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Association of melatonin, green propolis and paclitaxel promotes inhibition of Ehrlich tumors in mice

Associação de melatonina, própolis verde e paclitaxel promove inibição de tumores de Ehrlich em camundongos

Asociación de melatonina, propóleo verde y paclitaxel promueve la inhibición de tumores de Ehrlich en ratones

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ABSTRACT

Objective: To evaluate the antitumor action of Green Propolis and Melatonin. **Methods:** Ehrlich carcinoma cells were implanted in the subcutaneous tissue of male Swiss Webster mice, which were divided into experimental groups: Control, Animals that received 2 mg/kg of Melatonin; Animals treated with 50 mg/Kg of Propolis Verde; Animals treated with 5 mg/Kg of Paclitaxel; Animals treated with Melatonin and Green Propolis; Animals treated with Melatonin and Paclitaxel; Animals treated with Green Propolis and Paclitaxel; and Animals treated with Melatonin, Propolis Verde and Paclitaxel. **Results:** All protocols containing Green Propolis prevented metastatic and degenerative lesions in the lung and liver, minimizing the side effects of multidrug therapy. Although under the conditions applied in this study, Melatonin was not efficient in inhibiting EST, it attenuated myocarditis, renal tubular necrosis and pulmonary metastasis. **Conclusion:** Treatment with Melatonin, Propolis Verde and Paclitaxel showed the best results in reducing tumor weight, mitotic index and tumor inhibition, reinforcing the effectiveness of the association of natural compounds with chemotherapy in reducing tissue histopathological changes.

Keywords: Breast cancer, Antioxidants, Natural compounds, Histopathology, Multidrug therapy.

RESUMO

Objetivo: Avaliar a ação antitumoral da Própolis Verde e da Melatonina. **Métodos:** Células de carcinoma de Ehrlich foram implantadas no tecido subcutâneo de camundongos Swiss Webster machos, os quais foram divididos nos grupos experimentais: Controle, Animais que receberam 2 mg/kg de Melatonina; Animais tratados com 50 mg/Kg de Própolis Verde; Animais tratados com 5 mg/Kg de Paclitaxel; Animais tratados com Melatonina e Própolis Verde; Animais tratados com Melatonina e Paclitaxel; Animais tratados com Própolis Verde e Paclitaxel; e Animais tratados com Melatonina, Própolis Verde e Paclitaxel. **Resultados:** Todos os protocolos contendo Própolis Verde preveniram lesões metastáticas e degenerativas no pulmão e no fígado, minimizando os efeitos colaterais da poliquimioterapia. Embora nas condições aplicadas neste estudo, a Melatonina não tenha sido eficiente em inibir o EST, ela atenuou a miocardite, a necrose tubular renal e a

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metástase pulmonar. **Conclusão:** O tratamento com Melatonina, Própolis Verde e Paclitaxel apresentou os melhores resultados na redução do peso tumoral, índice mitótico e inibição tumoral, reforçando a eficácia da associação de compostos naturais com a quimioterapia na redução das alterações histopatológicas teciduais.

Palavras-chave: Câncer de mama, Antioxidantes, Compostos naturais, Histopatologia, Poliquimioterapia.

RESUMEN

Objetivo: Evaluar la acción antitumoral del Propóleo Verde y la Melatonina. **Métodos:** Se implantaron células de carcinoma de Ehrlich en tejido subcutáneo de ratones machos Swiss Webster, los cuales se dividieron en grupos experimentales: Control, Animales que recibieron 2 mg/kg de Melatonina; Animales tratados con 50 mg/Kg de Propóleo Verde; Animales tratados con 5 mg/Kg de Paclitaxel; Animales tratados con Melatonina y Propóleo Verde; Animales tratados con Melatonina y Paclitaxel; Animales tratados con Propóleo Verde y Paclitaxel; y Animales tratados con Melatonina, Propóleo Verde y Paclitaxel. **Resultados:** Todos los protocolos que contenían Propóleo Verde previnieron lesiones metastásicas y degenerativas en pulmón e hígado, minimizando los efectos secundarios de la polifarmacia. Aunque bajo las condiciones aplicadas en este estudio, la Melatonina no fue eficiente en la inhibición de EST, atenuó la miocarditis, la necrosis tubular renal y la metástasis pulmonar. **Conclusión:** El tratamiento con Melatonina, Propóleo Verde y Paclitaxel mostró los mejores resultados en la reducción del peso tumoral, índice mitótico e inhibición tumoral, reforzando la efectividad de la asociación de compuestos naturales con quimioterapia en la reducción de cambios histopatológicos tisulares.

Palabras clave: Cáncer de mama, Antioxidantes, Compuestos naturales, Histopatología, Terapia multidrogas.

INTRODUCTION

Neoplasms are the diseases that most affect human and domestic species. In humans, together with cardiovascular diseases, are the two biggest causes of death in the world. Considering cancer, approximately 18 million new cases were estimated worldwide in 2020, with breast cancer being the main type, and the second most common type in Brazil (SOUSA WO, et al., 2022). Cancer treatment varies according to the type and severity of the disease and may include surgery, radiation, hormones, immunotherapy, chemotherapy, or a combination of these (TSIMBERIDOU AM, et al., 2020). In this regard, the use of complementary therapies with the potential to attenuate the side effects of standard treatments has been increasing, as well as the ability to reduce the speed of tumor growth. Among these therapies, nutritional care and the use of substances of plant origin stands out, due to their antioxidant and anti-inflammatory properties (ALBALAWI AE, et al., 2022).

