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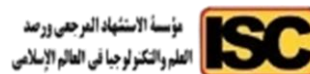
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Investigation of *Toxoplasma gondii* in women with breast cancer by using the Histopathology technique in Southern Iraq

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite; it has been proposed to play a carcinogenic role in various tissue types, such as the brain tissue. This study investigates the relationship between the infection of *T. gondii* and breast cancer among women employing the histopathology technique. Sixty-one women participated in this study. The biopsy of breast cancer tissues was collected from a private laboratory with the support of a specialist doctor (Advisor), from December 2023 to January 2025. The examination is conducted using the histopathology technique. The histopathology technique investigation reveals that the toxoplasmosis has resulted in extensive damage to the typical breast tissues, including necrosis and tissue destruction, as well as a high density of *T. gondii* at the site of infection.

Keywords: *Toxoplasma gondii*, Breast Cancer, Parasites, Histopathology, Histopathology technique, Southern Iraq.

المستخلص

المقوسة الغوندية *Toxoplasma gondii* طفيلي أولي إجباري داخل الخلايا، وقد رُئي أنه يلعب دورًا مسرطنًا في أنواع مختلفة من الأنسجة، مثل أنسجة المخ. تبحث هذه الدراسة في العلاقة بين الإصابة بالمقوسة الغوندية وسرطان الثدي لدى النساء، باستخدام تقنية علم الأمراض النسيجي Histopathology technique. شاركت إحدى وستون امرأة في هذه الدراسة. أُخذت خزعة من أنسجة سرطان الثدي من مختبر خاص بدعم من طبيب مختص (مشرف)، في الفترة من ديسمبر 2023 إلى يناير 2025. أُجري الفحص باستخدام تقنية علم الأمراض النسيجي. يكشف فحص تقنية علم الأمراض النسيجي أن داء المقوسات قد تسبب في تلف واسع النطاق لأنسجة الثدي النموذجية، بما في ذلك النخر وتدمير الأنسجة، بالإضافة إلى كثافة عالية من المقوسة الغوندية في موقع الإصابة.

Introduction:

Chemical pollutants interfere with vital cellular functions and directly facilitate the emergence of carcinogenic diseases. Furthermore, pathogens, including parasites, are involved in cancer induction (Callejas *et al.*, 2018; Tuama & Al-Moussawi, 2021; Atiya, 2024).

Toxoplasma gondii is an opportunistic obligate intracellular protozoan parasite (Alsaady *et al.*, 2021). It can infect any nucleated cell of endothermic vertebrates in more than 350 species (Alsaady *et al.*, 2021; Allamy & Alsaady, 2023; Hassoon *et al.*, 2023). It is organized as one of the most common parasites, affecting over a third of the world's population (Dupont *et al.*, 2023). This distribution is due to transmission modes; humans obtain toxoplasmosis through many transmission modes, such as ingesting oocysts or bradyzoites with contaminated food or water, transplacental, blood transfusion, or organ transplantation (Dubey, 2022; Allamy & Alsaady, 2023). *T. gondii* naturally exists in three distinct infectious stages: tachyzoites, bradyzoites, and oocysts (Dubey, 2022).

Breast cancer is the most common cancer among women worldwide, accounting for up to 36% of all cancer cases (WHO, 2024). Breast cancer incidence rates are rapidly rising worldwide, particularly in developed countries (Smolarz *et al.*, 2022). The infection rates of this disease predominantly affect women and typically develop in later life after puberty in both economically developed and developing countries; moreover, approximately 2.3 million women are diagnosed with breast cancer annually, resulting in around 670,000 deaths worldwide (WHO, 2024). The development of breast tumors is a complex process influenced by various factors, including environmental conditions and genetic predispositions (Cuthrell & Tzenios, 2023).

Multiple studies have indicated that toxoplasmosis may be linked to a higher risk of breast cancer, potentially because of ongoing inflammation and suppressed immune responses (Mostafa *et al.*, 2018; Salim *et al.*, 2022; Sangaré *et al.*, 2019). Research has shown that *T. gondii* infection occurs more frequently in cancer patients, including those with breast cancer, than in healthy individuals (Hagbin *et al.*, 2023). Women with breast cancer show a higher seroprevalence of toxoplasmosis compared to women without malignancy (Kalantari *et al.*, 2017).

Conversely, infection with *T. gondii* has been shown to inhibit the progression of various malignant tumors, including ovarian (Bolhassani & Zahedifard, 2012; Baird *et al.*, 2013), pancreatic (Sanders *et al.*, 2015), and breast tumors (Xu *et al.*, 2021; Ye *et al.*, 2024).

