Immunosuppressive Effect of Disseminated Breast Carcinoma on Severity of Hepatic Candidiasis

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Abstract: This study aims to demonstrate this relationship by observing the histopathological changes of the livers harvested from female Balb/c mice which were experimentally induced with breast cancer and inoculated with candida. The mice were randomly assigned to 5 different groups (n = 12). The first group (Group 1) was injected with Phosphate Buffer Solution (PBS), the second group (Group 2) with candida, third group (Group 3) with breast cancer and the final two groups, fourth and fifth group (Group 4, 5) having co-existence of candidiasis and breast cancer at 2 different doses of candidiasis, respectively. The prepared slides with the livers were stained with Haematoxylin and Eosin (H and E), Periodic Acid Schiff (PAS) and Gomori Methenamine Silver (GMS) stains for histopathology analysis. Grading of primary tumour and identification of metastatic deposits were done. Scoring of inflammation and congestion in the liver was done. Statistical tests done to compare group 2 and 4 showed that group 4 exhibited a highly statistically significant increase in inflammation and congestion (p<0.01). The median severity of candidiasis was also increased in group 4 as compared to group 2. In conclusion, based on the above evidences, hepatic candidiasis was significantly increased in mice with breast cancer.

Key words: Hepatic candidiasis, breast cancer, Candida albicans, immunosuppression

INTRODUCTION

Candidiasis is a disease caused by Candida sp., which are part of the normal flora found in the upper respiratory, gastrointestinal and female genital tract of the human body. Most cases of Candida infection result from Candida albicans, which is an opportunistic infection as it does not induce disease in immunocompetent individuals but can only do so in those with an impaired host immune defenses. Its infection is generally classified into superficial and deep. It commonly infects the nails, skin and mucous membranes, especially the oropharynx, vagina, oesophagus and gastrointestinal tract. Occasionally, they invade
the bloodstream and spread to other deep structure organs in the body such as kidneys, lungs, brain or other structures, causing systemic candidiasis (Levinson, 2006).

As of late, even though bloodstream infection has seen a decline in cases, yet the number of risk factors which could eventually lead to candidiasis has been increasing steadily (Richardson, 2005). The risk factors for candidiasis include immunosuppression due to chemotherapy or corticosteroid therapy, diabetes mellitus, low birth weight in neonates, broad spectrum antibiotics, long term catheterization, haemodialysis and parenteral nutrition. However, it has mainly been observed that the 3 main group of patients associated with candidiasis are those with neutropenic cancer, organ or stem cell transplant patients and those undergoing intensive care procedures.

Breast cancer is the most common cancer among Malaysian women. Approximately 1 in 20 women in the country develop breast cancer in their lifetime (Yip et al., 2006). There is a marked geographical difference in the worldwide incidence of breast cancer, with a higher incidence in developed countries compared to developing countries. In a survey done in 2 prominent hospitals in Malaysia, the age incidence was similar and it was discovered that on average, half of the cases are delayed in presentation. This was possibly attributed to a strong belief in traditional medicine, the negative perception of the disease, poverty and poor education, coupled with fear and denial (Hisham and Yip, 2004).

While the exact mechanism leading to candidiasis is not known, the initiation and progression of candidiasis can be viewed as an imbalance in the host-pathogen relationship in favour of Candida albicans. Recent studies have shown that invasive candidiasis is a common and serious complication of cancer and its therapy (Di Nubile et al., 2005). In cancer patients, it has been hypothesized that it develops from initial gastrointestinal colonization with subsequent translocation into the bloodstream. It is unclear what components of the innate immune system are necessary for preventing Candida albicans dissemination from the GI tract, but it is hypothesized that both neutropenia and GI mucosal damage are critical for allowing widespread invasive Candida albicans disease (Koh et al., 2008).

Very few studies have documented the co-existence and plausible relationships between breast cancer and candidiasis (Gottfredsson et al., 2003; Ghoneum and Gollapudi, 2004; Anderson et al., 2000; Safdar et al., 2001). However, there have been no authentic studies on hepatic candidiasis and its relationship with breast cancer in experimentally induced mice. This study hopes to establish a hypothetical relationship between the most common cancer in women in Malaysia and hepatic candidiasis by using a mouse breast cancer model with Candida inoculation. Results from this study will provide a groundwork from which further studies such as immunology studies can be carried out to better understand the pathogenesis of Candida in cancer patients. It may also help bring better insight into the current treatment and pathophysiology of cancer which has itself been shown to be a risk factor to the predisposition of candidiasis.

