferative effect of XN was shown not to be related to the ability of this compound in inhibiting the cellular uptake of glutamine [34].

Roehrer *et al.* reported that the antiproliferative effect of XN was related to changes in proteins involved in cell cycle, DNA replication and type I interferon pathway [42]. Finally, the effects of XN were reported to be achieved by reducing the expression of Notch1 and ABCG2 [46].

Importantly, the antiproliferative effects of XN were described to be highly selective for breast cancer cell lines [46,50].

3.1.1.2. Effects of XN on cellular viability

Several works clearly established that XN can reduce breast cancer cell viability [26,27,29,31,36-38,42,43], and several works relate that XN reduces not only proliferation, but also the viability of breast cancer cells [29,34, 46,47,49,50-52] (Table 1).

Gieroba *et al.* showed that XN can reduce cell viability by inducing disturbances to the cell membrane and by inducing morphological changes to the breast cancer

cells [26]. Additionally, viability was concluded to be reduced by XN by reducing the expression levels of NFKB2/1, SURVIVIN [27], reducing DNA synthesis [29] and reducing SURVIVIN and c-myc expression [31]. Additionally, XN was reported inhibit hormone-dependent stimuli vital for cell viability [37].

Another report concluded that the cytotoxic effect of XN was related to changes in proteins participating in type I interferon pathway (SQSTM1, ISG15, OAS3, IRF9, STAT1, PARP9 and DTX3L) and JAK/STAT signaling [42].

Another way through which XN reduces breast cancer cells viability is through direct DNA damage as reported by Liu *et al.*, after observing an increase in γ-H2AX. In the same work, XN was found to potentiate the cytotoxic effect of doxorrubicin in doxorubicin-resistant MCF-7/ADR cells [43].

In a recent paper, XN was found to reduce both ASCT2-mediated and non-ASCT2-mediated glutamine uptake by breast cancer cells, being the effect more pronounced in the MDA-MB-231 cell line than in the MCF-7 cell line. Interestingly, the cytotoxic effect of XN on MDA-MB-231 cells was concluded to be related to ASCT2 inhibition [34].

The cytotoxic effect of XN was associated with an increase in apoptosis rates, through multiple pathways [26,27,28,31,36,39,43,51]. Some studies found that XN modulates apoptotic signaling by upregulating pro-apoptotic proteins, including BAX, p21, and p53, while concurrently downregulating anti-apoptotic proteins such as NF-κB1/2, Bcl-2, FAK, MDR1, and Survivin [27,28,43,36]. Furthermore, XN appears to enhance apoptosis via the extrinsic pathway by increasing the expression of death receptors DR4 and DR5 [39], and via activation of caspase-dependent apoptotic cascades [31,36,43]. Moreover, mitochondrial-mediated apoptosis has also been implicated in XN-induced cell death [36]. However, some studies showed that XN reduces cell viability, leading to both necrosis and apoptosis [35,47], by disrupting the cellular membrane and inducing morphological alterations [35].

3.1.2. Effects of XN on cell cycle

By analysis of the effects of XN it is clear that it does also influence the cell cycle of breast cancer cells (Table 1).

XN was concluded to reduce cell cycle progression in the T47D cell line, supported by the tighter packing of DNA molecules [26]. Gholizadeh *et al*. also reached the same conclusion as the number of MCF-7 cells in the sub-G1 phase increased and the number of cells in G1 and S phases decreased [27]. Interestingly, Liu *et al*. reported an increase in the number of MCF-7/ADR cells in S and G2/M phase [43]. XN treatment also led to an increase of cells in Sub G0/G1

phase in MDA-MB-231 cells [36] and caused the accumulation of MDA-MB-435 cells in the S phase [46].

Table 1. Effects of XN on *in vitro* breast cancer cell viability, proliferation, cell cycle and invasive ability.

Cell line	Main conclusions	Main Findings	Specific findings	References
T47D	• XN possesses anti-proliferative and cytotoxic properties • Apoptosis and necrosis induced by XN may contribute to these effects	↓ cell proliferation ↓ cell viability ↑ necrosis ↑ apoptosis ↓ cell cycle progression	• Induces disturbances of the cellular membrane and morphological changes in the cancer cells • The decrease in cancer cell viability is mainly related to the induction of necrotic cell death, but XN also induces apoptosis • ↓ nucleic acid content, indicating tighter packing of DNA molecules • Cells pass the S phase but it is unknown if they enter further cell cycle stages and start the transcription process.	[26]
MCF-7	• XN can be introduced as an adjuvant anti-cancer agent for breast cancer	↓ cell proliferation ↓ cell viability ↓ cell invasion ↓ arrested cell cycle progression ↑ apoptosis and necrosis	↓ cell growth in both 2D and 3D cell cultures ↓ actin-microfilaments network intensity ↑ number of cells in sub-G1 phase, ↓ number of the cells in G1 and S phases ↑ late apoptosis and necrosis ↑ BAX, p21 and p53 ↓ MMP9, MMP2, NFKB2/1, BCL-2, FAK, MDR1, SURVIVIN and ABCG2	[27]
MDA-MB-231	• XN can potentially be used as anticancer agent alleviating malignant progression of TNBC	↓ proliferation ↑ induced apoptosis ↓ cell migration	↓ Bcl-2 expression ↓ gene expression levels and gelatinolytic activity of MMP-9	[28]
MCF-7	• XN shows antiproliferative and cytotoxic effect	↓ cell proliferation and viability	↓ DNA synthesis	[29]
MCF-7 MDA-MB-231	• XN may potentially be useful as a chemopreventive agent during breast hyperplasia and carcinogenesis, acting via the regulation of Notch associated apoptotic regulators *in vivo* and *in vitro*	↓ cell proliferation ↓ cell viability ↑ G_0/G_1 cell cycle arrest ↑ apoptosis ↓ cell migration (MDA-MB-231)	↓ c-myc, SURVIVIN ↑ p21WAF1/CIP1 ↓ in CDK4 and cyclin D1 ↑ caspase-mediated apoptosis ↓ EGFR; ↑ MIF ↓ Notch1, ↓ Hes1 and Hey1	[31]
MCF-7 KPL-3C	• XN may be a promising natural compound to suppress the growth of luminal-type breast cancer	↓ E2-induced cell proliferation • No cell phenotypic alterations	↓ ERα-positive breast cancer tumour growth ↑ number of cells in G1 phase • XN binds to the tumour suppressor protein prohibitin 2 (PHB2), disrupting BIG3-PHB2 interaction and acting as a suppressor of	[32]

	• This effect is related to specific disruption of the BIG3-PHB2 interaction regardless of VCP (valosin-containing protein) function		E2/ERα signalling pathways in ERα-positive breast cancer cell growth. This effect is independent of VCP function.	
MCF-7	• XN in low dose (0.01 μM) improves mitochondrial function, whereas a high dose of XN (5 mM) worsens the functionality of this organelle.	• Biphasic effects of XN on cell proliferation, ROS production, catalase, SOD, glutathione reductase, protein carbonylation, SIRT1 and OXPHOS complexes expression	Biphasic effects of XN: ↑ cell proliferation at lower concentrations ↓ cell proliferation at higher concentrations ↑ ROS production at lower concentrations ↓ ROS production at higher concentrations ↓ catalase, SOD and glutathione reductase at lower concentrations ↑ glutathione reductase and protein carbonylation at higher concentrations ↑ SIRT1 only at lower concentrations ↑ OXPHOS complexes expression only at lower concentrations	[33]
MCF-7 MDA-MB-231 MCF-12A	• The cytotoxic effect of XN is related to ASCT2 inhibition • Targeting glutamine uptake might constitute a potential interesting strategy for triple-negative breast cancer	↓ proliferation and viability of MDA-MB-231 cells ↓ cellular uptake of glutamine • The cytotoxic effect of xanthohumol, but not its anti-proliferative effect, is related to ASCT2 inhibition • Combination of xanthohumol with the breast cancer chemotherapeutic agent doxorubicin results in an additive anti-proliferative, but not cytotoxic effect.	↓ total and Na^+-dependent glutamine uptake in MDA-MB-231 cells; it shows a less marked inhibitory effect on glutamine uptake by MCF-7 cells In MDA-MB-231 cells: • XH is as an uncompetitive inhibitor of Na^+-dependent glutamine uptake • ↓ both ASCT2 (alanine, serine, cysteine transporter 2)-mediated and non-ASCT2-mediated glutamine uptake • does not interfere with the transcription rates of ASCT2 • ↑ SNAT1 and SNAT2 mRNA levels	[34]
MCF-7	• Modulation of ALP by XN might provide therapeutic strategies against hormone-dependent breast cancer	↓ ALP activity ↓ RNA and protein expression of IALP, but not TNS-ALP ↓ cell viability	E2 caused: ↓ ALP activity ↓ RNA and protein expression of IALP and TNS-ALP ↑ cell viability	[37]
MDA-MB-231 MDA-MB-436	XN shows cytotoxic effects	↓ cell viability		[38]
MCF-7	• The antiproliferative and cytotoxic effect of XN is related to changes in proteins participating on cell cycle and DNA replication as well as of type I interferon signaling pathway	↓ proliferation and viability ↓ type I interferon signaling pathway proteins ↑ proteins involved in cell cycle and DNA replication	↓ proteins of the type I interferon signaling pathway (including SQSTM1, ISG15, OAS1, OAS3, IRF9, STAT1, PARP9, and DTX3L) and JAK/STAT signaling ↑ proteins involved in cell cycle and DNA replication (including kinesin family members, minichromosome maintenance complexes (MCM), and aurora kinases (AURK))	[42]
MCF-7 MCF-7/ADR	• XN may be a potent chemo- and radio-sensitizer	• XN ↑ radiation-induced cell death and apoptosis in	• XN induced more apoptosis in MCF-7/ADR than in MCF-7 cells	[39]

	• Its actions are mediated through STAT3 and EGFR inhibition	both MCF-7 and MCF-7/ADR cells • The synergistic effect of XN with radiation treatment was much greater in MCF-7/ADR cells compared with MCF-7 cells • XN restored sensitivity of MCF-7/ADR cells to doxorubicin and radiation therapies	• ↑ death receptor (DR4 and DR5 expression) in MCF-7/ADR cells • ↓ Multi-drug resistance 1 (MDR1), epidermal growth factor receptor (EGFR) and signal transducer and activator of transcription 3 (STAT3) expression levels in MCF-7/ADR cells • These XN-induced changes in MCF-7/ADR cells were synergistically increased by radiation	
MCF-7 MCF-7/ADR	• XN sensitizes the inhibitory effect of doxorubicin on MCF-7/ADR cells • XN is a promising compound targeting doxorubicin-resistant breast cancer cells • XN regulates their stemness	↓ cell viability of MCF-7 and MCF-7/ADR ↑ apoptosis of MCF-7/ADR ↑ the number of MCF-7/ADR cells in S and G2/M phases ↑ the cytotoxic effect of doxorubicin on MCF-7/ADR cells ↓ the stemness of MCF-7/ADR cells	• ↑ DNA damage leading to an ↑ in γ-H2AX • ↓ bcl-2 and pro-caspase3, ↑ bax • ↓ in the colony formation and growth, cell migration, the percentage of side population cells, the self-renewal ability of cancer stem-like cells, the sphere formation of cancer stem-like cells, and stemness-related biomarkers • ↓ Notch1 and ABCG2 expression in MCF-7/ADR cells.	[43]
MCF-7 SK-BR-3 T47D	• XN is a selective antiproliferative agent against breast cancer cells	↓ cell proliferation	• Selectivity for breast cancer cell lines in comparison with non-cancerous cell lines	[46]
MCF-7	• XN shows antiproliferative effects	↓ cell proliferation		[49]
MCF-7 T47D MDA-MB-231	• XN shows antiproliferative effects	↓ cell proliferation	• Selectivity for breast cancer cell lines in comparison with non-cancerous cell lines	[50]
MCF-7/6 MCF-7/AZ T47D	• XN inhibits invasion, indicating a possible role as an antiinvasive agent in vivo as well	↓ invasive properties of MCF-7/6 and T47-D cells ↓ cell growth/proliferation of MCF-7/6 and T47-D cells ↑ apoptosis of MCF-7/6 and T47-D cells	↑ function of the E-cadherin/catenin complex	[51]
MCF-7	XN is an antiproliferative agent with potential chemopreventive activity against breast cancer in humans	↓ cell proliferation	↓ DNA synthesis	[52]
MDA-MB-231	XN might be a novel therapeutic drug for breast cancer	↓ cell viability ↑ cell cycle arrest ↑ Apoptosis	↑ percentage of cells in SubG0/G1 phase ↑ Bax, caspase-9 and caspase-3 (the apoptotic effect was mitochondria and caspase-dependent through the intrinsic pathway)	[36]
MCF-7	XN showed cytotoxic effects	No selective effect was found		[27]