Therefore, this study aims to investigate the potential association between *Toxoplasma gondii* infection and breast cancer by analyzing breast tissue samples employing the histopathology technique. This investigation seeks to clarify an unconventional pathway that may enhance understanding of breast cancer development mechanisms.

Materials and Methods:

Sixty-one women participated in this study. Forty-six women are found to be infected with breast cancer, five are with benign breast tumors, and ten women are non-infected with breast cancer or benign breast tumors. The biopsy of breast cancer tissues was collected from a private laboratory with the help of a specialist doctor (Advisor), from December 2023 to January 2025.

The chemical solutions:

The chemical solutions are summarized in Table 1.

Table 1: The chemical solutions.

No.	Materials and Solutions
1	Different Ethanol Changes (70%, 90%, 95%, 100%)
2	Harris's Hematoxylin
3	Mayer Hematoxylin
4	Acid Alcohol Solution
5	Ammonia Water Solution 0.2%
6	Formalin Solution 10%

Histopathology procedure:

Histopathological examination was performed according to Wick (2019), and as follows:

- 1- The breast tissue samples were collected and fixed in a 10% formalin solution for 48 hrs. To ensure optimal preservation of the samples, the formalin solution was replaced after the initial 24 hrs post-collection.
- 2- After the tissue samples were meticulously sectioned to a thickness of 0.5 cm, they were subsequently placed into plastic cassettes for dehydration and clearing, utilizing an automated tissue processor (Histo-Line ATP700, Italy). After fixation, tissues undergo gradual dehydration using changes of ethanol (70%, 90%, 95%, and 100%) to remove water and minimize distortion. Absolute ethanol is used at the end to ensure complete dehydration. Clearing is accomplished by immersing tissues in xylene 100%. Subsequently, the samples were embedded in paraffin using the standard paraffin embedding technique with a tissue embedding system (HESTION TEC2800-C, China).
- 3- Tissue samples were processed using a semiautomatic microtome (Histo-Line, Italy) to ensure precise trimming and sectioning at a thickness of 4 μ m. The tissue sections were then carefully placed in a water bath (FALC BI, Italy) before being mounted onto glass slides using a hot plate (K & K HYSH11, Korea).
- 4- Tissue samples were deparaffinized using two changes of xylene for two min each. Subsequently, the samples were rehydrated through a series of ethanol dilutions (100%, 90%, and 70%) for two min each. Following rehydration, the breast sections were rinsed with tap water, stained with Harris's hematoxylin for 5 to 8 min, and washed again with tap water for two min.
- 5- To improve the clarity of the staining process, the tissue sections were initially differentiated in a 1% acid alcohol solution for 20 sec. This step effectively removed any excess hematoxylin stain, followed by a thorough wash in tap water for 1 min. Next, the breast tissue sections were immersed in a 0.2% ammonia water solution for 1 min to bleach the hematoxylin stain further.

Finally, the sections were rinsed in running tap water for 5 min to ensure all residues were removed.

- 6- Breast tissue sections underwent a 20 The tissue sections were then dehydrated using a series of ethanol dilutions, first with 70% ethanol, then 90%, and concluding with 100% ethanol, each for 2 min. Finally, the samples were cleared using two changes of xylene for 2 min each, ensuring thorough preparation for examination.
- 7- Eventually, to facilitate detailed observations and examinations, tissue sections were thoroughly examined using a light microscope at various magnifications, including 40X, 100X, 200X, and 400X.

Results and discussion:

The identification of *T. gondii* was accomplished by applying the histopathology technique using H&E. The study demonstrates the presence of *T. gondii* in malignant tissue samples obtained from women diagnosed with breast cancer and demonstrates that the infection with *T. gondii* causes severe damage to the typical breast tissues (Fig. 1).



Figure 1: Normal microscopic appearance of the breast tissue. The ducts in the lobules with collagenous stroma extend between these structures. The adipose tissue can be seen as highly visible and admixed with these elements. H&E stain, 100X.

The microscopic examination of breast tissue sections has demonstrated the following findings:

1) Breast invasive ductal carcinoma with positive toxoplasmosis:

Histopathological section stained with H&E at (A: 40X, B: 100X, C: 400X) magnification showed invasive ductal carcinoma (IDC) of the breast with noted positive toxoplasmosis. The milky duct contains dense collagenous fibrous tissue, among which lie small irregular nests and cords, or glands of cancer cells. The ducts and the veins incorporated into the tumor often become encased in a thick layer of elastic tissue. Dense fibrotic stroma indicates a desmoplastic

response, and myofibroblasts and collagen deposition give the cancer a firm consistency. The typical lobular and ductal structures are replaced by invasive tumor cells.