An example is Green Propolis, a resin collected by bees from different plant sources, which has more than 300 components in its constitution, such as polyphenols, steroids, amino acids, micronutrients, and vitamins; highlighting flavonoids, phenolic acids, and their esters as the main biologically active components. These compounds account, among others, for the antitumor and immunostimulatory properties of propolis, and their potential to reduce the side effects of chemotherapy treatments has also been pointed out (KOCOT J, et al., 2018).

Such properties have been demonstrated in experimental protocols for the treatment of various types of cancer, where the antitumor activity of the aqueous or ethanolic extract of Propolis on breast, lung, bladder, and melanoma, among other types, was observed. The administration of Propólis Verde, or compounds extracted from it, promoted a significant decrease in the growth of tumor cells, inhibition of tumor angiogenesis, and an increase in the cytotoxic activity of natural killer cells (CARDOSO EO, 2022).

The anti-tumor potential has also been associated with Melatonin. This hormone has its production reduced in patients with different types of cancer, especially during the initial phase of tumor growth (BARTSCH CBHKM, 2002). Its involvement in different anticancer mechanisms, such as modulation of the immune system, induction of apoptosis of tumor cells, inhibition of cell proliferation, reduction in tumor growth and

metastases, reduction in side effects associated with chemotherapy and radiotherapy, decrease in drug resistance used in cancer therapy and increase the therapeutic effects of conventional treatments, and its administration in parallel with these treatments has been suggested.

METHODS

Animals and accommodation

Forty-six male Swiss albino mice (Mus musculus) were used, at 45 days of age, weighing approximately 30.0g ± 5.0g, which were submitted to visual clinical evaluation and kept in the vivarium of the Pharmacology laboratory of the Federal Rural University of Pernambuco – UFRPE (Recife-PE, Brazil). The animals were kept at room temperature of 22ºC ± 2ºC, exposed to a photoperiod of 12 hours of light for 12 hours of darkness, and with access to water and feed (Purina rodent Labine), *ad libitum*.

Ehrlich tumor induction

Ehrlich tumor cells maintained in the peritoneal cavity of Swiss mice were used in the in vivo assay. Ascitic fluid was collected, submitted to cell counting in a Neubauer chamber, and diluted in 150mM NaCl until obtaining a concentration of 5.0 x 106 cells in 100µL. This solution was inoculated subcutaneously in the right dorsolateral region in healthy mice for induction of solid Ehrlich Tumor (TSE). Tumor growth was measured daily using a caliper. Its value in cm was converted to g, and its volume was presented in g/cm3.

Experimental draw

The experimental period lasted 7 days and the weight of the animals in each group was obtained daily. Ethanol extract of Própolis Verde was added to the animals' water at a concentration of 50 mg/kg of live weight (Araújo et al., 2010). New solutions were prepared every two days. Melatonin (Sigma-Aldrich) was dissolved in ethanol (0.02mL) and diluted in 0.9% saline solution to obtain a dose of 200µg per 100g of body weight (Dair et al. 2008). Melatonin applications were performed intraperitoneally, always between 18:00 and 19:00 hours. Paclitaxel (TAXOL®) was injected intraperitoneally at a dose of 5.0 mg/kg body weight (Ghoneum et al. 2019) and administered as a single dose on the first day of treatment. The animals in the control group received a 0.9% sodium chloride placebo solution.

To investigate the isolated or combined effects of Propolis Verde, Melatonin, and Paclitaxel, the following experimental groups were established: Control – mice with tumors without any treatment (n = 7); M – Melatonin-treated mice (n = 5); PV – mice treated with Propolis Verde (n = 6); PTX – Paclitaxel-treated mice (n = 5); M+VP – mice treated with Melatonin and Propolis Verde (n = 6); M+PTX – mice treated with Melatonin and Paclitaxel (n = 5); PV+PTX – mice treated with Propolis Verde and Paclitaxel (n = 5); and M+PV+PTX – mice treated with Melatonin, Propolis Verde and Paclitaxel (n=7). All experiments procedures were approved by the UFPE Ethics Committee on Animal Use (Protocol 115/2022).

Tissue collection and analysis

After the end of the treatments, the animals were submitted to euthanasia procedures to collect tissue fragments, using sodium thiopental solution (100mg/mL) intraperitoneally. At the time of euthanasia, blood, lung, kidneys, liver, spleen, heart, and tumors were collected. These organs and the tumor mass were weighed, sectioned and their fragments were fixed in a 10% neutral formalin solution, histologically processed for inclusion in paraffin, cut in a microtome at 4µm thick, and stained with Hematoxylin-Eosin for analysis under an optical microscope.

Blood samples were obtained from each animal by cardiac puncture immediately after euthanasia. The blood obtained was placed in penicillin-type vials containing anti-coagulant. Blood counts were performed according to the methodology described by Coles (COLES EH, 1984). Visible hemolysis-free serum aliquots were used for the measurement of total proteins, using the Biuret reaction method (DIAS FCR et al. 2017).

The percentage of weight gain of the animals during the experimental period was estimated by obtaining their average weight at the beginning (Day 1) and end of the experiment (Day 7), then dividing the weight on day 7 by the weight on day 1, the value obtained is subtracted from 1 and multiplied by 100 (El-GUENDOUZ S et al. 2016).