**MATERIALS AND METHODS**

The study was conducted between September 2008 and August 2009 in the Research Laboratory and animal holding facilities of International Medical University, after prior approval of the research and ethical committees. The statistical analysis was done using SPSS software version 15.0 using Mann Whitney Paired t-test, Kruskal-Wallis test and Spearman’s rho test.
Experimental Animals

Female Balb/c mice were used for the research, after prior approval from the Ethical committee. The mice were divided into 5 groups (Table 1). Dosing began when the mice were 10 weeks old and weighed between 15-25 g. They were housed in groups of 6 mice for each metal cage located within the Animal Housing Facility in International Medical University. The mice were fed with standard mice chow and were given free access to water. The weight of the mice was recorded at the start, once every week thereafter and finally at the end of the experiment.

Culture of Candida Yeast Cells

The Candida yeast cells were obtained from patient clinical isolates (IMU Research Lab.). Usage of sample was done with prior permission from the researcher. The cells were then subcultured onto a solid media of Sabouraud agar by streaking methods and stored in an incubator at 37°C. Before harvesting the colonies for inoculation, one of the Candida colonies was subcultured into the YPD broth and left for 72 h in a shaking incubator (Cerotechn S11) fixed at 100 rpm at a controlled temperature of 37°C. After 3 days or on the stipulated day of inoculation, serum was added into the broth to allow for germ tubes formation to occur and left in the shaking incubator for an additional of 3 h with similar settings. The colonies were then harvested by means of centrifugation. The volume and concentration needed for inoculation was prepared by dilutions and calculated using a haemocytometer.

Inoculation of Mice with Candidiasis

The mice were first placed inside a retainer and a 27G needle syringe was used to inject 0.1 mL of candida blastospores suspended in Phosphate Buffer Solution (PBS) with a concentration of 5x10⁶ cells mL⁻¹ via the tail vein made diluted by ethanol swap. This step was repeated with another group of mice with a concentration of 5x10⁶ cells mL⁻¹.

Culture of 4T1 Breast Cancer Cells

The breast cancer cells (4T1 cell line, IMU Research Lab.) were maintained and sub cultured into a 25 cm² culture flask until they were healthy and had achieved a steady replicative rate. They were then harvested by means of centrifugation and kept suspended in the culturing medium. The volume and concentration needed for inoculation was prepared by dilutions and calculated using a haemocytometer.

Inducing Mice with 4T1 Cancer Cells

The mice were first anesthetized with diethyl ether before injection of 0.1 mL of 1x10⁶ cells mL⁻¹ was administered subcutaneously into the mammary fatpad at the axilla of the right arm.

Table 1: Groups used in this research with their respective characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Group description</th>
<th>Concentration per dose of 0.1 mL</th>
<th>Duration before dissection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control group (Injected with PBS only)</td>
<td>0 mL candida blastospores/mL</td>
<td>2 weeks</td>
</tr>
<tr>
<td>2</td>
<td>Mice inoculated with Candida albicans</td>
<td>5x10⁶ cells mL⁻¹</td>
<td>2 weeks</td>
</tr>
<tr>
<td>3</td>
<td>Mice inoculated with breast cancer</td>
<td>1x10⁶ cells mL⁻¹</td>
<td>4 weeks</td>
</tr>
<tr>
<td>4</td>
<td>Mice inoculated with breast cancer and subsequently inoculated with Candida albicans</td>
<td>1x10⁶ cells mL⁻¹ of 4T1 breast cancer cells and 5x10⁶ cells mL⁻¹ for Candida albicans</td>
<td>3 weeks+1 week</td>
</tr>
<tr>
<td>5</td>
<td>Mice inoculated with breast cancer and subsequently inoculated with Candida albicans</td>
<td>1x10⁶ cells mL⁻¹ of 4T1 breast cancer cells and 5x10⁶ cells mL⁻¹ for Candida albicans</td>
<td>3 weeks+1 week</td>
</tr>
</tbody>
</table>
Sample Collection

The mice were weighed at the end of the experiment before being sacrificed with diethyl ether in a desiccator. The livers from all groups, primary breast tumours and spleen, kidneys, lungs, heart and brain were harvested. They were subsequently fixed in formalin 10% for at least 2 days.