The results observed the presence of chronic inflammation and tissue changes, which may be due to toxoplasmosis, which is a known contributor to tumor progression, where the infected tissues may contain tachyzoites or tissue cysts, though usually these are difficult to detect in routine H&E unless extensive. The tumor grade is moderate to high based on nuclear atypia and mitotic count observed in tissue sections and *T. gondii* evidence, which is not more visible histologically in H&E staining; the implication of aggressive behavior and potential immune modulation if co-infected with toxoplasmosis (Fig. 2).

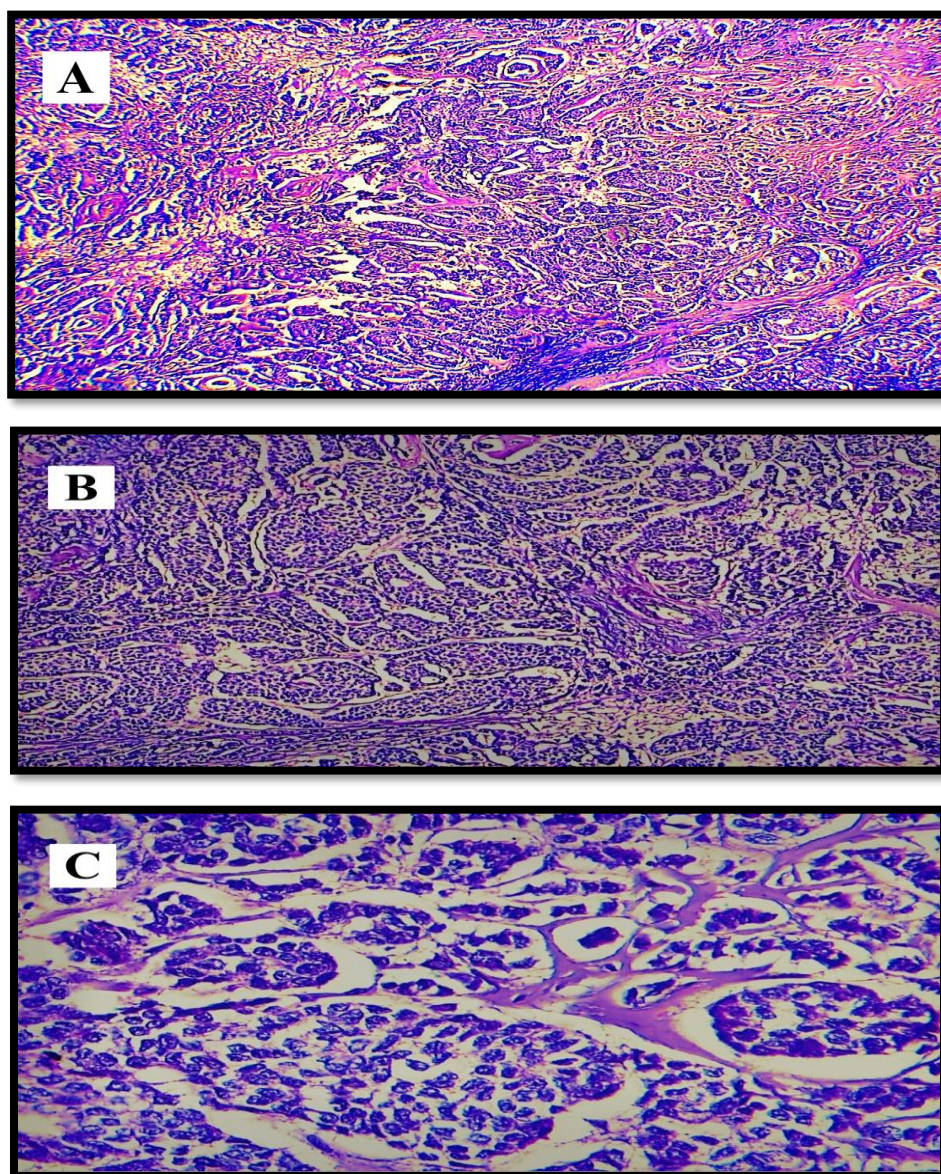


Figure 2: Breast invasive ductal carcinoma with positive toxoplasmosis. Infiltrative ducts and nests of malignant ductal epithelial cells within desmoplastic stroma. H&E. A: 40X, B: 100X & C: 400X.