The cellular and nuclear area of the tumor cells were obtained from the measurement of 10 cells and their nuclei, in 5 fields per animal. The nucleus: cytoplasm ratio was obtained by dividing the mean nuclear area obtained in each group by the respective mean cell area, and the value obtained was multiplied by 100.

Tumor inhibition percentages were estimated from the knowledge of the estimated average tumor mass in each animal during each day of the experimental period. Thus, the average tumor mass of the control group was subtracted from the average tumor mass obtained in each treatment used, the value obtained is divided by the average tumor mass of the control group and multiplied by 100 (SHARAWI ZW 2020).

The mitotic index of TSE cells was obtained from the quantification of metaphase figures, in 10 fields per animal, using a 40x objective. After estimating the cell area and obtaining the number of tumor cells per 10,000 µm2, the mitotic index was then obtained by dividing the average number of tumor cells in mitosis by the number of tumor cells per 10,000 µm2, multiplying the value obtained by 100.

For the microscopic analysis of Organs collected organs, parameters were defined that varied according to the lesions in each organ, establishing a graduation table, where “0” represents the absence of lesion; “1” represents mild for low-intensity injuries; “2” moderate, with more intense disseminated or multifocal lesions and “3” for lesions of greater intensity.

Liver metastases were classified according to a specific grading table, where “0” indicates the absence of metastatic cells; “1” indicates the presence of 1 to 20 clusters; “2” indicates 21 to 40 clusters; “3” indicates 41 to 60 clusters; “4” indicates 61 to 80 clusters; “5” indicates 81 to 100 clusters; “6” indicates 101 to 120 clusters; “7” indicates 121 to 140 clusters; “8” indicates 141 to 160 clusters; “9” indicates 161 to 199 clusters, and “10” indicates the presence of metastatic cells forming more than 200 clusters.

Data with quantitative and qualitative characteristics were recorded in individual protocols, considering measures of central tendency and frequency dispersion analysis protocols for qualitative data, and paired comparisons by the non-parametric Kruskal-Wallis test. A significance level of 5% was considered to contrast measures of central tendency.

RESULTS

Hematological and biometric parameters

Data on the evaluation of hematological parameters are shown in **Table 1**. There was no statistically significant difference between the treatments used and the reference values for the hematological parameters evaluated, with trends of increase or decrease in some parameters having been found.

Table 1 - Hematological parameters of male swiss albino mice (Mus musculus), carriers of Ehrlich's solid tumor and treated with Melatonin, Green Propolis and Paclitaxel, alone or in association.

Groups	HTC (%)	PPT (g/dl)	LINF (%)	MONO (%)	BAST (%)	NEUT (%)	EOSI (%)	BASO (%)
C	37,7 ± 5,1	6,6 ± 0,3	55,6 ± 15,5	1,7 ± 1,4	2,0 ± 1,6	40,3 ± 14,0	0,1 ± 0,4	0,3 ± 0,5
M	38,0 ± 11,5	6,2 ± 0,4	66,7 ± 10,5	1,7 ± 1,3	1,7 ± 0,5	29,7 ± 12,2	0,0 ± 0,0	0,0 ± 0,0
PV	48,6 ± 3,6	6,5 ± 0,4	65,2 ± 7,8	2,4 ± 1,9	0,8 ± 0,8	31,4 ± 8,8	0,2 ± 0,5	0,0 ± 0,0
PTX	38,0 ± 4,8	6,5 ±0,4	65,7 ± 5,7	1,7 ± 1,0	1,5 ± 0,6	30,7 ± 6,2	0,0 ± 0,0	0,2 ± 0,5
M+PV	38,0 ± 1,4	6,2 ± 0,1	66,0 ± 5,6	1,0 ± 0,0	1,5 ± 0,7	29,5 ± 6,4	0,5 ± 0,7	0,5 ± 0,7
M+PTX	29,6 ± 9,5	5,2 ± 0,8	54,2 ± 5,5	2,2 ± 1,6	1,4 ± 1,3	41,8 ± 7,3	0,4 ± 0,9	0,0 ± 0,0
PV+PTX	35,0 ± 5,2	6,1 ± 0,6	68,0 ± 9,8	0,7 ± 0,5	1,7 ± 1,0	29,5 ± 8,8	0,0 ± 0,0	0,0 ± 0,0
M+PV+PTX	36,2 ± 7,3	6,3 ± 0,3	51,3 ± 13,4	1,2 ± 1,2	2,0 ± 1,4	45,3 ± 11,8	0,2 ± 0,4	0,2 ± 0,4
Reference *	35 – 50	5 – 7	55 – 80	5	0	26	3	0

C: Control; M: Melatonin; PV: Green Propolis; PTX: Paclitaxel; HTC: Hematocrit; PPT: Total Plasma Proteins; LINF: Lymphocytes; MONO: Monocytes; BAST: Rods; NEUT: Neutrophils; EOSI: Eosinophils; BASE: Basophils. *Reference values based on Viana (2007). **Source:** Pinto CF, et al., 2025.

The mean values referring to the animals' body weight at the end of the experiment, tumor weight, liver, spleen, kidneys, heart, and lung are shown in **Table 2**. The animals' weight did not differ statistically between the groups, nor during the experimental period. and not at the end of it. The percentage of weight gain was

significantly reduced in almost all treatments used when compared to the control group, except for the M group; as well as between different treatments, highlighting the highest percentage of reduction in the M+PV group and the lowest reduction in the group where Propolis Verde was administered alone.