Tissue Processing

The fixed organs were then sectioned and processed to paraffin blocks. Sections of 4 µm were taken on glass slides and were stained with Haematoxylin and Eosin (H and E), Periodic Acid Schiff (PAS) and Gomori Methenamine Silver (GMS) and were dehydrated, cleared and mounted with cover slips using DPX mountant media.

RESULTS

The slides were observed under the light microscope for grading of the primary tumour, presence of metastatic deposits and extent of candidiasis in the liver by comparatively examining the slides stained in H and E, PAS and GMS and extent of organ inflammation and congestion. Lastly, a correlation was made between the pathological lesions observed in the groups with that of the groups’ mean gross weight changes.

The histopathological scoring of inflammation and congestion changes in these livers was based on standard techniques used in earlier studies (Lee et al., 2008; Black et al., 1999). Scoring of candidiasis (Fig. 2) (Balish, 2009) and grading of the primary tumour was done using the conventional method of analyzing the similarity of the cells to its tissue of origin as poorly differentiated, moderately differentiated and severely differentiated (Kumar and Stanley, 2003) (Fig. 1).

Statistical Analysis

In this study, 60 samples were studied and analyzed. All analytical data were expressed in mean with standard deviation and with a 95% confidence interval. The level of significance was set at 0.05. Statistical tests that were used in the experiment were:

![Photomicrograph showing malignant cells with multiple mitotic figures in the primary breast cancer (400x, H and E)](image_url)

Fig. 1: Photomicrograph showing malignant cells with multiple mitotic figures in the primary breast cancer (400x, H and E)
Fig. 2: Photomicrograph under oil immersion showing a colony of Candida yeast cells and hyphae in the hepatic parenchyma (1000x, PAS)

Table 2: Results of paired t-test for gross weight of mice at initial and end of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean initial weight (g)</th>
<th>Mean final weight (g)</th>
<th>Asymptotic significance (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.71</td>
<td>19.09</td>
<td>0.001***</td>
</tr>
<tr>
<td>2</td>
<td>18.26</td>
<td>16.85</td>
<td>0.009**</td>
</tr>
<tr>
<td>3</td>
<td>19.00</td>
<td>20.00</td>
<td>0.000***</td>
</tr>
<tr>
<td>4</td>
<td>19.90</td>
<td>18.01</td>
<td>0.039*</td>
</tr>
<tr>
<td>5</td>
<td>20.25</td>
<td>18.04</td>
<td>0.032*</td>
</tr>
</tbody>
</table>

The mean gross weight in grams at initial and end was included. *Significant difference at p-value < 0.05; **Significant difference at p-value <0.01

- Paired t-test for comparison of initial and final mean weight of mice in each group
- Kruskal-Wallis test for global comparison of groups for all the parameters
- Non-parametric Mann-Whitney-U test for comparison between 2 groups for each parameter
- Spearman’s rho Test for correlation of candidiasis, cancer metastases, inflammation and congestion

The statistical tests were conducted with the aid of SPSS Statistical software version 16. For all the individual tests, a p-value of less than 0.05 (p<0.05) was taken and considered as significant. Paired T test is a parametric method to test for any significant difference between the means of the same or related subject over time or in differing circumstances. From the test conducted, it was found that the p-value was less than 0.05 (p<0.05) in all the groups with group 1, 2 and 3 showing p-value less than 0.01 (p<0.01). This means that there was a significant difference in the weight of the mice in all the groups at the initial and end of experiment (Table 2).

Based on the global comparison done for metastasis in each of the organs for all the groups, Mann-Whitney test for comparison between groups 3 and 4 showed a significant difference in all the organs except the brain (p<0.01) (Table 3). The kidneys showed a greater level of significance (p<0.01) as compared to the other organs. This shows that the presence of hepatic candidiasis as in group 4 has an effect on the extent of the metastatic growth in these organs.