2) Benign fibroadenosis with positive toxoplasmosis:

The high-resolution histological section (H&E stained, at 40X magnification) was found to be benign fibroadenosis with positive toxoplasmosis. Based on the histopathological changes, there is proliferation of small ducts or acini with dilated ducts or cysts within the lobules. A dense fibrous layer surrounds the glandular tissue, compressing and distorting it. The fibroblastic stroma found between the ducts may appear hyalinized.

Inflammatory changes observed by scattered mononuclear inflammatory infiltrates may include lymphocytes, plasma cells, and possibly macrophages in the interstitial stroma, which may be secondary to toxoplasmosis (Fig. 3).

The histological section (100X magnification) observed that the lobules are enlarged and consist of benign glandular and ductular elements, the ductules are found lined by a dual layer of epithelium (inner luminal epithelial and outer myoepithelial cells), which confirms the benign nature. Scatter some chronic inflammatory cells, primarily lymphocytes and plasma cells, are found around ducts and in the stroma (Fig. 3).

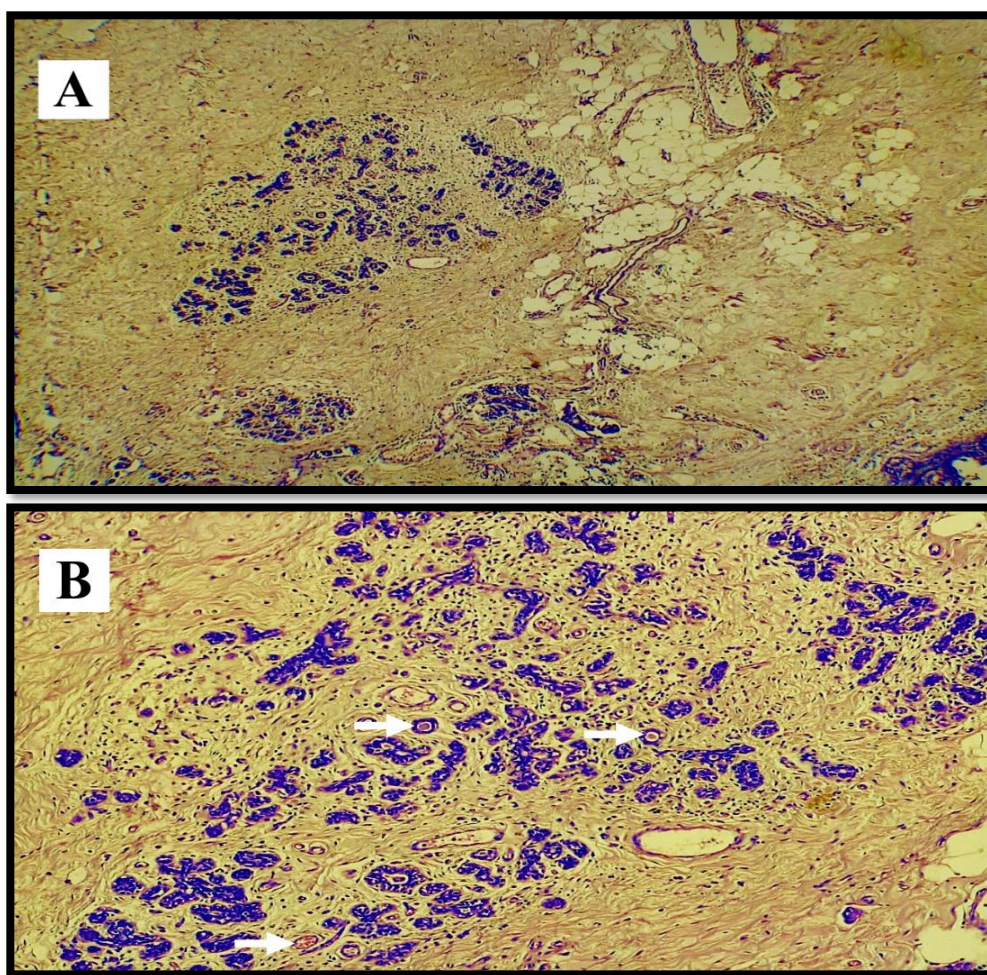


Figure 3: Benign fibroadenosis with positive toxoplasmosis. Expanded lobules of benign glands and ductules within fibrous stroma. H&E. A: 40X & B: 100X.

The histological section (400X magnification) shows that proliferation of lobules and ducts with regular arrangement, without cellular abnormalities or architectural distortions, indicates a tumor or malignant cells. The ductal epithelial cells were found cuboidal to columnar, with uniform, round nuclei and no pleomorphism, and the myoepithelial cells that support the epithelial cells are often present but less prominent.