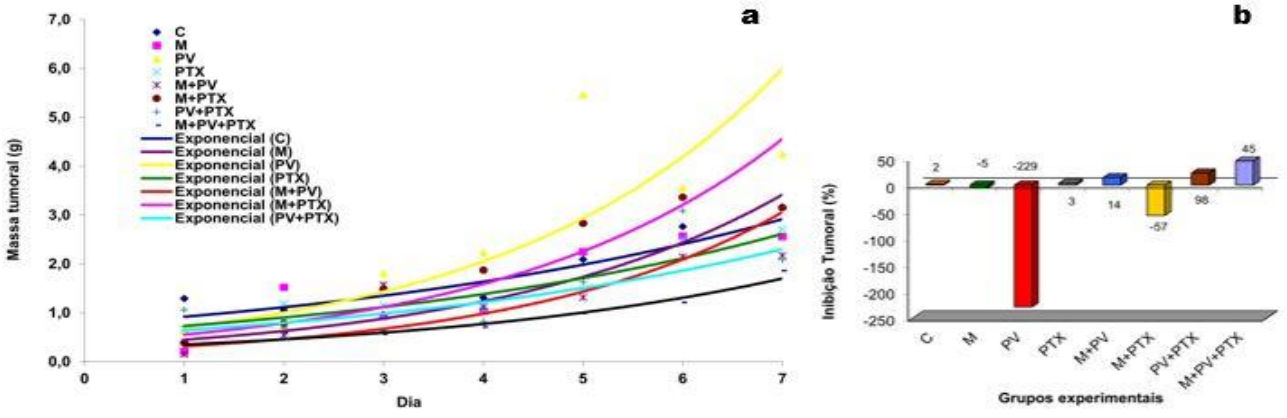
As for the tumor weight, it was observed that the animals in the groups treated with associations containing Green Propolis in the protocol obtained lower values, although the isolated treatment with Green Propolis has shown the opposite action, leading to the highest value of tumor weight observed between the groups. This isolated treatment promoted a 51.8% increase in tumor weight compared to the control group, and a 128.5% increase when compared to the M+PV+PTX group, which was the one that promoted the lowest mean tumor weight.

In the analysis of the weight of the other organs collected, there was a tendency of a 17% increase in the liver weight of the animals treated with Paclitaxel alone compared to the control group; although the variations observed between this and the other experimental groups were not statistically significant. As for the spleen, a tendency of increase of 47% and 31% was also observed in the animals of the groups treated with M+PV+PTX and PV, respectively, with the control group; and a trend of 60% reduction in their weight in the PV+PTX group compared to the control. Similarly, heart weight also tended to increase in the M+PV+PTX (127%), PTX (27%), and M+PTX (18%) groups compared to the control group; while lung weight showed a tendency to decrease by 41% in the PV+PTX group and a tendency to increase by 18% in the M group when compared to the control group. On the other hand, the kidneys of animals treated with PTX alone increased significantly (29%) with the control group; while in the animals treated with PV+PTX, there was a significant reduction of 8% in the weight of this organ with the control group (**Table 2**).

Tumor growth and inhibition

The tumor growth curves during the experimental period are shown in **Figure 1A**. The PV group showed the greatest tumor growth, while the M+PV+PTX group had the smallest growth curve. Tumor growth was also lower in the PV+PTX group, followed by the control. Among the treatments, the combination of Melatonin, Green Propolis, and Paclitaxel (M+PV+PTX) resulted in the greatest tumor reduction, whereas Green Propolis alone (PV) had the worst outcome. Although the differences were not statistically significant, the M+PV+PTX group showed a 56% reduction in tumor weight compared to the PV group. This protocol was also more effective than the control and other treatment combinations. **Figure 1B** highlights the superior tumor inhibition capacity of the M+PV+PTX group, showing 45.5% inhibition compared to the control. In contrast, the PV group had a negative inhibition rate of -229.04%. Tumor inhibition was also observed in the PTX, M+PV, and PV+PTX groups, while the M and M+PTX groups showed negative inhibition percentages.

Figure 1 - Development of solid Ehrlich tumor in male Swiss albino mice (Mus musculus) treated with Melatonin (M), Propolis green (PV) and Paclitaxel (PTX), alone or in association, as well as in the Control group (C), during the experimental period. A: Estimation of tumor mass and exponential curves of tumor evolution in the different groups throughout the experimental period. B: Percentage of tumor inhibition in the different groups at the end of the experimental period.



Source: Pinto CF, et al., 2025.

Table 3 presents quantitative data for Ehrlich's tumor. The total cell area of the tumor showed no correlation with the nucleus: cytoplasm ratio or the mitotic index. Among the treatments, the PTX group had the highest nucleus: cytoplasm ratio, while the M+PV+PTX group had the lowest, along with the control. Although there was no statistical difference in mitotic index, the M+PV+PTX group showed a 45.2% reduction compared to the control, the lowest rate among all treatments. This was followed by the M+PV (39%) and PV (37%) groups.

Table 3 - Morphometry of Ehrlich tumor cells in male swiss albino mice (Mus musculus) treated with Melatonin (M), Propolis Verde (PV) and Paclitaxel (PTX), alone or in association.