MDA-MB-231	XN is cytotoxic to MDA-MB-231 cells XN inhibits CXCR4 expression	XN inhibits CXCR4 expression at a transcriptional level	• This effect seems to be specific to XN, as IXN and 8-PN did not show the same effect • XN could block endogenous activation of nuclear factor kappa b, a key transcription factor that regulates the expression of CXCR4	[31]
MDA-MB-435	XN shows antiproliferative effects XN shows cytotoxic effect	• XN shows antiproliferative effects by reducing DNA synthesis • XN causes cell cycle arrest in S phase • XN induces apoptosis		[46]

3.1.3. Effects of XN on breast cancer cell invasion

One of the most important steps in disease progression is the breast cancer cells invading the hosts body and metastasizing in other locations, as it is commonly known that breast cancer cells frequently metastize to the bones, lungs, liver, brain and lymph nodes (ref[a]). The growth of a new solid mass may compromise the function of several vital organs and progressively reduce the patient's quality of life. Because cell invasion is a critical factor in the progression of cancer, including breast cancer, identification of therapeutic agents that inhibit this process may contribute to improved patient outcomes.

In this context, XN was proved to reduce cell migration [28,31,43,51,45], invasion [27,45,51] and colony-formation ability [43] (Table 2). Some of these results are next detailed.

Gholizadeh *et al.* reported that XN exerts its anti-invasive effects by downregulating the expression of matrix metalloproteinases MMP9 and MMP2, as well as focal adhesion kinase (FAK) [27].

Similarly, in triple-negative breast cancer (TNBC) cell lines, XN was found to reduce MMP9 gene expression and gelatinolytic activity, further supporting its role in invasion suppression [28].

Additionally, XN mitigates cell invasion by downregulating epidermal growth factor receptor (EGFR) expression while upregulating macrophage migration inhibitory factor (MIF) [31].

Moreover, Vanhoecke *et al.* demonstrated that XN disrupts cytoskeletal organization, further contributing to its anti-invasive properties [51].

Table 2. Effects of XN on *in vitro* breast cancer cells migration, invasion, metastasis and angiogenesis.

Cell line	Main findings	Detailed findings	References

MCF-7	↓ cell invasion	↓ actin-microfilaments network intensity in MCF-7 spheroids ↓ MMP2, MMP9 and FAK gene expression levels	[27]
MDA-MB-231	↓ cell migration	↓ gene expression levels and gelatinolytic activity of MMP-9	[28]
MDA-MB231	↓ cell migration	↓ EGFR; ↑ MIF ↓ Notch 1 signaling pathway	[31]
MCF-7 MCF-7/ADR	↓ colony formation and growth, cell migration ↓ the stemness of MCF-7/ADR cells	↓ colony formation and growth, cell migration, the percentage of side population cells, the self-renewal ability of cancer stem-like cells, the sphere formation of cancer stem-like cells, ↓ stemness-related biomarkers ↓ Notch1 and ABCG2 expression in MCF-7/ADR cells.	[43]
MCF-7	↓ the formation of ‘‘circular chemorepellent-induced defects’’ (CCIDs) ↓ NF-kB, CYP1A1 and selectin E ↓ ICAM-1 expression and adherence of MCF-7 cells to LECs, which is a prerequisite for CCID formation ↑ 12(S)-HETE production ↓ markers of epithelial-to-mesenchymal transition (EMT) and of cell mobility	MCF-7 breast cancer spheroids placed on lymphendothelial cells (LECs) induce ‘‘circular chemorepellent-induced defects’’ (CCIDs) in the LEC monolayer, which resemble gates for intravasating tumor bulks at an early step of lymph node colonization. XN: ↓ CCIDs formation through two distinct pathways: one dependent of NF-kB (at higher concentrations) and the other independent of NF-kB (at low concentrations). ↓ markers of EMT and of cell mobility (such as paxillin, MCL2 and S100A4)	[45]
MCF-7/6 T47D	↓ invasive properties of MCF-7/6 and T47-D cells	↑ the function of the E-cadherin/catenin complex (E-cadherin-mediated cell-cell adhesion)	[51]

3.1.4. Effects against resistance to therapy

In the treatment of cancer, one major obstacle for therapy efficacy and success is the development of an innate ability of cancer cells to resist conventional chemotherapy drugs or radiation therapy. In this context, Table 3 summarizes the reported results of XN activity against radiotherapy and chemotherapy resistance by breast cancer cells.

Analysis of this Table shows that XN is probably able to inhibit breast cancer cells from expelling chemotherapeutic drugs from the cell, as it was found to downregulate the expression of multidrug resistance-associated genes MDR1 and ABCG2 by breast cancer cells [27,43].

Moreover, by combining XN with doxorubicin (a widely used chemotherapy drug) resulted in a synergistic effect against breast cancer cells [34,43], thus giving the option of using lower doses of chemotherapeutic drugs that are known for their highly cytotoxic effects against healthy human cell lines. Additionally, XN can restore breast cancer sensitivity to chemotherapy (doxorubicin) [34,43] and radiotherapy [39]. This is particularly important in the case of radiotherapy

resistance, for which the mechanisms of resistance are still pretty much unknown.

Table 3. *In vitro* effects of XN against resistance to chemotherapy and radiotherapy.

Cell line	Main findings	Specific observations/mechanisms of action	References
MCF-7	XN downregulated multidrug resistance-associated genes	↓ MDR1 and ABCG2	[27]
MCF-7 MDA-MB-231	Combination of xanthohumol with the breast cancer chemotherapeutic agent doxorubicin results in an additive anti-proliferative, but not cytotoxic effect.	↑ the anti-proliferative effect of doxorubicin Does not change the cytotoxic effect of doxorubicin	[34]
MCF-7 MCF-7/ADR	XN may be a potent chemo- and radio-sensitizer, and its actions are mediated by STAT3 and EGFR inhibition	• XN ↑ radiation-induced cell death and apoptosis in both MCF-7 and MCF-7/ADR cells • The synergistic effect of XN with radiation treatment was much greater in MCF-7/ADR cells compared with MCF-7 cells • XN restored sensitivity of MCF-7/ADR cells to doxorubicin and radiation therapies	[39]
MCF-7 MCF-7/ADR	• XN sensitizes the inhibitory effect of doxorubicin on MCF-7/ADR cells	↑ the cytotoxic effect of doxorubicin on MCF-7/ADR cells ↓ ABCG2 expression in MCF-7/ADR cells.	[43]

3.2. In vivo and *ex vivo* effects of XN

Evaluation of the *in vivo* effect of XN allows not only to obtain confirmation of its efficacy in the whole (complex) organism, but allows evaluation of putative toxicity and side effects and takes also into account the influence of the tumor microenvironment on its efficacy.

Some data on the *in vivo* effect of XN on experimental animal (mouse) models have been reported. In agreement with the antitumoral effects described for XN *in vivo*, this compound exhibits an antitumoral effect against breast cancer *in vivo* (Table 4). However, XN was only tested against ER-positive breast cancer xenografts, and it would be very interesting to test it also against triple-negative breast cancer cell xenografts.

Table 4. *In vivo* and *ex vivo* effects of XN.

Experimental model	Main conclusions	Main findings	References
Breast cancer xenografts (MCF-7 cells) in NIH (s) II nude mice	• XN ↓ tumor progression • XN is able to target both breast cancer and host cells, namely inflamatory and endothelial cells, suggesting its potential	**In vitro** (MCF-7 cells) • XN possesses cytotoxic and antiproliferative effects **In vivo:** • XN did not change tumour size • XN ↓ tumour mean weight • XN ↑ central necrosis area within tumour • XN ↓ inflamatory cell number and focal proliferation areas	[29]

	use as a double-edge anti-cancer agent	• XN ↑ the percentage of apoptotic cells • XN ↓ microvessel density (anti-angiogenic effect) • XN ↓ immunostaining for NFkB, phosphorylated-inhibitor of kappa B and interleukin-1b and decreased also NFkB activity	
Breast cancer xenografts (4T1 cells) in BALB/c nude mice	•XN ↓ tumor growth •XN may potentially be useful as a chemopreventive agent during breast hyperplasia and carcinogenesis, acting via regulation of Notch associated apoptotic regulators in vivo and in vitro.	**In vitro** (MCF-7 and MDA-MB-231 cells)**:** • XN ↓ cell viability • XN ↑ and apoptosis • XN caused G0/G1 cell cycle arrest • XN ↓ the Notch signaling pathway, Notch 1 and Ki-67 **In vivo:** • XN ↓ tumor size and weight • XN ↓ of the Notch signaling pathway and apoptotic regulators B-cell lymphoma-2 (Bcl-2), Bcl-extra large and caspase-3	[31]
Breast cancer xenografts (KPL-3C cells) in BALB/c nude mice	• XN ↓ ERα-positive breast cancer tumour growth • XN may be a promising natural compound to suppress the growth of luminal-type breast cancer	**In vitro:** • XN ↓ ERα-positive breast cancer cell (MCF-7 and KPL-3C) growth • XN caused G1-arrest of MCF-7 cells **In vitro and in vivo:** • XN binds to the tumour suppressor protein prohibitin 2 (PHB2), disrupting BIG3-PHB2 interaction and acting as a suppressor of E2/ERα signalling pathways in ERα-positive breast cancer cell growth	[32]
Breast cancer xenografts (4T1 cells) in BALB/c nude mice	• XN ↓ cancer tumour growth • XH regulated Th1/Th2 balance drift to Th1 polarization • STAT4 may play a positive role in the regulation of Th1/Th2 cytokines by XN	**In vivo:** • ↓ tumor growth • ↑ secretion of perforin and granular enzyme B (Granzyme B) • ↑ the ratio of CD8+/CD25+ • ↑ Th1 cytokines IL-2 and IFN-γ • ↓ Th2 cytokines • ↑ expression of T-bet, a Th1-specific transcription factor • ↑ activation of STAT4	[35]
Mouse mammary gland ex vivo organ culture	• XN inhibited DMBA-induced preneoplastic lesion formation		[46]

3.1.4. The effects of XN derivatives

As stated above, XN is efficiently metabolized both by the gut microbiota and by human enzymes, and so it is evident that the derivatives generated may play an important role in its activity against cancer cells. Also, because of the difficulties inherent to XN use as a therapeutic agent, related to its low oral absorption rates, XN derivatives have been synthetized, by editing the molecule skeleton, and tested, aiming at obtaining a greater efficacy and at understanding in detail how to improve its activity. The results obtained so far are shown in Table 5.

Among the tested compounds, Compound 8, 8-PN, XNC, PEG-GO@XN nanocomposite appear to have higher potencies than XN, and Semisynthetic aurone derivative of XN appear to be more selective, while the results obtained with IXN are less consistent (Table 5).

Table 5. Effects of XN derivatives on breast cancer cells *in vitro* or *in vivo*.