Some ductules show increased epithelial layers, consistent with benign epithelial hyperplasia, usually seen in fibroadenosis. The interlobular stroma contains scattered lymphocytes and plasma cells, which indicate chronic inflammation. This inflammatory response may be due to *T. gondii* infection, which can stimulate a localized immune reaction (Fig. 4).

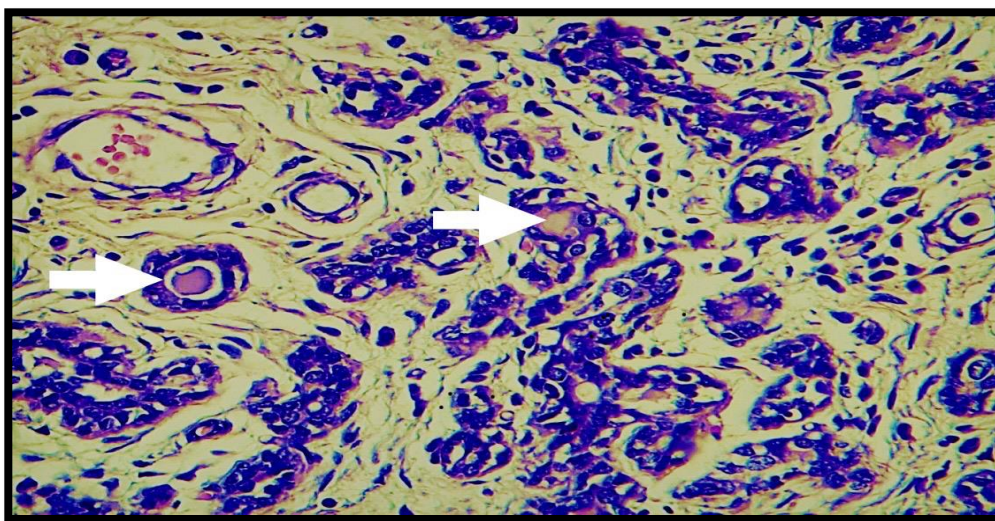


Figure 4: Benign fibroadenosis with positive toxoplasmosis. Expanded lobules of benign glands and ductules within fibrous stroma with eosinophilic secretion in the lumens. H&E. 400X.

3) Breast invasive ductal carcinoma with negative toxoplasmosis:

The Figure (5A) observed breast invasive ductal carcinoma with negative toxoplasmosis (40X magnification), characterized by malignant ductal epithelial cells arranged in irregular ducts, tubules, and nests infiltrating the surrounding breast tissue. The surrounding stroma is fibrotic and reactive, forming desmoplasia, which is considered a typical response to invasive carcinoma. The malignant epithelial cells show pleomorphism with hyperchromatic nuclei and high nuclear-to-cytoplasmic ratios, with mitoses that may be visible, indicating active proliferation. No granulomatous changes or surrounding inflammatory infiltrate, suggestive of *T. gondii* infection, which supports a histological toxoplasmosis-negative result.

The (Fig. 5B) provided shows classic features of breast invasive ductal carcinoma with negative toxoplasmosis (100X magnification), which shows infiltrating malignant cells into the ducts and tubules, invading the surrounding breast parenchyma with irregular shapes, and showing a randomized arrangement, indicating loss of standard ductal architecture. The stroma is markedly desmoplastic with dense, pink, eosinophilic collagen deposition and scattered fibroblasts with myofibroblasts. Histologically, the absence of granulomatous inflammation or

necrosis indicates that no *T. gondii* cysts or tachyzoites are seen, supporting the negative toxoplasmosis status.

The (Fig. 5C) provided shows classic features of invasive ductal carcinoma of the breast with negative toxoplasmosis, which is characterized by infiltrative nests and irregular ducts composed of malignant ductal epithelial cells. The tumor cells have hyperchromatic, pleomorphic nuclei and prominent nucleoli, indicating high-grade malignancy. The tumor cell nests are surrounded by desmoplastic stroma of dense fibrous tissue, which appears as pinkish, eosinophilic fibrotic areas that separate the nests of tumor cells.