Groups	Cell area (μm^2)	Nuclear area (μm^2)	N:C ratio (%) *	N:C ratio μm^2 **	Mitoses/group	Mitotic index (%)
C	93,49 ± 18,76	41,21 ± 8,39	44,07	110,44 ± 20,77	2,18 ± 1,00	1,88 ± 0,95
M	82,14 ± 10,34	40,66 ± 4,45	49,55	123,25 ± 15,09	1,58 ± 0,13	1,30 ± 024
PV	78,14 ± 12,20	37,53 ± 7,85	47,99	130,43 ± 19,43	1,56 ± 0,38	1,18 ± 0,24
PTX	75,82 ± 23,06	40,94 ± 19,82	53,95	148,34 ± 68,92	1,62 ± 0,87	1,27 ± 0,97
M+PV	74,46 ± 6,20	33,27 ± 3,19	44,72	135,07 ± 11,14	1,66 ± 0,34	1,15 ± 0,19
M+PTX	91,52 ± 11,74	42,28 ± 6,55	46,22	110,89 ± 15,94	1,52 ± 0,58	1,40 ± 0,56
PV+PTX	88,21 ± 12,76	43,80 ± 8,69	49,66	115,06 ± 14,64	1,70 ± 0,32	1,52 ± 0,45
M+PV+PTX	88,65 ± 9,75	39,74 ± 4,77	44,78	114,03 ± 13,04	1,16 ± 0,35	1,03 ± 0,35

* Nucleus:Cytoplasm ratio of tumor cells; ** Number of tumor cells/10,000 µm2; C: Control; M: Melatonin; PV: Green Propolis; PTX: Paclitaxel.
Source: Pinto CF, et al., 2025.

Histopathological analysis by treatment

The main histopathological findings observed in each organ are quantified in **Figure 2**. In the control group, lower pulmonary congestion was noted, but with signs of hypertension, emphysema, and metastases (**Fig. 2A**). The kidneys showed glomerular atrophy, hydropic degeneration, coagulative necrosis, and protein casts (**Fig. 2C-D**). The liver had the highest degree of metastasis, steatosis, periportal hepatitis, and necrosis (Fig. 2B). The spleen displayed replacement of red and white pulp by neoplastic tissue and marked hemorrhage (**Fig. 2F**).

In the Melatonin-treated group, no pulmonary metastases were found, and the lowest myocarditis level was observed, though with severe myocardial changes (**Fig. 2E**). Kidney analysis showed reduced glomerular atrophy and tubular degeneration, despite metastasis and fibrosis (**Fig. 2C-D**). The liver showed high necrosis (**Fig. 2B**), and the spleen had fewer metastases, increased congestion, more hemosiderocytes, and lowest germinal center apoptosis (**Fig. 2F**).

In the Green Propolis group, kidneys had pronounced chronic interstitial nephritis, glomerular atrophy (**Fig. 2C**), and congestion (**Fig. 2D**). The liver showed reduced metastasis and periportal inflammation (**Fig. 2B**). The heart presented with high myocarditis (**Fig. 2E**), and the spleen had less hypoplasia, but increased congestion and metastasis (**Fig. 2F**). In the Paclitaxel group, the lungs had the highest peribronchiolitis, but the lowest interstitial pneumonia and emphysema (**Fig. 2A**). The liver showed minimal coagulative necrosis, with increased congestion and periportal hepatitis (**Fig. 2B**). Kidneys had diffuse tubular necrosis, fibrosis, and hydropic degeneration, but no metastases (**Fig. 2C-D**). The heart displayed myocarditis and metastasis (**Fig. 2E**), and the spleen showed increased congestion and germinal center apoptosis (**Fig. 2F**).

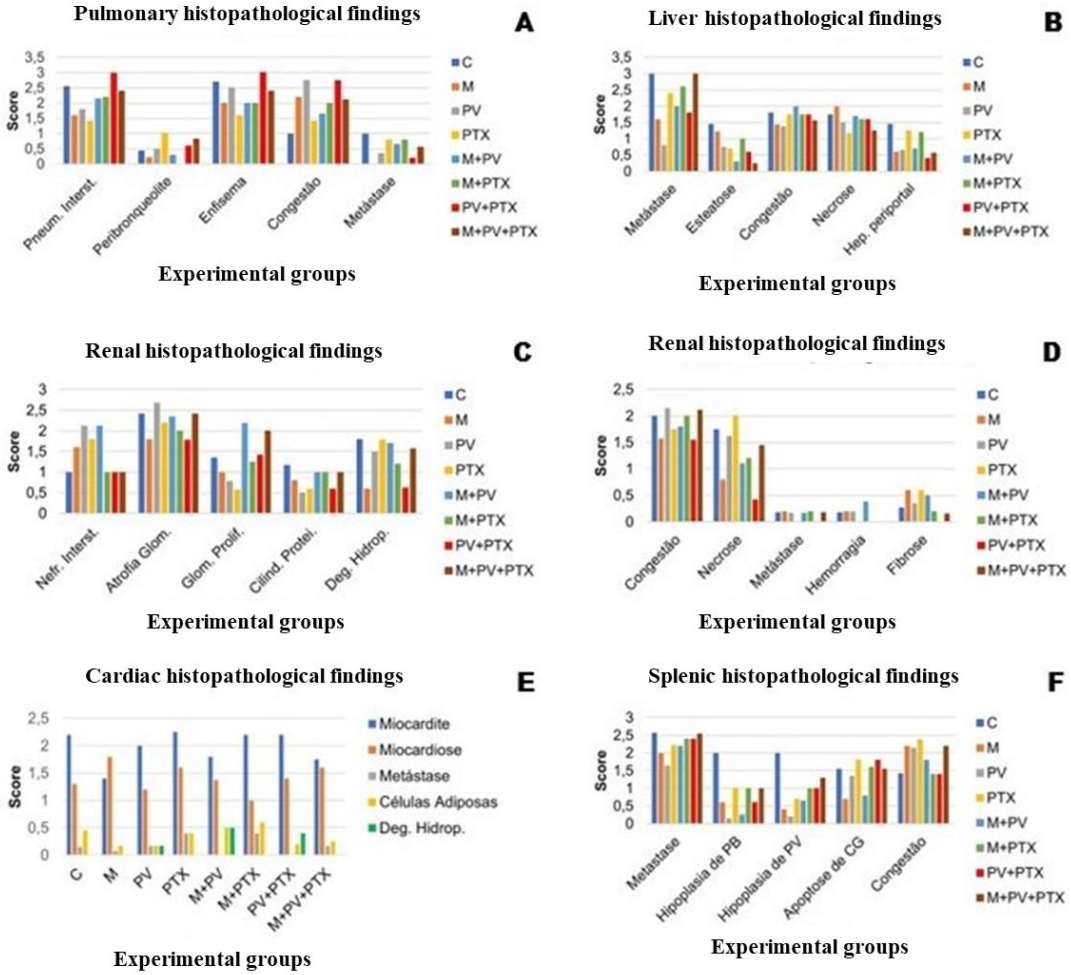
The Melatonin + Green Propolis combination reduced interstitial pneumonia and emphysema (**Fig. 2A**), hepatic steatosis, hepatitis, and metastasis (**Fig. 2B**). However, kidneys showed high levels of interstitial nephritis, glomerular atrophy, glomerulonephritis, and hemorrhage (**Fig. 2C-D**). There was severe myocardial degeneration, no cardiac metastases (**Fig. 2E**), and decreased spleen hypoplasia, apoptosis, and fibrosis (**Fig. 2F**).

