INTRODUCTION
Breast cancer is a global health problem and a leading cause of female morbidity and mortality. It is the second most common cancer after the lung cancer and the fifth most common cause of death associated with this disease. The involvement of breast cancer patients in a regular physical activity has been frequently associated with a reduction of morbidity, an increase in quality of life and a better clinical outcome. Appropriate animal models are essential to not only the diagnosis but also the therapeutic approaches for this disease. The model of N-methyl-N-nitrosourea (MNU)-induced mammary tumors in rats has been frequently used to study this disease; these tumors have some similarities with the human breast cancer, namely the estrogen dependence and the aggressiveness. The determination of the proliferation index (PI) of a tumor is normally evaluated by immunohistochemistry (IHC) using the marker Ki-67. It is a nuclear protein essential for the progression through the cell cycle, being an excellent marker of the proliferative state of a cell population. Ki-67 is expressed during the late G1, S, G2, and M phase of the cell cycle, whereas rest cells (G0 phase) do not express it. In order to clarify the effects of physical exercise on mammary cancer, it was evaluated the Ki-67 PI of mammary tumors chemically-induced in a recognized rat model.

MATERIALS AND METHODS
Twenty-one female Sprague-Dawley rats (Harlan Interfauna, Barcelona, Spain) were used in this study. The animals were kept in polycarbonate cages (1500U Eurostandard Type IV S, Tecniplast, Italy) in the facilities of the University of Trás-os-Montes e Alto Douro, under controlled conditions of temperature (23±2°C), humidity (50±10%), system of renovation and filtration of air (10-20 ventilations/hour) and a cycle of 12 hours of light and 12 hours of darkness. The animals were fed with a commercial diet (4RF21®, Mucedola, Italy) and tap water ad libitum. All procedures were performed according to the National and European legislation for the utilization of animals for experimental purposes (European Directive 2010/63/UE and Decree-Law 113/2013) and were approved by the Direção Geral de Alimentação Veterinária (Approval no. 005961).

The animals were divided into two experimental groups: sedentary (n=11) and exercised (n=10). At seven weeks of age, all animals received an intraperitoneal injection of the carcinogen agent N-methyl-N-nitrosourea (MNU) (50 mg/kg body weight). The rats from exercised group were subjected to exercise protocol 5 days/week, 60 min/day, at a speed of 20 m/min for 35 weeks. At sacrifice, mammary tumors were collected, fixed and histologically evaluated. The sections were incubated with the primary antibody for Ki-67 and the Ki-67 PI was determined.

Experimental Pathology and Health Sciences
2015:7 (2): 5-6
After fixation, the mammary tumors were cut; their surface was carefully evaluated and they were processed for inclusion in paraffin. Paraffin sections with 2 μm of thickness were made in an automated microtome (Leica® RM 2035) and stained with hematoxylin and eosin (H&E). The sections were evaluated by a pathologist and classified according to the classification previously proposed by Russo and Russo.11 In each section were considered the histological patterns with greater proportion (≥50%).

For IHC evaluation, it was obtained the monoclonal primary antibody Ki-67 (clone MIB-5, DAKO®). It was used the indirect method (NovoLink™ kit, Leica Biosystems®), the antibody was incubated at a dilution of 1:50, overnight at 4°C. To evaluate the cellular Ki-67 PI it was considered the brown labelled nuclei by the Ki-67 antigen, independently of the invasiveness of the neoplasia. With a magnification of 100x were located the more labeled areas, and then under the magnification of 400x were counted 1000 neoplastic cells in each section identifying the brown labelled nuclei.

Statistical analysis was performed using JMP® Starter 5.0.1 (SAS Institute Inc, North Caroline, USA). The histological patterns were compared between groups using the χ² test, the ANOVA test was used to compare Ki-67 PI among lesions and between the groups. The results were considered statistically significant when p<0.05.

RESULTS
In sedentary group were identified 36 neoplasms, while in exercised group were identified 32 neoplasms. In both groups, the neoplasms were mostly epithelial, being the epithelial malignant the most prevalent (88.57%). The histologic study also revealed that the papillary pattern was the most frequently identified in both groups, followed by cribriform and the cystopapillary (one type of papillary form) that was only identified in exercised group. Additionally, in sedentary group were identified two intraductal proliferations, two comedocarcinomas and two tubular carcinomas.

At the IHC study, it was observed a punctual nuclear staining for Ki-67 in both groups sedentary and exercised. There was no significant statistical differences in Ki-67 PI among lesions (p=0.07); however the cribriform and papillary lesions had the highest mean values of PI with 9.70% and 4.20%, respectively. Although the difference did not reach the level of statistical significance, the Ki-67 PI was lower in exercised group than in sedentary one (4.13% and 5.41%, respectively) (p=0.79).

DISCUSSION
In this study the proliferation marker Ki-67 showed expressiveness in almost all types of lesions found in both groups. In general, the immunoreactivity for Ki-67 was scarce, clearly inferior to the expected; it was probably related with the type of neoplasms identified in both groups which were predominantly of low grade of malignancy. The highest immunexpression of Ki-67 in cribriform and papillary lesions can be related to the histological conformation that show high cellularity. Inversely to that observed in a study performed by Malicka et al.,6 where no changes were observed in cell proliferation, in this work the Ki-67 PI was higher in sedentary group than in exercised one (p>0.05). These different results may be related with the duration of the exercise protocol, in the study performed by Malicka et al.12 the animals were exercised for 12 weeks while in the present study they were exercised for a longer period of 35 weeks.

CONCLUSION
The exercise training reduced the immunexpression of Ki-67 in MNU-induced mammary tumors, suggesting that the practice of physical exercise may decrease tumor proliferation. In future studies, it will be necessary to correlate the Ki-67 PI with the expression of estrogen receptors in order to establish the real progression of the tumors and acquire a broader knowledge about the effects of exercise training on tumors’ progression.

REFERENCES
[8]: Sysel AM, Vail VE, Nagle RB, Bauer JA. Immunohistochemical Quantification of the Vitamin Immunohistochemical Quantification of the Vitamin B12 Transport Protein (TCII), Cell Surface Receptor (TCII-R) and Ki-67 in Human Tumor Xenografts. Anticancer Res. 2013:33; 4203–4212