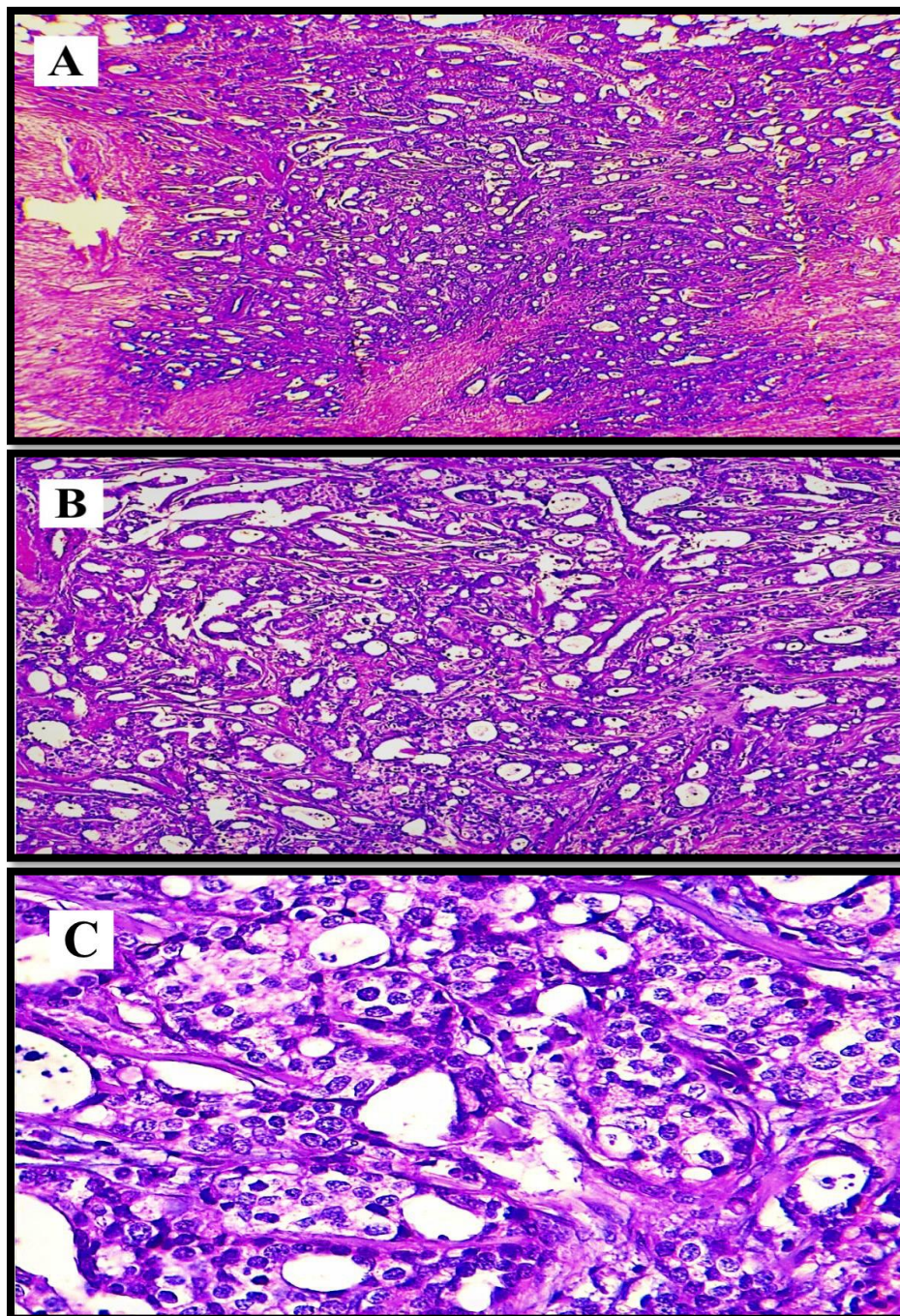


Figure 5: Breast invasive ductal carcinoma with negative toxoplasmosis. H&E. A: 40X, B: 100X & C: 400X.

4) Benign fibroadenosis with negative toxoplasmosis:

The Figure (6A) observed breast with benign fibroadenosis or fibrocystic changes with negative toxoplasmosis (40X magnification). Where found, lobular units are enlarged, showing proliferation of glandular structures (benign acini and ductules), and this expansion is typical of fibrocystic changes. The ductules are lined by two layers of luminal epithelial cells and myoepithelial cells, indicating benign architecture without cellular atypia, mitotic activity, and necrosis. There is increased dense collagenous stroma between the glandular tissues; there are no granulomas, necrosis, or parasitic cysts, which supports the negative toxoplasmosis status, as there is no histological evidence of *T. gondii* infection or inflammation.