By comparing the median severity of hepatic candidiasis between group 2 and 4, it was observed that there was significant difference in its severity. In group 2, the severity of
Table 3: Results of Mann-Whitney test for comparison between group 3 and 4 for extent of organ metastases

<table>
<thead>
<tr>
<th>Organs</th>
<th>Kruskal-Wallis test for global comparison of organ metastases among groups</th>
<th>Mann-Whitney test for comparison between group 3 and 4 for extent of organ metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.000**</td>
<td>0.000**</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.000**</td>
<td>0.016*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.000**</td>
<td>0.015*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.000**</td>
<td>0.016*</td>
</tr>
</tbody>
</table>

*Significant difference at p-value ≤0.05; **Significant difference at p-value ≤0.01

Table 4: Histopathological scoring of candidiasis in H and E

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Median of severity of candidiasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2: Mice with  Candidida (5x10^9 cells mL^-1)</td>
<td>+</td>
</tr>
<tr>
<td>Group 4: Mice with breast cancer + Candida (5x10^9 cells mL^-1)</td>
<td>+++</td>
</tr>
</tbody>
</table>

- Absent, +: Mild, ++: Moderate, +++: Severe

Table 5: Results of Kruskal-Wallis for global comparison between groups and Mann-Whitney Test for comparison between group 2 and 4 for liver parameters

<table>
<thead>
<tr>
<th>Liver</th>
<th>Asymptotic significance (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kruskal-Wallis test of global comparison</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.000**</td>
</tr>
<tr>
<td>Congestion</td>
<td>0.000**</td>
</tr>
<tr>
<td>Candidasis</td>
<td>0.000**</td>
</tr>
<tr>
<td>Cancer metastasis</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

*Significant difference at p-value ≤0.05; **Significant difference at p-value ≤0.01

candidiasis was mild while that in group 4 was moderate (Table 4). These observations were also observed in slides stained in Periodic Acidic Schiff (PAS) and Gomori Methenamine Silver (GMS) stains.

Kruskal-Wallis test for global comparison between the groups for inflammation and congestion showed that there was a significant difference with p-value less than 0.01 (p<0.01) between these groups in all the livers (Table 5). Mann-Whitney test for comparison between group 2 and 4 for inflammation response showed a significant difference in all the livers. This shows that the co-existence of both candidiasis and cancer in the mice had a heightened effect on the severity of inflammation as compared to mice with candidiasis alone.

Mann-Whitney test for comparison between group 4 and 5 for extent of candidiasis showed that the increase in Candida dosage inoculated in group 5 with a concentration of 5x10^9 cells mL^-1 compared to 5x10^9 cells mL^-1 in group 4 exhibited statistical significant difference. This shows that the increased dose in group 5 showed a statistically significant effect on the inflammatory response seen in the liver. The correlation made between hepatic candidiasis and cancer metastases was significant.

**DISCUSSION**

Few studies have been done on experimental candidiasis in mice. This provided the research team the information on the necessary dosages and previous observations from which this experiment can draw comparison (De Repentigny, 2004; Wong et al., 2008; Ashman and Papadimitriou, 1987). Some of these studies have been dedicated to the
observations of the correlation between candidiasis and other forms of immunosuppression such as chemotherapy, steroid therapy, antibiotic therapy and some other form of malignancies such as leukemia and oesophageal cancer.

Response to Editors comment on to give possible reasons where results differ from previous study:

No change has been made for this, since there is no similar previous study as ours is a pioneer study focusing on the relationship between hepatic candidiasis and breast cancer by comparing the behaviour of candidiasis when the body is subjected to a chronic disease state. Though previous studies have been done on candidiasis alone (without breast cancer), we used those studies for obtaining information on culturing and dosage only.

However, there has never been an exclusive study on hepatic candidiasis and its relationship with breast cancer even though few epidemiological studies have shown a co-existence of breast cancer and systemic candidiasis in humans (Gottfredsson et al., 2003; Saffar et al., 2001; Talamin et al., 2009). Hence, this study aims to focus on the relationship between hepatic candidiasis and breast cancer by comparing the behaviour of candidiasis when the body is subjected to a chronic disease state. This study was done, bearing in mind that breast cancer was not only chosen as an ideal representation of a chronic illness but also one that is capable of suppressing the host immune system (Mandeville et al., 1982; Semiglasov et al., 1978; Ortiz and Stolar, 1988; Das et al., 1985).