Cell line	Main conclusions	Main effects	XN derivative	Ref.
MDA-MB-231 MCF-7	• Compound 8 has a more potent cytotoxic effect than XN • Compound 8 has antimigratory and antiinvasive effects • Compound 8 has proapoptotic effect	• Concentration-dependent cytotoxic effect in MCF-7 cells MDA-MB-231cells • In MDA-MB-231 cells: ↓ migration and invasion by downregulation of the expression of HIF-1alfa, MMP-2 and MMP-9 ↑ apoptosis by increasing Bax/Bcl-2 ratio and downregulating Akt protein expression	**Compound 8** (C5 guanidino-substituted XN) (2-((E)-2,4-dihydroxy-5-((E)-3-(4-hydroxyphenyl)acryloyl)-6-methoxy-3-(3- methylbut-2-en-1-yl)benzylidene)hydrazine-1-carboximidamide) (↑ water solubility and improved lipid-water partition coefficient than XN)	[30]
MCF-7	• XN in low dose (0.01 μM) and 8-PN at all assayed doses (0.001–20 μM) improve mitochondrial function, whereas a high dose of XN (5 μM) worsens the functionality of this organelle	8-PN: ↑ cell number ↓ ROS production ↓ CAT, SOD and glutathione reductase No effect on protein carbonyl levels ↑ Sirt1 expression levels, no effect on sirt3 levels ↑ expression of complexes II, III, and V XH: A biphasic effect on cell number: an increase (0.001-1 μM) and a decrease (10–20 μM) A biphasic effect on ROS production: decrease (0.001-0.01 lower conc) and increase (1-15 higher) ↓ CAT, SOD and Glutathione Reductase ↑ protein carbonyl levels ↑ Sirt1 expression levels, no effect on Sirt3 levels ↑ expression of complexes II and V	The XN metabolite **8-PN** (8-prenylnaringenin)	[33]
MDA-MB-231 MDA-MB-436 Breast cancer xenografts (MDA-MB-231 cells) in BALB/c nude mice	• PEG-GO@XN has enormous potential to suppress metastatic breast cancer by selective targeting oxidative phosphorylation and epithelial-mesenchymal transition of cancer cells	**In vitro:** ↓ intracellular ATP levels ↓ mitochondrial respiration ↓ the assembly of F-actin cytoskeleton ↓ cell migration and invasion Does not interfere with the proliferation and metabolism of non-cancerous cells **In vivo:** ↓ metastasis of cells to lungs (both lung surface and internal metastatic nodules) in nude mice ↓ TGF-β1-induced downregulation of E-cadherin and up-regulation of N-cadherin, vimentin, Snail and Twist, i.e., caused the maintenance of “epithelial-like” rather than the “mesenchymal-like” features ↓ decreased the motility potential of cells	A **PEG-GO@XN nanocomposite** with good stability and biocompatibility	[38]
MDA-MB-231 4T1	• The biotinylated derivatives showed slightly higher antiproliferative activity		**4-O-biotinylxanthohumol** and **4,4´-di-O-biotinylxanthohumol** (vitamin fragment in the C-4	[40]

MCF-7	towards all types of tested breast cancer cells than XN • The double biotinylated XN derivative proved to be the most effective in inhibiting cell growth		position and C-4´) (↑ hydrophilicity than XN)	
MCF-7	• XNC has more potent cytotoxic and antiproliferative effect than XN • XNC and XN possess different modes of action/target structures: XNC causes ER stress and seems to be involved in cell-cell adhesion, whereas XN influences cell cycles and DNA replication as well as type I interferon signaling pathway.	XNC: • ↑ expression of Heat shock proteins (HSPA5, HSPA13, HSP90B1, HSP40) • ↑ expression of microtubule-associated protein 1 light chain 3 α and β, and β2 (MAP1LC3A / MAP1LC3B / MAP1LC3B2), which are involved in the cellular response to nitrogen level, autophagy of mitochondrion, and mitochondrion disassembly • ↓ expression of proteins part of tubulin (TUBA1A,TUBA1B, TUBA1C, TUBA3D, TUBB6), myosin (MYL12A, MAL12B, MYL6, MYL9, MYH7B, MYH9), and various kinases (CDKs, MAPKs, PAK, PKC), affecting cell-cell contacts XN: • ↓ Type I interferon signaling pathway (including QSTM1, ISG15, OAS1, OAS3, IRF9, STAT1, PARP9, and DTX3L)	• **XNC** (xanthohumol C) (dehydrocycloxanthohumol)	[42]
MCF-7 SK-BR-3 T47D	• The higher selectivity of the aurone in comparison to XN shows that the modification of the flavonoid skeleton, prenylated chalcone, into its aurone ↑ the selectivity against cancer cell lines	• Both XN and its derivate showed potent (MCF-7, T47D) to moderate (SK-BR-3) antiproliferative activity; their potency was similar • The aurone exhibited a higher selectivity against breast cancer cell lines	Semisynthetic aurone derivative of XN **((Z)-6,4´-dihydroxy-4-methoxy-7-prenylaurone**)	[46]
SK-BR-3	• XN, IXN and 8-PN induced: - ↓ of aromatase activity - ↓ cellular proliferation - ↑ apoptosis • The anti-breast cancer effects of these compounds are related to their ability to decrease estrogen synthesis	• The potency in ↓ culture growth was: XN > 8-PN > IXN • The potency in ↓ DNA synthesis was: XN > 8-PN > IXN • All compounds ↓ cell proliferation • All compounds ↑ apoptosis, 8-PN displayed the most striking effect • The potency in ↓ aromatase activity was: 8-PN > XN > IXN	**IXN** **8-PN**	[47]
MCF-7	• Reduction of the a,β-double bond → ↑ antiproliferative properties • Prenyl moiety modification → ↓ antiproliferative properties • Isomerization of chalcone to flavanone → ↓ in cytotoxic activity against MCF-7 cancer cells	• All compounds presented cytotoxic effect against MCF-7 • The cytotoxic potency of the biotransformation products was similar or lower than that of XN	**XND (xanthohumol D)** **IXN** Six products of XN biotransformation by fungal organisms: - **4,2´,4´-trihydroxy-6´-methoxy-3-prenyl-α,β-dihydrochalcone** - **2"-(2"'-Hydroxyisopropyl)-dihydrofurano[4",5":3',4']-4,2'-dihydroxy-6'-methoxychalcone** - **2"-(2"'-Hydroxyisopropyl)-dihydrofurano-[4",5":3',4']-4',2-dihydroxy-6'-methoxy-α,β-dihydrochalcone**	[49]

			- **Xanthohumol H (3'-[3"-hydroxy-3"-methylbutyl]-4,2',4'-trihydroxy-6'-methoxychalcone)** - **mixture of diastereoisomers of (2*S*,2"*S*) and (2*S*,2"*R*) 2"-(2"'-hydroxyisopropyl)-dihydrofurano-[4",5":7,8]-4'-hydroxy-5-methoxyflavanone and (*Z*)-2"-(2"'-hydroxyisopropyl)-dihydrofurano-[4",5"-6,7]-3',4'-dihydroxy-4-methoxyaurone**	
MCF-7 T47D MDA-MB-231 MCF-10A	• The observation that the antiproliferative effect of XN and 2HXN>>8-PN and 6-PN indicates that it highly depends on the flavonoid skeleton • The observation that 8-PN exerts weaker antiproliferative effect than 6-PN suggests that the position of the prenyl group is an important determinant • Except for the hydrogenation of the α,β-double bond in xanthohumol, most of the structural modifications, such as isomerization of XN to IXN or demethylation of IXN to 8PN led to reduction of the antiproliferative activity • The higher antiproliferative activity was found against breast cancer cell lines with high levels of estrogen receptor expression	• All compounds ↓ cell proliferation • α,β-dihydroxanthohumol and XN have the most potent antiproliferative effect against all the cell lines • T47D was the most sensitive of the BC cell lines (and has the highest level of estrogen receptor expression) • The least sensitive was MDA-MB-231 cell lines (these cells have a very low level/absence of the estrogen receptor expression) • All compounds showed selectivity towards breast cancer cell lines in relation to MCF-10A cells, especially 2HXN and XN, which showed very high selectivity (SI=5.16-9.91)	**2HXN** **IXN** **8-PN** **6-PN** **NG** – non prenylated naringenin	[50]
MCF-7	• As antiproliferative agents, XN (chalcone) and IX (flavanone isomer of XN) may have potential chemopreventive activity against breast and ovarian cancer in humans.	• Culture growth inhibitory effects were found for all; XN was the most potent • At 100 μM, all the compounds were cytotoxic • XN and IXN caused a concentration-dependent ↓ in DNA synthesis (the potency of XN>IXN for 1 and 2 days treatment, the potency of IXN>XN for 4 day treatment) • Neither XN nor IXN induced internucleossomal DNA fragmentation	**IXN** **TP: 2´,4´,6´,4-tetrahydroxy-3´prenylchalcone** **TG: 2´,4´,6´,4-tetrahydroxy-3´geranylchalcone** **DX: Dehydrocycloxanthohumol** **DH: Dehydrocycloxanthohumol hydrate** **8-PN** **6-PN**	[52]
MCF-7	• With high probability, these compounds will not be metabolized by gastrointestinal microbiome like XN to 8-PN, which besides its assumed beneficial activities may promote mammary and endometrial cancers	• All compounds ↓ cell proliferation, with different potencies • Flavonoids containing chromane- and chromene-like moieties, especially chalcones, are potent antiproliferative agents • The most promising antiproliferative agents seem to be the minor hops prenylchalconoids XNC and	**XNC (xanthohumol C)** **1",2"-Dihydroxanthohumol C** **1",2"-Dihydroxanthohumol K** **IXN** **XNK (xanthohumol K)** **4,4´-Dimethoxymethyl xanthohumol** **1",2",α,β,-tetrahydroxanthohumol C** **1",2",α,β-tetrahydroxyxanthohumol k** **1",2"-Dihydroisoxanthohumol C**	[53]

		1”,2”-dihydroxanthohumol K and non-natural 2,3-dehydroisoxanthohumol, which exhibited activity comparable to cisplatin	**5,4'-Dihydroxy-6",6"-dimethyl-4",5"-dihydropyrano-[2",3":7,8]flavanone** **5,4'-dihydroxy-6",6"-dimethyl-4",5"-dihydropyrano-[2",3":6,7]flavanone** **2,3-Dehydroisoxanthohumol C** **2,3-Dehydroisoxanthohumol** **1”,2”-Dihydro-2,3-dehydroisoxanthohumol C**	
MCF-7 MCF-10A	• PN preferentially enhance the nontoxic estrogen 2-hydroxylation pathway through AhR mediated up-regulation of P450 1A1,	↓ potentially genotoxic estrogen 4-hydroxylation pathway ↓ estradiol induced colony formation in MCF-10A • 6-PN Preferentially Induced Estrogen 2-Hydroxylation Metabolism in MCF-10A and MCF-7 Cells • 6-PN Induced P450 1A1/1B1 Activity in MCF-10A and MCF-7 Cells. • 6-PN Increased XRE Activation and Acted as an AhR Agonist.	**IXN** **8-PN** **6-PN**	[54]
MCF-7 (ATCC(HTB22))	• The compounds stimulated proliferation, decreased viability and colony formation	• All compounds similarly stimulated proliferation • The compounds were cytotoxic: IXN>6-PN>8-PN> XN • The compounds inhibited colony-formation: IXN>6-PN>8-PN	**8-PN** **6-PN** **IXN**	[57]
MCF-10A	Hops extracts possess cancer chemopreventive activity through attenuation of estrogen metabolism mediated by 8-PN	• No significant reduction in the formation of 2- and 4-MeOE1 was observed with XN • ↓ formation of 2- and 4-MeOE1 in the presence of nanomolar amounts of 8-PN • ↓ in E2-induced colony formation by 8-PN	**8-PN**	[56]
MCF-7	• XN possesses cytotoxic effects that disappear after 70h of treatment	• The fiber combination seem to possess cytotoxic effect that does not disappear	**Resveratrol/polyethylene oxide (PEO) (50/50)+XN/Poly(lactide-glycolide) (PLGA) (10/90) fibers**	[17]
MDA-MB-231	• IXN strongly reduced TGF- β expression • IXN antagonized the cellular effects of TGF-β • IXN blocked vascular mimicry of cancer cells	• IXN blocked completely TGF-β-induced increase of mRNA levels of PAI-1, MMP-2, PDGF (angiogenesis related markers), ANGPTL-4 (angiopoietin-like 4) and SMAD7 • IXN strongly reduced the TGF-β-induced elevation of expression of chemokines (CCL-3, CCL-2, CXCL-16), angiogenesis promoting growth factors (angiopoietin-1 and –2, GMCSF) and matrix metalloproteinases (MMP-9 and –8) • IXN decreased the formation of capillary-like tubules on Matrigel, this effect was not related to a cytotoxic effect	**IXN**	47

4. DISCUSSION

XN has demonstrated significant potential as an anticancer agent against breast cancer through various mechanisms, including cell cycle arrest, apoptosis induction, and inhibition of metastasis. Its ability to interfere with multiple cellular pathways, such as the Notch signaling pathway and NF-κB, suggests a broad-spectrum anticancer effect. Moreover, XN has been shown to modulate the tumor microenvironment by reducing inflammatory responses and altering immune system activity. These findings indicate that XN may be particularly beneficial for aggressive and treatment-resistant breast cancer subtypes.