The (Fig. 6B) observed benign fibroadenosis or fibrocystic changes with negative toxoplasmosis under (100X magnification), which are characterized by expanded lobules with benign glands and ductules and observed clusters of glandular and ductal epithelial structures arranged in lobular patterns. The epithelial cells lining those ducts were uniform, lacked significant atypia, and had round to oval nuclei with regular chromatin. The stroma is abundant and appears collagenous and hypocellular, which is typical of fibroadenosis, where the dense stroma surrounds and separates the ductal and glandular structures. Given the absence of inflammatory or infectious features, with no granulomas, necrosis, or visible organisms, and the diagnosis of negative toxoplasmosis, there are no signs of the parasitic infection.

The (Fig. 6C) shows benign fibroadenosis or fibrocystic change with negative toxoplasmosis (400X magnification) which is a common benign breast condition and characterized by enlarged mammary lobules with increased numbers of acini, the lobules appear more prominent than normal breast tissue, multiple small ducts and ductules are visible throughout the tissue, the glands are lined by benign epithelial cells and these epithelial cells maintain their standard outer structure. Dense fibrous connective tissue appears to surround and separate the glandular structures with a pink to eosinophilic stain. This fibrosis is a characteristic feature of fibroadenosis and confirms the benign nature of the lesion with no evidence of atypia, malignancy, or infectious organisms. No granulomas, necrosis, or parasitic cysts are present, which supports the negative toxoplasmosis status, as there is no histological evidence of *T. gondii* infection or inflammation.

The present study demonstrated the presence of *T. gondii* in malignant breast tissue among women and identified particular histological alterations resulting from its impact; consistent with our results, numerous studies have reported that *T. gondii* is present in malignant breast tissue (Kalantari *et al.*, 2017; Ameer *et al.*, 2022; Al-eadani *et al.*, 2025).

The results of this study indicate that chronic inflammation and tissue alterations are due to the presence of *T. gondii* in the tissue, and these indications resemble those in malignant brain tissue (Yang *et al.*, 2024). Also, the study found implications of aggressive behavior and potential immune modulation of toxoplasmosis, as Jung and colleagues reported, *T. gondii* can modulate host biological processes for its survival strategy in the malignant brain tumors (Jung *et al.*, 2022).