The Melatonin + Paclitaxel group showed no peribronchiolitis but no significant improvement in other lung findings (**Fig. 2A**). The liver had severe chronic hepatitis and high metastases (**Fig. 2B**). Metastases were also present in the kidneys, heart, and spleen (**Fig. 2C-F**), along with moderate myocarditis and adipose infiltration in the heart (**Fig. 2E**). The Green Propolis + Paclitaxel group exhibited the highest pneumonia, emphysema, congestion, and pulmonary hemorrhage (**Fig. 2A**). The liver showed the highest congestion, with reduced

hepatitis (**Fig. 2B**). Kidneys had lower nephritis, glomerular atrophy, degeneration, and the lowest necrosis levels (**Fig. 2C-D**). The heart had severe hydropic degeneration (**Fig. 2E**), and the spleen showed high apoptosis and metastasis (**Fig. 2F**).

Lastly, in the group treated with Melatonin + Green Propolis + Paclitaxel, lungs had peribronchiolitis, perivasculitis, and fibrosis (**Fig. 2A**). The liver showed high metastasis and hepatitis, but the lowest steatosis among groups (**Fig. 2B**). Although no renal hemorrhage was found, no major improvements in other kidney parameters were noted (**Fig. 2C-D**). The heart showed moderate myocarditis and cardiomyolysis (**Fig. 2E**), and the spleen had the highest splenitis and red pulp hypoplasia (**Fig. 2F**).

Figure 2 - Score of the main histopathological alterations found in the lungs, liver, kidneys, heart and spleen of male albino swiss mice (Mus musculus) with Ehrlich tumor and treated with Melatonin (M), Green Propolis (PV) and Paclitaxel (PTX), isolated or in association, as well as the control group (C). tire Interst.: Interstitial Pneumonia; hep. Periportal: Periportal Hepatitis; Nephr. Interst.: Interstitial Nephritis; Glomerular Atrophy: Glomerular Atrophy; Glom. Prolif.: Proliferative Glomerulonephritis; Cylinder Protein.: Protein Cylinders; Deg. Hydrop.: Hydropic Degeneration; BP Hypoplasia: White Pulp Hypoplasia; PV Hypoplasia: Red Pulp Hypoplasia; GC Apoptosis: Germination Center Apoptosis.