However, despite its promising *in vitro* and *in vivo* effects, several limitations hinder the clinical application of XN. One of the major challenges is its poor bioavailability due to high hydrophobicity, rapid metabolism, and short half-life. Most XN is rapidly metabolized and excreted before reaching therapeutic concentrations in the bloodstream. Additionally, XN undergoes extensive biotransformation, producing metabolites like isoxanthohumol (IXN) and 8-prenylnaringenin (8-PN), which may contribute to or alter its biological effects. Therefore, understanding the pharmacokinetics and metabolism of XN in humans is crucial for developing effective therapeutic strategies.

Extensive research has already been done relating to the effects of XN against other cancer cell lines, where its ability to influence the hormonal metabolism are particularly interesting. XN has also been shown to exert significant estrogenic activity [63,64]. Moreover, the anti-inflammatory effects that XN exerts may lead to better outcomes and increase the quality of life of patients [29,35,45].

While preclinical data support XN anticancer effects, human trials are necessary to validate its efficacy, determine optimal dosages, and assess potential side effects. Moreover, while XN appears to selectively target cancer cells, its long-term safety and interactions with existing chemotherapies require further investigation.

FUTURE PERSPECTIVES

To overcome the limitations of bioavailability and stability of XN, future research should focus on innovative drug delivery systems. Nanoparticle-based formulations, liposomes, and polymeric micelles have shown promise in enhancing the solubility, stability, and targeted delivery of XN to tumor sites. Additionally, structural modifications of XN, such as biotinylation and synthetic derivatives, have demonstrated improved anticancer activity and bioavailability in experimental studies. Further exploration of these approaches could lead to more effective therapeutic applications.

Another important area of research is the role of XN metabolites in breast cancer treatment. While XN itself is unstable, its metabolites, including IXN and 8-PN, may retain or even enhance anticancer properties. Investigating their mechanisms of action and potential synergy with XN could provide deeper insights into its therapeutic potential.

Additionally, future studies should explore the combination of XN with conventional chemotherapy or immunotherapy. Preliminary evidence suggests that XN can enhance the efficacy of doxorubicin and sensitize resistant cancer cells to treatment. Investigating combination therapies could lead to more effective and less toxic breast cancer treatments.

Finally, clinical trials are essential to confirm the efficacy and safety of XN in humans. Well-designed phase I and II trials should focus on determining its pharmacokinetics, optimal dosage, and potential adverse effects. If successful, XN could become a valuable addition to anticancer therapies.

CONCLUSION

Xanthohumol exhibits strong anticancer potential against breast cancer, with effects on proliferation, apoptosis, and metastasis. However, its low bioavailability, together with metabolic and chemical instability pose significant challenges for clinical application. Advances in drug delivery systems, structural modifications, and combination therapies offer promising solutions to enhance its therapeutic potential. While preclinical studies strongly support the efficacy of XN in relation to breast cancer, further research, particularly clinical trials, is essential to establish its role in breast cancer treatment. If these challenges are addressed, XN could emerge as a novel, natural-based therapeutic option for breast cancer patients.

LIST OF ABBREVIATIONS

(XN) Xanthohumol

(IXN) Isoxanthohumol

(6-PN) 6-prenylnaringenin

(8-PN) 8-prenylnaringenin

CONSENT FOR PUBLICATION

Not applicable

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or Otherwise.

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The importance is not justified. — 0
The importance is alluded to, but not explicitly justified. — 1
The importance is explicitly justified. — 2

2

2) Statement of concrete aims or formulation of questions

No aims or questions are formulated. — 0
Aims are formulated generally but not concretely or in terms of clear questions. — 1
One or more concrete aims or questions are formulated. — 2

2

3) Description of the literature search

The search strategy is not presented. — 0
The literature search is described briefly. — 1
The literature search is described in detail, including search terms and inclusion criteria. — 2

2

4) Referencing

Key statements are not supported by references. — 0
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2

5) Scientific reasoning

(e.g., incorporation of appropriate evidence, such as RCTs in clinical medicine)

The article's point is not based on appropriate arguments. — 0
Appropriate evidence is introduced selectively. — 1
Appropriate evidence is generally present. — 2

2

6) Appropriate presentation of data

(e.g., absolute vs relative risk; effect sizes without confidence intervals)

Data are presented inadequately. — 0
Data are often not presented in the most appropriate way. — 1
Relevant outcome data are generally presented appropriately. — 2

2

Sumscore 12

Fig. 1 SANRA - Scale

SANRA – explanations and instructions

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Consider how well the manuscript outlines the clinical problem and highlights unanswered questions or evidence gaps – thoroughly (2), superficially (1), or not at all (0).

Item 2 – Statement of concrete/specific aims or formulation of questions

A good paper will propose one or more specific aims or questions which will be dealt with or topics which will be reviewed.
Please rate whether this has been done thoroughly and clearly (2), vaguely or unclearly (1), or not at all (0).

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A convincing narrative review will be transparent about the sources of information on which the text is based. Please rate the degree to which you think this has been achieved. To achieve a rating of 2, it is not necessary to describe the literature search in as much detail as for a systematic review (searching multiple databases, including exact descriptions of search history, flowcharts, etc.), but it is necessary to specify search terms, and the types of literature included. A manuscript which only refers briefly to its literature search would score 1, while one not mentioning its methods would score 0.

Item 4 – Referencing

No manuscript references all statements. However, those that are essential for the arguments of the manuscript – "key statements" – should be backed by references in all or almost all cases. Exceptions could reasonably be made for rating purposes where a key statement has uncontroversial face-validity, such as "Diabetes is among the commonest causes of chronic morbidity worldwide."
Please rate the completeness of referencing: for most or all relevant key statements (2), inconsistently (1), sporadically (0).

Item 5 – Scientific reasoning

The item describes the quality of the scientific point made. A convincing narrative review presents evidence for key arguments. It should mention study design (randomized controlled trial, qualitative study, etc), and where available, levels of evidence.
Please rate whether you feel this has been done thoroughly (2), superficially (1), or hardly at all (0). Unlike item 6, which is concerned with the selection and presentation of concrete outcome data, this item relates to the use of evidence and of types of evidence in the manuscript's arguments.

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This item describes the correct presentation of data central to the article's argument. Which data are considered relevant varies from field to field. In some areas relevant data would be absolute rather than relative risks or clinical versus surrogate or intermediate endpoints. These outcomes must be presented correctly. For example, it is appropriate that effect sizes are accompanied by confidence intervals. Please rate how far the paper achieves this – thoroughgoingly (2), partially (1), or hardly at all (0). Unlike item 5, which relates to the use of evidence and of types of evidence in the manuscript's arguments, this item is concerned with the selection and presentation of concrete outcome data.

Fig. 2 SANRA—explanations and instructions document

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The length of a published comprehensive review article is from 6000-10000 words with 100 or more references, excluding figures, structures, photographs, schemes, tables, *etc*.

Mini-Reviews

Mini-reviews should be 3000- 6000 words with 75 or more references, excluding figures, structures, photographs, schemes, tables, *etc*.

Systematic Reviews

Systematic Reviews include systematic updates on review protocols, methods, research, and results from all relevant fields for any studies and updates on already published issues. The total number of words for a published systematic review is from 4000 to 6000 words with 100 or more references, excluding figures, structures, photographs, schemes, tables *etc*. Systematic reviews

and meta-analyses must be reported according to PRISMA guidelines (www.prisma-statement.org).

Registration of Systematic Reviews

Bentham Science Publishers supports retrospective registration of systematic reviews in a suitable registry (such as PROSPERO). The registered systematic review must include the registration number as the last line of the manuscript abstract.

Research Articles

Research articles should be of 4000-6000 words with 75 or more references excluding figures, structures, photographs, schemes, tables, *etc*.

Short Communications

Short Communications are concise articles that present significant findings or innovative ideas in a brief and focused format. They are intended to communicate preliminary results, novel methods, or theoretical advancements that may not require the extensive background, methodology, and discussion of a full-length research article. Short communications should be 2000-3000 words with 25 or more references excluding figures, structures, photographs, schemes, tables, *etc*.

Current Frontiers

The articles should be contributed by eminent experts on cutting-edge recent developments. They should be written in the format of mini-reviews (about 4 to 5 pages, approximately 800 to 850 words per composed page excluding tables, structures, graphics, figures and captions) with about 70 references to recent literature. All pages should be numbered sequentially.

Randomized Drug Clinical Trial Studies

Trial studies should be 4000 to 6000 words with 50 or more references excluding figures, structures, photographs, schemes, tables etc.

Editorials

Editorials are short papers on important topics related to the journal. The total number of words in an editorial should not exceed 1000 to 1500, and it should contain only 10-15 references. An abstract is not required.

Commentaries

Commentaries present an analysis by scientists on different important issues related to the publications in the journal. Commentaries should contain less than 3000 words, including the abstract, main text, references, and figure legends. However, an abstract is not necessary.

Perspectives

A perspective provides a short overview of a research topic relevant to the field. The length of a published perspective ranges from 1500 to 1800 words, with 20 or more references, excluding figures, structures, photographs, schemes, tables, *etc*.

Industry News

Industry News should provide important developments in industries related to the scope of the journal, that could be of interest to the readers. The length of the submission should be about 1000 words, and it should ideally have 10 or more references (an abstract is not required).

Patent News

Patent News may present important information about recent important patents that have been granted, relevant to the scope of this journal. The length should be about 1000 words, and it should ideally have 10 or more references (abstract is not required).

There is no restriction on the number of figures, tables or additional files *e.g.* video clips, animation and datasets, that can be included with each article online. Authors should include all relevant supporting data with each article (Refer to Supplementary Material section).

MANUSCRIPT PREPARATION

The manuscript should be written in English in a clear, direct, and active style. All pages must be numbered sequentially, facilitating the reviewing and editing of the manuscript.

MICROSOFT WORD TEMPLATE

It is advisable that authors prepare their manuscript using the template available on the Web, which will assist in preparation of the manuscript according to journal's format. Download the Template.

SECTIONS IN MANUSCRIPTS

Manuscripts submitted for research and review articles in the journal should be divided into the following sections:

- Title
- Title page
- Structured Abstract
- Graphical Abstract
- Keywords
- Text Organization
- Conclusion
- List of Abbreviations (if any)
- Consent for Publication
- Availability of Data and Materials
- Funding
- Conflict of Interest

- Acknowledgements
- References
- Appendices
- Figures/Illustrations (if any)
- Chemical Structures (if any)
- Tables (if any)
- Supportive/Supplementary Material (if any)

Title

The title of the article should be precise and brief and must not contain be more than 120 characters. Authors should avoid the use of non-standard abbreviations and question marks in titles. The first letter of each word should be in capital letters except for articles, conjunctions, and prepositions.