This study is a pioneer work on the growth of hepatic candidiasis after inducing mice with breast cancer to study how the presence of a chronic illness such as breast cancer can by itself, be attributed to the increased severity of candidiasis. Scoring was done on the severity of candidiasis and grading of the primary tumour as well as identification of their metastatic deposits was conducted. Other parameters taken into consideration included gross weight of the mice at the beginning and end of experiment, inflammation and congestion in the liver which was studied by scoring on a semi-quantitative scale using an established technique as mentioned earlier.

**Hepatic Candidiasis**

In group 2, the mice were solely inoculated with candidiasis by intravenous injection via the tail vein for a duration of 2 weeks. During the course of the experiment, signs of the disease can be appreciated in these mice. Their eye balls were protruded, their fur was roughened and they were generally less active as compared to the normal group with increased group huddle and sleep. They also appear very weak and thin with the curvatures of the bony structures beneath the mice visible to the naked eye. In addition, the weight taken at the start and end of the experiment showed that there was a statistically significant reduction in their mean weight. This could be attributed to the possible loss of appetite and general cachexic state of the mice.

Histopathologically, good growth of *Candida* colonies in the form of hyphae, yeast cells and pseudohyphae were discovered in the liver parenchyma. This was attributed to the mild dose of 5x10^7 cells mL^{-1} *Candida* cells injected and the short duration of the experiment as also shown in few other works.

**Breast Cancer Study**

In group 3, the mice were injected at the mammary fatpad with 4T1 cancer cells in the right axilla region with a concentration of 1x10^6 cells mL^{-1} (Pulaski and Rosenberg, 2001). After 4 weeks of growth and metastases, the mice were sacrificed for analysis. During the course of experiment, the weights of the mice were reduced for the first week before gradually
increasing in the 3rd week. The growth of the primary tumour was detected as a palpable mass as early as the 10th day and latest by the 14th day. The mice were generally active for the first 2 weeks with no apparent deviations from that usually seen in the normal control group. However, by the 3rd week, they began to exhibit signs of lethargy and did not move quite as often and the mass of tumour began to appear significantly enlarged to the naked eye by the middle of 3rd week. Their general appetite was good. No distinct changes to the fur, eyes or prominent curvatures of the bony structures could be appreciated.

Grading done for the primary tumour showed it to be moderate to poorly differentiated with the majority presenting as poorly differentiated. Metastatic deposits were discovered in the lungs, liver and spleen with varying frequencies among the mice. Scoring for inflammation done showed that the median of severity of the entire group was moderate in the liver. The microabscesses that were observable in group 2, were not seen in this group. Therefore, in this group with mice having breast cancer, the severity of inflammation and congestion seen in the liver are mostly mild in severity with metastatic deposits found in the lungs, liver and spleen.

**Correlation between Hepatic Candidiasis and Breast Cancer**

In group 4, the mice were first induced with breast cancer for 3 weeks and subsequently inoculated with *Candida* with a concentration of $5 \times 10^6$ cells mL$^{-1}$ for 1 week. The time of induction with breast cancer was set at 3 weeks based on studies demonstrating that by this period, adequate metastases have occurred in all these organs (Tsao et al., 2008). The initial stages of tumour growth and changes in the mice were similar as to that seen in group 3 but subsequently when *Candida* was injected, changes seen in group 2 were exhibited within days instead of the 2nd week as seen in group 2. These changes include protruded eyes, roughened fur and they were generally inactive with increased huddle and sleep. Also, in the final stages of the experiment, a surge in the growth of tumour size could be observed.

Grading carried out for the primary tumour exhibited poorly differentiated tissue with atypical cells and high number of mitotic figures. Metastatic deposits were also discovered in the lungs, liver, spleen and even in the kidneys at a higher frequency as compared to that seen in group 3. These differences were statistically significant (p<0.05). This shows that there was an increased frequency of metastatic deposits in these organs in group 4 as compared to that in group 3. This suggests a possible role of *Candida* causing immunosuppression which by itself attributed to the increased metastatic deposits of the cancer seen in these organs. It also explains the late surge in tumour growth seen late in the experiment.