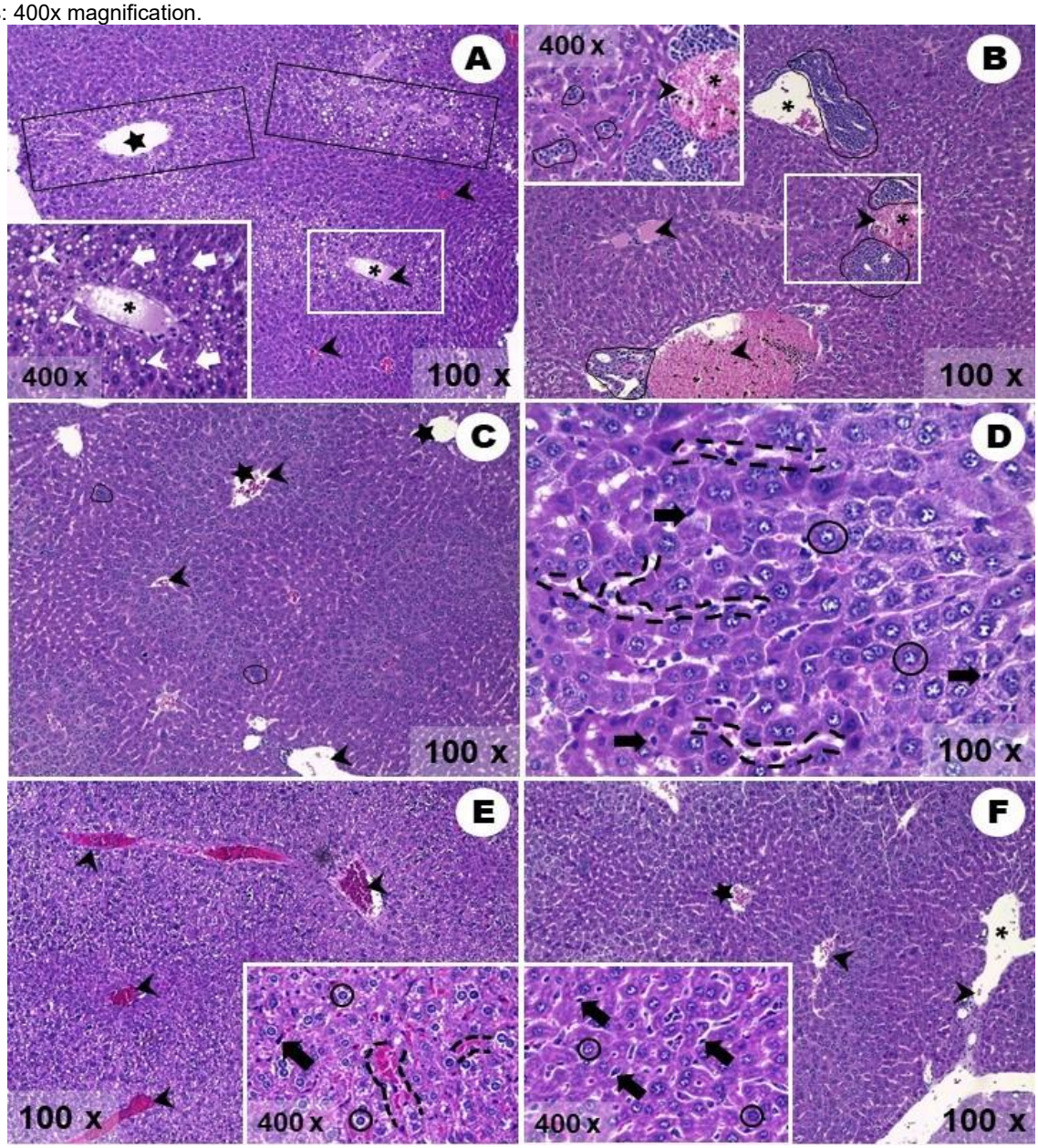
Source: Pinto CF, et al., 2025.

Hepatic histopathological analysis

Among the analyzed organs, the liver exhibited the most pronounced histopathological alterations (**Figure 3**). The control group showed extensive metastases in both periportal and interstitial regions, along with intense steatosis (macro- and microsteatosis) and vascular congestion. Treatment with green propolis alone resulted in a significant 80% tumor reduction, accompanied by a marked decrease in metastatic foci and vascular

congestion. Treatments with melatonin (M) and the green propolis + paclitaxel (PV+PTX) combination also reduced tumor burden by approximately 75%, although not statistically significant. The PV+PTX group displayed intense vascular congestion and increased Kupffer cell activation. The M+PV+PTX combination group showed the lowest degree of steatosis, reduced vascular congestion compared to the control, and Kupffer cell activation levels similar to those seen with paclitaxel alone.

Figure 3 - Histopathological aspects of the liver parenchyma of male mice (Mus musculus) with Ehrlich's tumor and submitted to different treatments. A and B: Control Group. A: Areas of intense hepatic steatosis **(rectangle)** and congested vessels **(black arrowhead)** are observed. Detail: Macrosteatosis **(white arrowhead)** and microsteatosis **(white arrow).** B: Foci of periportal metastasis **(outline)** and intense congestion in blood vessels **(black arrowhead).** Detail: Congestion in the portal vein **(asterisk)** and high-grade interstitial metastatic foci **(contour).** C and D: Green Propolis Group. C: A low degree of metastasis is observed with few metastatic foci **(outline)** and less vascular congestion **(black arrowhead).** D: Low degree of congestion in sinusoid capillaries **(dashed).** E: Green Propolis + Paclitaxel Group. Intense vascular congestion is observed **(black arrowhead).** Detail: Congested sinusoid capillaries (dashed) and active Kupffer cell **(black arrow)**. F: Melatonin Group + Green Propolis + Paclitaxel. There is an area free of hepatic steatosis and low vascular congestion **(black arrowhead).** Detail: Kupffer cell proliferation **(black arrow). Circle:** Hepatocytes. **Black arrow:** Kupfer cells. **Dashed:** Sinusoid capillaries. **Asterisk:** Port vein. **Star:** Centrilobular vein. Coloring: HE. A-F: 100x magnification. Details: 400x magnification.



Source: Pinto CF, et al., 2025.

DISCUSSION

To our knowledge, this is the first study using the Ehrlich carcinoma model to evaluate the combined effects of substances with known individual benefits, aiming to identify the most effective and least toxic treatment protocol for murine breast carcinoma. Given that Ehrlich's tumor is a well-established model for breast and lung cancer studies, the results here are promising for future therapeutic applications in humans.