As recommended by the Reporting guidelines, information about the study should be a part of the title (particularly for randomized or clinical trials, systematic reviews and meta-analyses).

Authors should also provide a short 'running title with no more than 80 characters'. Title, running title, byline, correspondent footnote, and keywords should be written as presented in the original manuscript.

Title Page

Title page should include paper title, author(s) full name and affiliation, corresponding author(s) names complete affiliation/address, along with phone, fax and email.

Structured Abstract:

The abstract of an article should be a clear, concise and accurate summary, having no more than 250 words, and including the explicit sub-headings (as in-line or run-in headings in bold). Use of abbreviations should be avoided, and the references should not be cited in the abstract.

All the original research articles, systematic reviews, and meta-analyses must be accompanied by a structured abstract. Ideally, each abstract should include the following sub-headings, but these may vary according to the requirements of the article.

Introduction/Objective: Summarize the objective or purpose of the research in a few sentences.

Methods: Give a brief description of the research design, methodology, and other relevant details about the conduct of the study.

Results: Outline the main conclusions or findings of the study, often with statistical data or significant findings.

Conclusion: Provide an overview of the study's key findings and any implications.

The headings can vary, but must state the purpose of the study, details of the participants, measurements, methods, main findings, and conclusion.

Graphical Abstract

A graphic should be included when possible with each manuscript for use in the Table of Contents (TOC). This must be submitted separately as an electronic file (preferred file types are EPS, PDF, TIFF, Microsoft Word, PowerPoint, CDX, etc.). A graphical abstract, not exceeding 30 words, along with the illustration, helps to summarize the contents of the manuscript in a concise, pictorial form. It is meant as an aid for the rapid viewing of the journals' contents and to help capture the readers' attention. The graphical abstract may feature a key structure, reaction, equation, etc., that the manuscript elucidates upon. It will be listed along with the manuscript title, authors' names, and affiliations on the contents page, typeset within an area of 5 cm by 17 cm, but it will not appear in the article's PDF file or print.

Graphical Abstracts should be submitted as a separate file (must clearly mention graphical abstract within the file) online *via* Bentham's Manuscript Processing System (MPS).

You can view a few examples of the Graphical Abstracts on our website.

Keywords

A total of 6 to 8 keywords must be provided. Choose important and relevant keywords that researchers in your field will be searching for so that your paper will appear in a database search. The keywords should be contained in the title, and they should appear several times in the article. In biomedical fields, MeSH terms are a good 'common vocabulary' source to draw keywords from https://www.nlm.nih.gov/mesh/meshhome.html.

Text Organization

The main text should begin on a separate page and should be divided into the title page, abstract, and the main text. The text may be subdivided further according to the areas to be discussed, which should be followed by the List of Abbreviations, Conflict of Interest, Acknowledgements and Reference sections. For review, the manuscript should be divided into title page, abstract and the main text. The Review Article should mention any previous important recent and old reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The authors are advised to present and discuss their observations in brief.

For Research Articles the manuscript should begin with the title page and abstract followed by the main text, which must be structured into separate sections as **Introduction, Materials and Methods, Results, Discussion, Conclusion, Ethics Approval and Consent to Participate, Human and Animal Rights, Conflict of Interest, Acknowledgements and References**.

All randomized clinical trials must include a flow diagram and authors should provide a completed randomized trial checklist (see CONSORT Flow Diagram and Checklist; www.consort-statement.org) and a trial protocol.

The manuscript style must be uniform throughout the text, and 10 pt Times New Roman font should be used. The full term for an abbreviation should precede its first appearance in the text unless it is a standard unit of measurement. The reference numbers should be given in square brackets in the text. Italics should be used for Binomial names of organisms (Genus and Species),

for emphasis, and for unfamiliar words or phrases. Non-assimilated words from Latin or other languages should also be italicized e.g., *in vivo, in vitro, per se, et al., etc.*

SECTION HEADINGS

Section headings should be numbered sequentially, left aligned, and have the first letter capitalized, starting with the introduction. Sub-section headings, however, should be in lowercase and italicized with their initials capitalized. They should be numbered as 1.1, 1.2, *etc*.

INTRODUCTION

The Introduction section should include the background and aims of the research in a comprehensive manner.

MATERIALS AND METHODS

This section provides details of the methodology used along with information on any previous efforts with corresponding references. Any details for further modifications and research should be included. Sufficient details should be provided to the reader about the original data source in order to enable the analysis, appropriateness, and verification of the results reported in the study.

It is important for the Methods Section to be sufficiently detailed in respect of the data presented, and the results produced from it. This section should include all the information and protocol gathered for the study at the time when it was being written. If the study is funded or financially supported by an organization to conduct the research, then it should be mentioned in the Methods Section. Methods must be result-oriented. The statement regarding the approval by an independent local, regional or national review committee (e.g. name of ethic committee and institutional review board) should be part of the Methods Section.

Transparent reporting on AI and AI-assisted Technologies

Authors who use AI tools for the production of images or graphical elements of the paper or in the collection and analysis of data must disclose the use of such tools in the Materials and Methods (or similar section) of the paper, stating how the AI tool was used and which tool was used.

Bentham Science Publishers will assess whether the manner in which AI was used and declared is reasonable and compliant with its published principles and practices. After publication, content may be rejected or changed due to inadequate declaration or the specific circumstances of its use.

EXPERIMENTAL

Repeated information should not be reported in the text of an article. A calculation section must include experimental data, facts, and practical development from a theoretical perspective.

RESULTS

Results should be precise.

DISCUSSION

This should explore the significance of the results of the work, present a reproducible procedure, and emphasize the importance of the article in the light of recent developments in the field. Extensive citations and discussion of published literature should be avoided.

This section of research articles should discuss the implications of the findings in the context of existing research and highlight the study's limitations. The authors should justify the sample size according to the study purpose and methods.

The Results and Discussion may be presented together under one heading of “Results and Discussion”. Alternatively, they may be presented under two separate sections (“Results” section and “Discussion” Sections). Short sub-headings may be added in each section if required.

CONCLUSION

A small paragraph summarizing the contents of the article, presenting the final outcome of the research, or proposing further study on the subject may be given at the end of the article under the Conclusion section.

FUNDING

The authors need to declare the funding sources of their manuscripts clearly by providing the name of the funding agency or financial support along with the allotted grant/award number in round brackets (if applied), for instance, "This work was financially supported by [Name of the funding agency] (Grant number XXX)".

Similarly, if a paper does not have any specific funding source and is part of the employment of the authors, then the name of the employer will be required. Authors will have to clearly state that the funder was involved in writing, editing, approval, or decision to publish the article.

Greek Symbols and Special Characters

Greek symbols and special characters often undergo formatting changes and get corrupted or lost during preparation of manuscript for publication. To ensure that all special characters used are embedded in the text, these special characters should be inserted as a symbol but should not be a result of any format styling (Symbol font face) otherwise they will be lost during conversion to PDF/XML.

Authors are encouraged to consult reporting guidelines. These guidelines provide a set of recommendations comprising a list of items relevant to their specific research design. Chemical equations, chemical names, mathematical usage, unit of measurements, chemical and physical quantity, and units must conform to SI and Chemical Abstracts or IUPAC.

All kinds of measurements should be reported only in the International System of Units (SI).

Appendices

In case there is a need to present lengthy but essential methodological details, appendices must be used, which can be a part of the article. An appendix must not exceed three pages (Times New Roman, 10-point fonts, 900 max. words per page). The information should be provided in a

condensed form, ruling out the need for full sentences. A single appendix should be titled APPENDIX, while more than one can be titled APPENDIX A, APPENDIX B, and so on.

Supportive/Supplementary Material (if any)

We do encourage appending supportive material, for example, a PowerPoint file containing details about the study, a PowerPoint file containing additional screenshots, a Word, RTF, or PDF document showing the original instrument(s) used, a video, or the original data (SAS/SPSS files, Ms Excel files, Access Db files, etc.), provided it is inevitable or endorsed by the journal's Editor.

Supportive/Supplementary material intended for publication must be numbered and referred to in the manuscript but should not be a part of the submitted paper. In-text citations as well as a section with the heading "Supportive/Supplementary Material" before the "References" section, should be provided. All Supportive/Supplementary Material must be listed and a brief caption line for each file describing its contents should be included.

Any additional files will be linked to the final published article in the form supplied by the author but will not be displayed within the paper. They will be made available in exactly the same form as originally provided only on our Website. Please also make sure that each additional file is a single table, figure, or video (please do not upload linked worksheets or PDF files larger than one sheet). Supportive/ Supplementary material must be provided in a single zipped file not larger than 4 MB.

Authors must clearly indicate if these files are not for publication but meant for the reviewers'/editors' perusal only.

List of Abbreviations

If abbreviations are used in the text, they should either be defined in the text where they first used, or a list of abbreviations can be provided.

RESEARCH ETHICS AND POLICIES

CONFLICT OF INTEREST

AUTHORS

All potential conflicts of interest (competing interests) that could have a direct or indirect influence on the work must be disclosed by the authors. Even if an author does not have a conflict, disclosing affiliations and interests allows for a more comprehensive and open approach, which leads to a more accurate and objective evaluation of the work. Conflicts of interest, whether genuine or imagined, are a perspective to which the readers are entitled.

The publication of a conflict statement in the article itself, as well as the submission of the conflict disclosure form, is required for all types of papers. It is not necessarily the case that a monetary relationship with examination support or funding for counseling work is inappropriate. Even if the authors do not have any conflict of interest, they still need to provide a confirmation statement in their manuscripts, *i.e.,* “The author(s) confirm(s) that there is no conflict of interest related to the manuscript.”

The following are some examples of potential conflicts of interest that are directly or indirectly related to the research:

Financial competing interests include (but are not limited to):

- Type of support/grant number
- Institutional Conflicts of Interest
- Funds received by the author
- Funds received by the institution
- Travel allowances for the research
- Funds received for article preparation and reviewing
- Funds for conducting review activities
- Support provided for article writing assistance, for drugs, equipment, etc
- Paid lectures
- Pending fund or grant

Financial conflicts of interest can be personal as well as institutional. A Personal conflict of interest occurs when a contributor involved in the publication process either receives an amount of money or expects to receive some financial help (including any other financial benefits such as patents or stocks, gifts, or services) that may impact the work related to a specific publication. More importantly, in academic research, such financial relationships can lead to institutional conflicts of interest (COIs) because the economic interests of the institution or institutional representatives may unsuitably affect the decision-making process.

An institutional conflict of interest arises in a situation when financial interests of an institution or any institutional official (e.g., investments held by the university in a company) have the potential to unduly influence the research conducted by its employees or students, or pose an unacceptable risk to human subjects. Such conflicts usually arise in a state of affairs where a research project directly offers assistance or a benefit to an external entity *via* evaluation, validation, trial, or test of an invention, product, drug, service, or technology, and the institution holds a financial interest with the external entity. Such financial interests incorporate, but are not limited to, the receipt of licensing payments or royalties from the external entity, or ownership interest with the external entity. When human subjects are involved in any research project, and the institution supports such a financial interest, the conflict of interest is speculated to be unreasonable.

Non-financial competing interests should include (but are not limited to) the following:

In addition, interests other than monetary and any funding (non-financial interests) should be declared if they are relevant to readers. Personal relationships or conflicting interests directly or indirectly related to research, as well as professional interests or personal opinions that may impact your research, are examples of these.

Intellectual property, in basic terms, refers to any intangible property that is the result of creativity, such as patents, copyrights, etc. Similarly, this section seeks to know about copyright and patent (licensed patent, pending or issued) and any payment received for intellectual property, including the following:

- Patent

- Licensed Patent
- Issued Patent
- Pending Patent
- Royalties
- Licensee
- Remarks

DISCLOSURE STATEMENT

All conflict of interest disclosure forms are collected by the corresponding author. It is sufficient for the corresponding author to sign the disclosure form on behalf of all authors in author collaborations when legal agreements for representation allow it. The templates of the form can be found here.