Notable changes in the kidneys include candidiasis involvement in the renal parenchyma, renal tubules and pelvis. Within the liver parenchyma and vasculature, distinct changes like microabscesses, chronic inflammation and congestion were observed at a greater level in this group as compared to that seen in group 2. This group also exhibited increased group median of severity in *Candida* infection in the kidneys and liver. The kidneys demonstrated moderate severity as compared to mild in group 2 while the liver showed moderate severity of candidiasis as compared to absence of candidiasis seen in group 2. Hence, this group showed an extra involvement of liver compared to just kidneys as seen in group 2. This observation holds true in scoring done for both PAS and GMS.

Scoring of inflammation showed moderate severity seen in the brain, kidneys and lungs while the liver showed severe changes as compared to just mild seen in all the organs in group 2. Comparison of inflammation severity between these 2 groups was statistically significant (p<0.01).
As for congestion, this group exhibited moderate congestion in the brain and kidneys as compared to mild in group 2 and while congestion in the lungs was not seen in group 2, this group showed mild congestion. Also, the liver showed severe congestion as compared to just moderate congestion seen in group 2. Comparison between group 2 and 4 for congestion were statistically significant (p<0.05). In conclusion, the severity of candidiasis, inflammation and congestion were seen at greater levels in breast cancer induced mice with candidiasis as compared to mice with only candidiasis.

Dose Dependent Study

In group 5, the mice were first induced with breast cancer and subsequently with candidiasis at a higher dose of \(5\times10^6\) cells mL\(^{-1}\). They were similar to group 3 at the initial stages of the cancer growth but subsequently when candidiasis was injected, the mice died within the first week of inoculation at varied timings compared to group 4 where the time of inoculation with candidiasis was 1 week and mice living till the end of experiment. The sudden immediate death could be attributed to sepsisemia.

Grading done on the primary tumour showed them all to be poorly differentiated. Metastatic deposits were found in the kidneys, lungs liver and spleen. Scoring of candidiasis done showed moderate to intense severity, which was highly significant. This means that with an increased dose, the liver exhibited candidiasis with increased levels of severity. Perhaps with a higher dose, the higher reaches of the body are much better accessible as the proportion eliminated by the liver or spleen is less.

In the scoring for inflammation the liver showed a statistical significant difference when compared to group 4. The inflammation is much less in severity compared to that in group 4 which could be attributed to the short period of inoculation time before the demise of the mice resulting in inadequate time for chronic inflammation to take place.

In the scoring for congestion, group 5 showed significant severe congestion in the liver and this could be attributed to the acute changes seen in host response to a foreign pathogen.

Correlation of Hepatic Candidiasis, Cancer Metastases, Inflammation and Congestion

The significant correlation between hepatic candidiasis and cancer metastases indicates that an increase in cancer metastatic deposits was accompanied by an increase in candidiasis severity. The statistically significant correlation of hepatic candidiasis with inflammation and congestion shows that increased levels of candidiasis is accompanied by increased levels of inflammation and congestion in the respective organs studied.

CONCLUSION

In summary, the mouse model of inducing breast cancer was successful and the method and the technique of inducing hepatic candidiasis was effective. Both these were also attributed to the efficient culturing methods. Also, growth of breast cancer and hepatic candidiasis were observable in all the relevant groups. The weight of the mice was also correlated with the pathology suffered by the mice. All the objectives were carried out with precision and successfully achieved. Analysis was done based on the scoring of hepatic candidiasis, grading of metastatic deposits, inflammation and congestion in all the groups. The inflammation and congestion parameters showed a statistically significant increase in severity in all the organs when comparing the group of mice with hepatic candidiasis and
breast cancer to the group of mice with hepatic candidiasis alone. The median severity of the entire group for candidiasis scoring in the kidneys and liver was also increased for the group of mice with hepatic candidiasis and breast cancer. Hence, based on these evidences, hepatic candidiasis appears more severe in experimentally induced mice with breast cancer than in mice without.

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