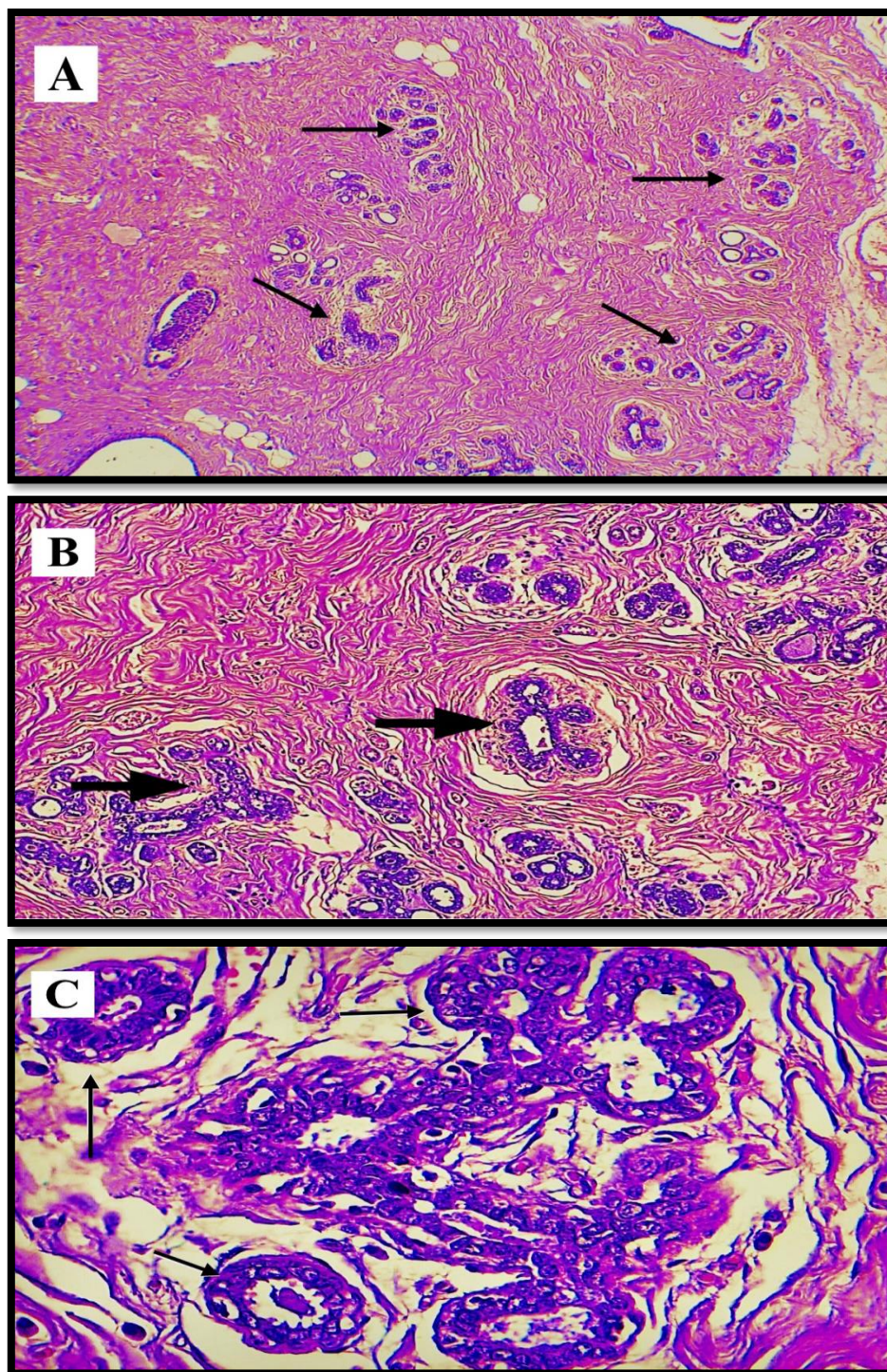


Figure 6: Benign fibroadenosis with negative toxoplasmosis. Expanded lobule of benign glands and ductules within fibrous stroma lined by benign ductal epithelial cells (black arrows). H&E. A:40X, B: 100X & C: 400X.

The study observes inflammatory changes represented by accumulation of lymphocytes and macrophages which are essential in defense against toxoplasmosis, and this agrees with studies have been showed that the infection with *T. gondii* presents a complex interaction between the parasite and the host's immune response, also indicates that CD4⁺ and CD8⁺ T cells play vital roles in managing the infection by releasing the cytokine IFN- γ , which stimulates macrophages to eliminate the parasite effectively (Dupont *et al.*, 2012; Sturge & Yarovsky, 2014; Khan *et al.*, 2019).

Certain ductules have been demonstrated to have increased epithelial layering, consistent with benign epithelial hyperplasia, commonly observed in fibroadenosis as reported by Stachs and colleagues (Stachs *et al.*, 2019).

The stroma may be fibrotic and reactive, producing desmoplasia, which is viewed as a typical response to invasive carcinoma (Fig. 5); as Kumar and colleagues (2021) reported, desmoplasia refers to the growth of dense fibrous connective tissue around a tumor or area of injury, it is characterized by the excessive production of collagen and extracellular matrix by activated fibroblasts, resulting in a fibrotic reaction; this process often forms a stiff tissue stroma around malignant cells and is considered an essential component of the TME; Desmoplasia can influence tumor progression, invasion, and response to therapy.

The absence of granulomatous inflammation and necrosis in the breast tissues indicates that *T. gondii* is not observed (Fig. 5), thereby supporting the negative diagnosis of toxoplasmosis in the breast tissue; these findings are supported by a study conducted by Weiss & Dubey (2009), which shows that the presence of granulomatous inflammation in histological examination of tissue samples suspected of being infected with *T. gondii* is an important indicator that the body is attempting to contain the infection and provides histopathological evidence supporting the diagnosis.

The current study demonstrate that the infection of *T. gondii* has caused severe damage to the typical breast tissues (Fig. 2), including necrosis, destruction in infected tissues, and *T. gondii* at the site of infection; these findings with the study of Xu and colleagues (2021), which declared the detection of *T. gondii* in malignant tissues and suggested its potential impact on the tumor microenvironment.

While our study revealed histological changes, Al-Eadani *et al.* (2025) reported that *T. gondii* infection did not induce significant alterations in the examined breast tissue sections.

Conclusions:

In light of the findings obtained from the current study, it can be concluded that there is a strong positive correlation between *T. gondii* infection and breast cancer, but no association with benign breast tumors. *T. gondii* can evade immune responses, inhibit apoptosis, promote the proliferation of infected cells, and alter the cellular microenvironment. histopathological examinations supported the role of *T. gondii* in inducing chronic inflammation, tissue damage, and desmoplastic reactions, which may contribute to tumor progression.

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Declaration of Competing Interest:

The researcher declares that there are no known financial interests or personal relationships that could have influenced the work reported in this paper.

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