- Disclosure form
- ICMJE disclosure form

Before the reference list, the corresponding author will include a summary statement in the text of the article that reflects what is reported in the potential conflict of interest disclosure form (s). Author(s) may declare(s) names of reviewers who they think might have a potential conflict of interest; therefore, the Editorial Office could avoid inviting such reviewers for an unbiased opinion.

UNDISCLOSED CONFLICT OF INTEREST

Undisclosed conflict of interest cases before or after the publication of an article are dealt with as per the guidelines of COPE.

a. Undisclosed conflict of interest in a submitted article (View COPE guidelines)
b. Undisclosed conflict of interest in a published article (View COPE guidelines)

For more information on COIs, see the guidance from the ICMJE.

PEER REVIEWERS

Bentham Science tries to conduct a transparent peer-review process with the help of the reviewers who do not have any conflict of interest with the authors. In this connection, reviewers who belong to the same institute or countries as authors are not invited to review manuscripts. However, it is not possible for the Editorial Office to be aware of all competing interests; therefore, it is expected from authors to submit a list of reviewers who they think have a conflict of interest to ensure a transparent and unbiased review process.

The Editorial Office expects reviewers:

- Not to accept manuscript review requests if they have any potential conflict of interest and inform the Editorial Office accordingly.
- To decline review requests if they have recently published or submitted an article with any of the authors listed in the manuscript.
- To inform the Editorial Office if they have any personal relationship with the authors or work in the same institutes as the authors, which could affect the review transparency.

- To inform the Editorial Office/Editor-in-Chief/Handling Editors about any scientific misconduct or fraud, plagiarism, conflict of interest, or any other unethical behavior related to the manuscript that they found while reviewing it and abstain from reviewing it further.

  During the submission of review comments, reviewers are asked to reconfirm that they do not have any conflict of interest related to the article. After confirming the below statement, they can submit their comments.

  "I hereby confirm that I don't have any conflict of interest related to the manuscript."

  If, however, there are still any remaining interests, then reviewers must mention those in the 'Confidential' section of the review form.

  Reviewers are not encouraged to contact authors directly regarding any of their conflicts of interest. Peer reviewers should follow journals' policies in situations they consider to represent a conflict to reviewing.

UNDISCLOSED CONFLICT OF INTEREST

If reviewers intentionally undisclosed any conflict of interest, then they will be blacklisted for any future peer-reviewing activity of the journal.

The Editorial Office always ensures that an author, if added after peer review activity of a manuscript, is not part of the reviewers' list who have conducted a peer review of the same manuscript.

EDITORS

Editors must not review submitted manuscripts if they have any personal, professional or financial involvement/conflict of interest with the authors of the manuscript. Every participant involved in the peer review process, including editorial board members, reviewers, and editors, must declare any potential conflicts of interest to ensure a transparent and unbiased review activity.

Editors-in-Chief or Editors who are responsible for the initial and final decision should recuse themselves from reviewing or making decisions on any manuscript that is written by authors affiliated with to the same institute as the editor or if they have been a family member, competitor, collaborator, or have published any manuscript in last 3 years with the authors associated with the manuscript. They can, however, nominate someone else on the Board who could provide a neutral opinion on the manuscript.

The Editorial office recommends editors to follow COPE and WAME guidelines to process such manuscripts which involve their personal relationships.

Manuscripts submission by an Editor/Editor-in-Chief

The initial and final decision on the manuscripts submitted by an Editor/Editor-in-Chief will be taken by any other member of the Board. The Editorial Office will identify members who do not have any potential conflict of interest with the Editor or Editor-in-Chief.

Acknowledgements

Any research assistants or other individuals who assisted with the research but did not meet all criteria for authorship, such as those who carried out the literature review, produced, computerized, and analyzed the data, or helped with the language, writing, or proofreading of the article, or offered any comments or suggestions, should be acknowledged. Briefly, everyone who has contributed significantly to the improvement of the paper should be acknowledged. It is recommended to mention the "Declared None" if there is no acknowledgement for the study.

Author Contributions/ CRediT Author Statement

The specific requirements for authorship have been defined by the International Committee of Medical Journal Editors (ICMJE). The examples of authors' contributions are 'designed research/study', 'performed research/study', 'contributed important reagents', 'collected data', 'analyzed data', 'wrote paper', etc. This information must be included in the submitted manuscript as a separate paragraph under the heading 'Authors' Contribution'. The corresponding author is responsible for obtaining permission from all co-authors for the submission of any version of the manuscript and making any changes in the authorship.

IMPORTANT: An Author Contribution CRediT (Contributor Roles Taxonomy) statement is now a strict requirement for all submissions to Bentham Science Publishers. This policy aims to address past inconsistencies in the provision of "Author Contribution" statements by authors. Authors are encouraged to clearly define and document their contributions to promote transparency and accountability in the research process.

HUMAN AND ANIMAL RIGHTS

Research Involving Humans

All clinical investigations should be conducted according to the Declaration of Helsinki principles. For all manuscripts reporting data from studies involving human participants, formal review and approval by an appropriate institutional review board or ethics committee are required.

Patient Consent

Compliance with the guidelines of the International Committee of Medical Journal Editors (www.icmje.org) is recommended, in accordance with the patient's consent for research or participation in a study as per the applicable laws and regulations regarding the privacy and/or security of personal information, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") and other U.S. federal and state laws related to confidentiality and security of personally distinguishable evidence, namely, the General Data Protection Regulation (GDPR) (EU) 2016/679 and member state implementing legislation, such as Canada's Personal Information Protection and Electronic Documents Act and India's Information Technology Act and related Privacy Rule, (together "Data Protection and Privacy Laws").

It is the responsibility of the author to ensure that:

- Patients' names, initials, or hospital numbers are not mentioned anywhere in the manuscript (including figures).

- Authors are responsible for obtaining the patient consent-to-disclose forms for all recognizable patients in photographs, videos, or other information that may be published in the Journal, in derivative works, or on the journal's website and for providing the manuscript to the recognizable patient for review before submission.
- The consent-to-disclose form should indicate specific use (publication in the medical literature in print and online, with the understanding that patients and the public will have access) of the patient's information and any images in figures or videos, and must contain the patient's signature or that of a legal guardian along with a statement that the patient or legal guardian has been offered the opportunity to review the identifying materials and the accompanying manuscript.
- If the manuscript has an individuals' data, such as personal details, audio-video material, *etc*., consent should be obtained from that individual. In case of children, consent should be obtained from the parent or the legal guardian.
- A specific declaration of such approval and consent-to-disclose form must be made in the copyright letter and in a stand-alone paragraph at the end of the article especially in the case of human studies where the inclusion of a statement regarding obtaining the written informed consent from each subject or subject's guardian crucial. The original form should be retained by the guarantor or the corresponding author. Editors may request to provide the original forms by fax or email.
- All such case reports require proper consent to be obtained prior to publishing. Please refer to COPE guidelines available at **https://publicationethics.org/resources/guidelines/journals%E2%80%99-best-practices-ensuring-consent-publishing-medical-case-reports**.

  Editors may request that authors provide documentation of the formal review and recommendation from the institutional review board or ethics committee responsible for oversight of the study. The editors reserve the right to reject manuscripts that do not comply with the above-mentioned requirements. The author will be held responsible for false statements or failure to fulfill the above-mentioned requirements.

Non-identifiable Images

Anonymous images that do not identify the individual directly or indirectly, such as through any identifying marks or text, do not require formal consent, for example, X-rays, ultrasound images, pathology slides, or laparoscopic images.

In case consent is not obtained, concealing the identity through eye bars or blurring the face would not be acceptable.

Research Involving Animals

For research involving animals, the authors should indicate whether the procedures followed were in accordance with the standards set forth in the eighth edition of "Guide for the Care and Use of Laboratory Animals" (grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals_prepub.pdf published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

Research work on animals should be carried out in accordance with the NC3Rs ARRIVE Guidelines. For *In Vivo* Experiments, please visit https://www.nc3rs.org.uk/arrive-guidelines

Authors should clearly state the name of the approval committee, highlighting that legal and ethical approvals were obtained prior to initiation of the research work carried out on animals and that the experiments were performed in accordance with the relevant guidelines and regulations stated below.

- US authors should cite compliance with the US National Research Council's "Guide for the Care and Use of Laboratory Animals"
- The US Public Health Service's "Policy on Humane Care and Use of Laboratory Animals" and "Guide for the Care and Use of Laboratory Animals"
- UK authors should conform to UK legislation under the Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039).
- European authors outside the UK should conform to Directive 2010/63/EU.
- Research on animals should adhere to the ethical guidelines of The Basel Declaration and the International Council for Laboratory Animal Science (ICLAS), which has also published ethical guidelines.
- The manuscript should clearly include a declaration of compliance with the relevant guidelines (*e.g.*, the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europe) and/or relevant permissions or licenses obtained by the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Animal Ethics Guidelines for Studies Involving Animal Subjects

Ethics Approval Exemption:
If a study is exempted from ethics approval, authors must indicate the reasons for exemption in the ethical statement.

Following is an example of Ethical Statements:

"This study involving animal subjects is exempted from ethics approval for (specific reasons). The exemption was evaluated and authorized by (Full name of ethics committee), ensuring adherence to ethical standards".

Client-Owned Animals:
Client-owned animals (non-commercially available animals such as pets or livestock) should be studied exercising best practices in veterinary care. Authors must confirm that the owner(s) (or their legal representatives) have provided written consent for this purpose.

Following is an example of an Ethical Statements:

"The animal study was evaluated and authorized by (Full name of the ethics committee). The owners provided written informed consent for their animals' involvement in this study, ensuring ethical treatment and compliance with standards."

International Standards and 3Rs Principle:
Studies involving animals must comply with internationally accepted standards and adhere to the 3Rs principles (Replace, Reduce, Refine).

- **Replace:** Replacing animals with alternatives whenever possible.

- **Reduce:** Reducing the number of animals used.
- **Refine:** Refining experimental settings can reduce animal damage.
  Authors are encouraged to follow the ARRIVE guidelines (Reporting in Vivo Experiments) for reporting experiments involving live animals.

  Example of an Ethical Statements:

  "This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices."

Euthanasia **Protocols:**
Studies on euthanasia, including chloral hydrate, ether, and chloroform overdose, are severely discouraged. Authors should include an in-depth description of any anesthetic, surgical, or euthanasia procedures conducted throughout the study.

If the experimental details explained in the study violate the standard animal research procedure, editors may seek extra documentation, such as approval forms and relevant literature citations.

Research Involving Plants

All experimental research on plants (either cultivated or wild) should comply with international guidelines. The manuscript should include a declaration of compliance of field studies with relevant guidelines and/or relevant permissions or licenses obtained by the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Hazard Study

Any unusual risks associated with the use of any chemicals, procedures, or equipment used in the work must be explicitly stated by the author in the manuscript, preferably in both the materials and methods section and the declaration section. For more information, visit The World Medical Association (https://www.wma.net/what-we-do/public-health/chemicals)

SEX AND GENDER EQUITY IN RESEARCH (SAGER) GUIDELINES

We strive to promote gender and sex equity in research and adhere to the guidelines of Sex and Gender Equity in Research (SAGER) to ensure inclusivity. All authors submitting research papers are required to follow the Sex and Gender Equity in Research (SAGER) guidelines. These guidelines are intended to encourage the inclusion of sex and gender considerations in research in order to improve the rigor and relevance of our publications.

The SAGER guidelines for reporting sex and gender information in methodology or study design, data analysis, results, and interpretation of findings are strongly encouraged. Authors of review articles are advised to address the methods used for selecting, locating, extracting, and synthesizing data. In addition, systematic reviews are also required to follow these guidelines.