Hematological and Biometric Parameters

Hematological and biochemical markers are widely used in cancer diagnostics (GONZALEZ-GRONOW M, et al., 2021), though they do not always correlate with tumor progression or treatment efficacy. Similar to other studies using complementary therapies for Ehrlich tumors, few alterations were observed in these parameters despite histopathological changes (GONZALEZ-GRONOW M, et al., 2021).

Green Propolis (PV) alone caused the greatest reduction in body weight gain, a possible indication of antitumor activity, since tumor ascites from Ehrlich tumors typically leads to excessive weight gain (MANGUEIRA VM, et al., 2017). However, the PV-only group exhibited the highest tumor weight and lowest tumor inhibition percentage. This discrepancy may stem from variations in propolis chemical composition, bee species, and botanical origin (JOSÉ M et al.), and also its concentration-dependent bioactivity, being pro- or antioxidant depending on flavonoid content (MUNARI CC, et al., 2008).

Melatonin (M) alone was not effective in tumor control, contrary to previous findings. Yet, combinations with PV or PV+Paclitaxel (PTX) showed a trend toward tumor weight reduction, supporting its role as a safe adjuvant in chemotherapy (AMIN AH, et al., 2019).

Renal hypertrophy in the PTX-only group suggests nephrotoxicity, a known side effect of chemotherapeutic drugs (ALMEER RS, et al., 2021). This effect was mitigated when PV was co-administered, likely due to its antioxidant properties (ABD ELDAIM MA, et al., 2019). Heart, liver, and lung weights also tended to increase with PTX, which aligns with its known toxicity (ELGHARABAWY RM, et al., 2021), whereas PV co-treatment attenuated these changes.

Tumor Growth and Inhibition

Animals treated with PV alone showed the fastest tumor growth and highest negative inhibition, suggesting limited efficacy. However, PV in combination enhanced therapeutic outcomes, consistent with previous findings that PV combined with chemotherapy reduces carcinoma damage more effectively than either treatment alone (SAMENI HR, et al., 2021). This synergy also reduces cytotoxic side effects and increases drug efficacy (BENKOVIC V, et al., 2007), with recommendations to administer PV prior to chemotherapy (EL-KHAWAGA OAY, et al., 2003a).

The mitotic index analysis highlighted the M+PV+PTX protocol, with 45.2% fewer mitoses than controls. M+PV and PV alone also showed reductions of 39% and 37%, respectively. These results align with Suzuki I, et al. (2002), who reported enhanced tumor regression and reduced chemotherapy-induced cytopenia with propolis. El-Khawaga OAY, et al. (2003a) attributed mitotic index reductions to G0/G1 phase arrest and decreased tumor cell viability. Melatonin likely enhances therapeutic outcomes through apoptosis induction, reduced proliferation, and metastasis inhibition (LIN PH, et al., 2020; TALIB WH, et al., 2021), underscoring its adjuvant potential.

Histopathological Analysis

Melatonin alone prevented lung metastasis, consistent with its role in inhibiting epithelial-mesenchymal transition (POURHANIFEH HM, et al., 2019; CHAO CC, et al., 2019). PV alone or in combination also effectively reduced lung metastasis, especially with PTX. PV significantly reduced liver metastasis and parenchymal damage, attributable to its antioxidant effects (EL-KHAWAGA OAY, et al., 2003b). Orsolic and Basic (2005) suggested that propolis enhances macrophage tumoricidal activity against Ehrlich carcinoma. Despite its nephroprotective potential (CHANG JF et al., 2020), PV alone caused marked renal damage. In contrast, melatonin reduced necrosis and degeneration, confirming its protective effects against chemotoxicity

(ALI BH, et al., 2020; MALEKI DANA P, et al., 2020). PTX induced myocarditis, which was not alleviated by PV or M, likely due to the heart's susceptibility to oxidative stress during chemotherapy (SIMBRE II et al., 2005). Splenomegaly in the M+PV+PTX group coincided with splenitis and hemorrhage (SEGURA JA, et al., 1997). Melatonin-treated groups showed reduced apoptosis in germinal centers and less hypoplasia, with enhanced white pulp activation, suggesting lymphocyte protection. These effects may stem from melatonin's dual action: inhibiting apoptosis in immune cells while promoting it in tumor cells (ANSELMO J, et al., 2005).

In conclusion, neither PV nor M alone significantly reduced tumor damage from Ehrlich carcinoma. Although propolis has documented immunomodulatory and chemoprotective effects (CHIU HF, et al., 2020), its isolated use did not impact Ehrlich ascitic cells (ORŠOLIĆ N, et al., 2010). Melatonin's anticancer potential also appears dose-dependent. El-Missiry MA and El-Aziz AF reported antitumor effects at doses 96% higher than used here. However, even at lower doses, melatonin attenuated renal and cardiac damage, supporting its role in reducing free radical-induced cell injury (KARBOWNIK M and REITER RJ, 2000).

CONCLUSION

The combined treatment of Melatonin, Propolis Verde, and Paclitaxel can attenuate the tissue changes associated with Paclitaxel. Because of the data obtained regarding the effects of Propolis Verde, it is recommended the development of new studies that compare this compound produced from different sources. Our results also prove the importance of the association of Melatonin with conventional cancer treatment, and it is recommended to investigate the association presented here using higher doses of this hormone. The results obtained regarding the association M+PV+PTX reinforce the need for the development of new studies that can unveil the possible mechanisms of antitumor action from treatments combining drugs and natural substances.

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