RESEARCH CONDUCTED IN SPECIAL OR CRITICAL SITUATIONS

Bentham Science expects all contributors to respect the values of justice, benevolence, and autonomy when conducting research. We understand that certain situations, such as medical emergencies or humanitarian crises may differ from non-emergency scenarios. Bentham Science recommends that research efforts should not hurt human subjects/respondents or the researchers and should be conducted with sufficient scientific rigor as permissible in these situations, respectively. Care should be taken to address potential problems faced by persons who may be victims of disasters or involved in a medical emergency. These are vulnerable individuals, and their privacy and dignity should be respected. Researchers should make note of this in their research and identify potential issues in their work that may arise because of such situations. Research directed in emergency circumstances should prioritize the well-being of the survivors involved in the research and with the goal of minimizing any future casualties. For guidance, the essential requirements of research in emergency situations are the preservation of human life, well-being and security, along with the rights to protection, privacy and confidentiality of subjects.

UNETHICAL BEHAVIOR

Unethical behavior and misconduct may be pointed out by anyone to the Editor and Publisher with sufficient evidence. The Editor, in consultation with the Publisher, will initiate an investigation against this unethical misconduct, complete the procedure till an unbiased decision is reached, and maintain confidentiality throughout the process of the investigation. The author should be given the opportunity to reply to all minor or major accusations.

In case of serious breaches, the employer may be informed, where appropriate, by the Editor/Publisher after reviewing all available information and evidence or after seeking help from experts in that field.

Conclusion

- Author(s) and Reviewers must be informed in case of misinterpretation or mishandling of International Acceptable Standards
- A strict notice should be sent to the author and reviewer to avoid future unethical misconduct
- An Editorial on the reported misconduct should be published, or an official notice of unethical behavior should be posted on the website
- An official letter about this misconduct should be issued to the Head of Departments, Funding Agencies of the accused author and the reviewer, as well as Abstracting & Indexing Agencies.
- Where required, retraction and withdrawal of publication may be undertaken from the Publisher's journal in discussion with the Head of the Department of the author or reviewer, and other higher authorities should be informed
- The Publisher may impose restrictions for some period on future publications from the accused author in the journals

Consent for Publication

If the manuscript has an individual's data, such as personal detail, audio-video material etc., consent should be obtained from that individual. In the case of children, consent should be obtained from the parent or the legal guardian.

A specific declaration of such approval and consent-to-disclose form must be made in the copyright letter and in a stand-alone paragraph at the end of the article especially in the case of human studies where the inclusion of a statement regarding obtaining the written informed consent from each subject or subject's guardian is a must. The original should be retained by the guarantor or corresponding author. Editors may request to provide the original forms by fax or email.

All such case reports should be followed by a proper consent prior to publishing.

RANDOMIZED DRUG CLINICAL TRIAL STUDIES

Randomized drug clinical trial studies are biomedical or health-related interventional and/or observational research studies conducted in phases in human beings who are randomly allocated to receive or not receive a preventive, therapeutic, or diagnostic intervention that follows a pre-defined protocol. The study is intended to determine the safety and efficacy of approaches to disease prevention, diagnosis and treatment.

Authors of randomized controlled trials are encouraged to submit trial protocols along with their manuscripts. All clinical trials must be registered (before recruitment of the first participant) at an appropriate online public trial registry that must be independent of for-profit interest (e.g.,www.clinicaltrials.gov). If you wish the editor(s) to consider an unregistered trial, please explain briefly why the trial has not been registered.

- All randomized clinical trials should include a flow diagram and authors should provide a completed randomized trial checklist (see CONSORT Flow Diagram and Checklist; www.consort-statement.org) and a trial protocol. For further details, please visit complete guidelines at: http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html
- Studies of diagnostic accuracy must be reported according to STARD guidelines; (www.stard-statement.org)
- Observational studies (cohort, case-control, or cross-sectional designs) must be reported according to the STROBE statement, and should be submitted with their protocols; (www.strobe-statement.org).
- Genetic association studies must be reported according to STREGA guidelines; (www.medicine.uottawa.ca)
- Systematic reviews and meta-analyses must be reported according to PRISMA guidelines; (www.prisma-statement.org)
- To find the reporting guidelines see (www.equator-network.org)

Important points to remember while submitting clinical trials:

- Each manuscript should clearly state an objective or hypothesis; the design and methods (including the study setting and dates, patients or participants with inclusion and exclusion criteria, or data sources, and how these were selected for the study); the essential features of any interventions; the main outcome measures; the main results of the study; a comment section placing the results in context with the published literature and addressing study limitations; and the conclusions. Data included in research reports must be original.
- Trial registry name, registration identification number, and the URL for the registry should be included at the end of the abstract and also in the space provided on the online manuscript

submission form. If your research article reports the results of a controlled health care intervention, list the trial registry, along with the unique identifying number (Please note that there should be no space between the letters and numbers of your trial registration number). Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials), are exempted.

- All reports of randomized trials should include a section entitled “Randomization and Masking”, within the Methods section.
- The manuscript must include a statement identifying the institutional and/or licensing committee that has approved the experiments, including any relevant details.
- The SI system of units and the recommended international non-proprietary name (rINN) for drug names must be used. Kindly ensure that the dose, route, and frequency of administration of any drug you mention are correct.
- Please ensure that the clinical trials sponsored by pharmaceutical companies follow the guidelines on good publication practice: (https://www.ismpp.org/gpp2)

The editors reserve the right to reject manuscripts that do not comply with the above-mentioned requirements. The author will be held responsible for false statements or failure to fulfill the above-mentioned requirements.

AVAILABILITY OF DATA AND MATERIALS

The source of data and materials should be mentioned in the manuscript, in support of the findings. Sharing research data is integral to its transparency and reproducibility. Data sharing involves the citation and availability of data that support the findings of the research.

Bentham Science encourages authors to share the source of data and materials in the manuscript in support of the findings.

Research Data Policy Types:

The four types of research data policies are mentioned below.

- **Case 1:** Data sharing and data citation
- **Case 2:** Data sharing and its evidence
- **Case 3:** Statement for Data sharing and data availability
- **Case 4:** Data sharing, evidence of data sharing and data for peer-review

Case 1: Data Sharing and Data Citation

Wherever appropriate and possible, the journal encourages authors to publish data to support their research findings in a public repository. Any datasets mentioned in the article that are available in external repositories should be cited.

How to Cite the Data?

Whether the data were developed by the author(s) or researcher(s), all publicly available data referenced in the preparation of an article should be cited in the text and reference list. The references relating to the data availability should be presented in the following format:

Example: Name of author(s), the title of the data set, data repository, document version (e.g., most recent updated version), Digital Object Identifier (DOI), and Bentham Science reference style should be included in data citations.

Case 2: Data Sharing and Its Evidence

When authors submit a paper to a journal, the authors agree that the data provided in the publication, including the relevant raw data, will be freely available to any researcher who wants to use these for non-commercial reasons without jeopardising participant anonymity.

Case 3: Statement for Data Sharing and Data Availability

Data availability declarations are required under Bentham Science research data policy types.

The statement relating to the data availability should be presented in the following format under a separate section for 'Availability of Data and Materials' in the manuscript:

1. *The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.*
2. *The data that support the findings of this study are available from the corresponding author, [author initials], on special request.*
3. *The datasets generated or analysed during the current study are not publicly available due to [mention the reason(s)].*
4. *Authors who do not wish to share their data should clearly state that the data will not be shared and thus mention it as 'Not applicable'.*
5. *The statement relating to the data should be presented in the following format:*

"The data supporting the findings of the article is available in the [repository name] at [URL], reference number [reference number]".

Additional Data Availability Statements

Authors can add or change the statement(s) above to fit their work the best. Depending on the nature of the research, several assertions may need to be merged.

Case 4: Data Sharing, Evidence of Data Sharing, and Data for Peer-Review

All datasets on which the paper's conclusions are based must be made accessible to reviewers and readers, according to the journal's rules. Prior to peer review, authors must either deposit their datasets in publicly accessible repositories or provide them as supplementary materials with their submission. For further details, please visit the complete guidelines at: **http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html**

Data Access and Retention

Authors may provide the raw data in connection with a paper for editorial review and should be prepared to provide public access to such data, if practicable. Thet should, in any event, be prepared to retain such data for a reasonable time after publication.

STANDARDS OF REPORTING

The Authors are encouraged to use industry-recognized reporting guidelines for biomedical and biological research, if applicable, to explain that all requirements for reporting have been adopted.

All authors must strictly follow the reporting guidelines below for preparing the study for publication.

- CONSORT: All randomized clinical trials must include a flow diagram and authors should provide a completed randomized trial checklist (see CONSORT Flow Diagram and Checklist; www.consort-statement.org) and a trial protocol.For further details, please visit complete guidelines at: http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html
- STARD and TRIPOD: Studies of diagnostic accuracy must be reported according to STARD guidelines; (www.stard-statement.org) and TRIPOD guidelines; (www.tripod-statement.org)
- STROBE: Observational studies (cohort, case-control, or cross-sectional designs) must be reported according to the STROBE statement, and should be submitted with their protocols; (www.strobe-statement.org).
- CARE: Case report must be reported according to CARE guidelines; (www.care-statement.org)
- COREQ: Qualitative research must be reported according to COREQ guidelines; (academic.oup.com/intqhc/article/19/6/349/1791966)
- CHEERS: Economic evaluations must be reported according to CHEERS guidelines; (www.bmj.com/content/346/bmj.f1049)
- STREGA: Genetic association studies must be reported according to STREGA guidelines; (www.medicine.uottawa.ca)
- PRISMA: Systematic reviews and meta-analyses must be reported according to PRISMA guidelines; (www.prisma-statement.org)
- MOOSE: Meta-analyses of observational studies in epidemiology must be reported according to MOOSE guidelines (http://www.ijo.in/documents/14MOOSE_SS.pdf)
- EQUATOR: To find the reporting guidelines see (www.equator-network.org)

POST-PUBLICATION DISCUSSIONS

Post-publication discussions are well-timed and engaging scientific remarks and justifications on research articles published in the journal. These remarks must be based on the information concurrent with the original study and not on the scientific advancements being made subsequently.

Manuscript Preparation, Submission, and Editorial Process:

- Post-publication discussion should commence with a short paragraph that outlines the summary of the article.
- Authors are advised to avoid using inciting tone in the comments and keep the message clear and concise.
- The main text should not exceed 1200 words with up to 15 references and may include one or two figures and/or tables.
- References should be submitted in the ACS or Vancouver style.

- The correspondents are recommended to contact the original authors first prior to submitting their comments to the journal, as this may resolve the issues that may have arisen due to some misunderstanding.
- The correspondence that has been done with the authors should also be submitted as an attachment with the manuscript.
  Any queries therein should be addressed to info@benthamscience.net

FIGURES/TABLES

Figures/Illustrations (if any)

All authors must strictly follow the guidelines below for preparing illustrations for publication in the journal. If the figures are found to be sub-standard, then the manuscripts will be rejected.

The authors are expected to submit good quality figure(s) in PDF, PPT, MS Word, TIFF or JPEG versions, which, if required, should be improved yourself or by professional graphic designers of your organization/ country. You may even consider approaching our contracted service provider, Eureka Science, for Graphics Enhancement Services.

The Graphics Designing team at Eureka Science can assist in improving the quality of your images at affordable rates. Eureka Science has offered special rates of **US $155** for improvement of up to five figures, with any additional figures being charged at **US $25** each.

The quality of Graphic Enhancement Services offered by Eureka Science can be viewed at http://www.eureka-science.com/images/Binder1.pdf, along with valuable feedback on their services at http://www.eureka-science.com/testimonials.php. You may contact Eureka Science at info@eurekascience.net

Note: Availing Graphics Enhancement Services does not guarantee acceptance of the manuscript for publication. The final acceptance/decision on the manuscript is taken by the EiC.

Guideline for Figures/Illustrations

Illustrations must be provided according to the following guidelines:

- Illustrations should be embedded in the text file and must be numbered consecutively in the order of their appearance. Each figure should include only a single illustration, which should be cropped to minimize the amount of space occupied by the illustration.
- If a figure is in separate parts, all parts of the figure must be provided in a single composite illustration file.
- Photographs should be provided with a scale bar, if appropriate, as well as high-resolution component files.
- All the numbers, symbols and letters in figures should be consistent and clear throughout and large enough to remain readable when the size is reduced for publication.
- It must be ensured to cite each figure in the text in sequence.

Scaling/Resolution

Line Art image type is normally an image based on lines and text. It does not contain tonal or shaded areas. The preferred file format should be TIFF or EPS, with the color mode being Monochrome 1-bit or RGB, in a resolution of 900-1200 dpi.

Halftone image type is a continuous tone photograph containing no text. It should have the preferred file format TIFF, with color mode being RGB or Grayscale, in a resolution of 300 dpi.

A combination image type is an image containing halftone, text, or line art elements. It should have the preferred file format, TIFF, with color mode being RGB or Grayscale, in a resolution of 500-900 dpi.

Formats

Illustrations may be submitted in the following file formats:

- **Illustrator**
- **EPS** (preferred format for diagrams)
- **PDF** (also especially suitable for diagrams)
- **PNG** (preferred format for photos or images)
- **Microsoft Word** (version 5 and above; figures must be a single page)
- **PowerPoint** (figures must be a single page)
- **TIFF**
- **JPEG** (conversion should be done using the original file)
- **BMP**
- **CDX** (ChemDraw)
- **TGF** (ISISDraw)

Bentham Science Publishers does not process figures submitted in GIF format.

For TIFF or EPS figures with considerably large file size restricting the file sizes, in online submissions is advisable. Authors may, therefore, convert to JPEG format before submission, as this results in significantly reduced file size and upload time, while retaining acceptable quality. JPEG is a 'lossy' format. However, in order In order to maintain acceptable image quality, it is recommended that JPEG files be saved at high or maximum quality.

Zipit or Stuffit tools should not be used to compress files prior to submission, as the resulting compression through these tools is always negligible.

Please refrain from supplying:

1. Graphics embedded in word processor (spreadsheet, presentation) document.
2. Optimized files optimized for screen use (like GIF, BMP, PICT, WPG) because of the low resolution.
3. Files with too low a resolution.
4. Graphics that are disproportionately large for the content.

Technical requirements for graphic/ figure submissions.

Requirement
Width = 8.5 inches (In-between the required size)
Height = 11 inches (In-between the required size)
Pixels/Inches = 300 (minimum dpi)
All figures should be in vector scale (except half tone, photograph.)

Image Conversion Tools

There are many software packages, many of them freeware or shareware, capable of converting to and from different graphics formats, including PNG.

General tools for image conversion include Graphic Converter on the Macintosh, Paint Shop Pro for Windows, and ImageMagick, available on Macintosh, Windows and UNIX platforms.

Bitmap images (*e.g.,* screenshots) should not be converted to EPS as they result in a much larger file size than the equivalent JPEG, TIFF, PNG, or BMP, and poor quality. EPS should only be used for images produced by vector-drawing applications such as Adobe Illustrator or CorelDraw. Most vector-drawing applications, can be saved or exported in EPS format. If the images are originally prepared in an Office application, such as Word or PowerPoint, original Office files should be directly uploaded to the site, instead of being converted to JPEG or another format of low quality.

Chemical Structures (if any)

Chemical structures must be prepared in ChemDraw/CDX and provided as separate files.

Structure Drawing Preferences

[As according to the ACS style sheet]

Drawing Settings	
Chain angle	120°
Bond spacing	18% of width
Fixed length	14.4 pt (0.500cm, 0.2in)
Bold width	2.0 pt (0.071cm, 0.0278in)
Line width	0.6 pt (0.021cm, 0.0084in)
Margin width	1.6 pt (0.096cm)
Hash spacing	2.5 pt (0.088cm, 0.0347in)
Text settings	
Font	Times New Roman
Size	10 pt
Under the Preference Choose	
Units	points
Tolerances	3 pixels
Under Page Setup Use	
Paper	US letter
Scale	100%

Tables (if any)

- Data Tables should be submitted in Microsoft Word table format.
- Each table should include a title/caption being explanatory in itself with respect to the details discussed in the table. Detailed legends may then follow.
- Table number in bold font *i.e.,* Table **1**, should follow a title. The title should be in small case with the first letter in caps. A full stop should be placed at the end of the title.
- Tables should be embedded in the text exactly according to their appropriate placement in the submitted manuscript.
- Columns and rows of data should be made visibly distinct by ensuring that the borders of each cell are displayed as black lines.
- Tables should be numbered in Arabic numerals sequentially in order of their citation in the body of the text.
- If a reference is cited in both the table and text, please insert a lettered footnote in the table to refer to the numbered reference in the text.
- Tabular data provided as additional files can be submitted as an Ms Excel spreadsheet.
- It is adequate to present data in Tables to avoid unnecessary repetition and reduce the length of the text.
- The citation of each table in the text must be ensured.
- Symbols and nonstandard abbreviations should be explained at the end of the text.
- All references should be numbered sequentially [in square brackets] in the table and listed in the same numerical order in the reference section.

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AUTHORSHIP CRITERIA

Bentham Science Publishers requires that all individuals listed as authors must have made a substantial contribution to the design, performance, analysis, or reporting of the work. The role of authors is judged on the basis of **ICMJE** and **COPE guidelines.**

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The Corresponding Author must provide a final list of authors at the time of submission, ensuring the correct sequence of the names of authors, which will not be considered for any addition, deletion, or rearrangement after the final submission of the manuscript. The email address of the principal author should be provided with an asterisk. However, the complete address, business telephone numbers, fax numbers and e-mail address of the corresponding author must be stated to receive correspondence and galley proofs. Bentham Science Publishers recommends that all contributors regularly update their profiles on SCOPUS/ORCID and other databases.

The corresponding author must have the approval of all other listed authors for the submission and publication of all versions of the manuscript.

AUTHOR IDENTIFICATION

Authors are strongly recommended to use their ORCID ID when submitting an article for consideration. Alternatively, they can acquire an ORCID ID via the submission process. For more information about ORCID IDs, visit here.

CHANGES TO AUTHORSHIP

At the time of initial submission, the finalized list of authors in correct sequence should be provided, which will not be changed once the publication process has started.

If any change is essential, then it can only be done after the approval of the Editor-in-Chief upon receiving the following details from the corresponding author:

1. The reason for the change in the author list and/or their sequence
a. A proper justification should be provided for changes in authorship.
b. Correction of existing names should be accompanied by a notice to the Editor-in-Chief of the journal.
2. A written confirmation from all the co-authors is a prerequisite for any amendment or removal. Any amendment to the authors' list will only be considered and approved by the Editor-in-Chief after complete verification. Publication of the manuscript will be withheld during consideration of the request. However, if the manuscript has already been published online, requests approved thereafter by the Editor-in-Chief will result in an erratum or corrigendum. The corresponding author is responsible for obtaining permission from all co-authors for any changes in the authorship.

Here is some advice by COPE on authorship issues. Bentham strives to follow these guidelines.

AUTHORSHIP AND AI TOOLS

Bentham Science Publishers recognizes that authors use a variety of tools for preparing articles related to their scientific works, ranging from simple ones to very sophisticated ones.

According to the COPE (Committee on Publication Ethics) guidelines, *"AI tools cannot meet the requirements for authorship as they cannot take responsibility for the submitted work. As non-legal entities, they cannot assert the presence or absence of conflicts of interest nor manage copyright and license agreements"*.

The pertinence of such tools may vary and evolve with public opinion, due to which the use of AI-powered language tools has led to a significant debate. These tools may generate useful results, but they can also lead to errors or misleading results; therefore, it is important to know which tools were used for evaluating and interpreting a particular scientific work.

Considering the above, we require that:

1. The authors should report any significant use of such tools in their works, such as instruments and software along with text-to-text generative AI consistent with subject standards for methodology.
2. All co-authors should sign a declaration that they take full responsibility for all of its contents, regardless of how the contents were generated. Inappropriate language, plagiarized and biased contents, errors, mistakes, incorrect references, or misleading content generated by AI language tools and the relevant results reported in scientific works are the full and shared responsibility of all the authors, including co-authors.
3. AI language tools should not be listed as an author; instead, authors should follow clause (1) above.

General Advice:

Advice on how to spot authorship problems

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Corresponding author requests the addition of an extra author before publication

Corresponding author requests the removal of the author before publication

After publication:

Request for addition of extra author after publication

Request for removal of author after publication

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PROOF CORRECTIONS

Authors will receive page proofs of their accepted papers before publication. To avoid delays in publication, proofs should be checked immediately for typographical errors and returned within **48 hours**. Major changes are not acceptable at the proof stage.

The corresponding author will be solely responsible for ensuring that the revised version of the manuscript incorporating all the submitted corrections receives the approval of all the co-authors of the manuscript.

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To extend the scope and visibility of your research by creating an animated abstract. Bentham Science has collaborated with Focus Medica, one of the world's largest publishers of expert animated atlases and videos in medicine and science.

An animated abstract will help summarise the essential discoveries/ key findings of your published research or review article. Each professionally produced full-coloured animated abstract in video format (length 3 – 5 minutes) is accompanied by an English-spoken or foreign language commentary. The animated abstract will be published online along with the published article.

The payment for an animated abstract will be **US $1320**. Initially, an advance amount of **US $800** will be payable to the Publisher to start work on the Animated Abstract, while the balance of **US $520** will be payable upon completion of the Animated Abstract.

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REVIEWING AND PROMPTNESS OF PUBLICATION

All papers submitted to Bentham Science for publication are immediately subjected to preliminary editorial scrutiny by the Editorial Staff and Editor-in-Chief in connection with their suitability. The Editor-in-Chief determines if the manuscript:

(a) falls within the scope of the journal and

(b) meets the editorial criteria of Bentham Science Publishers in terms of originality and quality.

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Before sending the manuscripts to reviewers, Bentham Science seeks consent from potential reviewers about their availability and willingness to review. Correspondence between the editorial office of the journal and the reviewers is kept confidential. The reviewers are expected to provide their reports in a timely fashion since a prompt review leads to the timely publication of a manuscript which is beneficial not only to the authors but to the scientific community as well.

The editorial process and peer-review workflow for each journal are taken care of by a team of Senior Editors, Editorial Board Members (EBMs), and dedicated Journal managers who have the required expertise in their specific fields.

Bentham Science Publishers carries out independent reviews of all articles. The reviewers are selected according to their expertise from our regularly updated referee database.

On the basis of reviewer comments, the Editors may recommend acceptance, revision, or rejection of a manuscript.

After a review of the manuscript by at least two or three independent experts, in addition to the views of the Editor, the decision is relayed to the authors, which may be categorized as:

- Requires minor changes
- Requires major changes
- Rejected with no resubmission

If an article receives two contradictory reports, the Editor-in-Chief retains the right to request additional comments and the discretion to make the final decision without waiting for additional reports, taking into consideration the content and conclusions presented in all reports. This proactive approach ensures promptness in conveying the Editor's decision, thereby facilitating swift communication with the author.

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The authors are usually requested to resubmit the revised paper within 15 days and it will then be returned to the reviewers for further evaluation. The publishers normally allow one round of revision, and, in exceptional cases, a second round of revision may be allowed. If further revision is needed, then the manuscript is rejected and the author is requested to resubmit the manuscript for fresh processing.

The final decision regarding acceptance or rejection is that of the Editor-in-Chief, depending on the quality of the revision and assessment of the quality of the manuscript. In rare cases, manuscripts recommended for publication by the referees may be rejected in the final assessment by the Editor-in-Chief.

The time frame for revision of any article may vary from one to four weeks, depending on the nature of the revision required (minor or major). However, authors who need extra time for revision should consult the Editor-in-Chief/Handling Editor with valid reasons and the submission date of the revised manuscript may be extended if the request is genuine.

After the successful completion of the review and acceptance of the article, the articles are typeset, and proofs are dispatched to authors for any corrections prior to final publication.

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