In vivo model of colorectal cancer: impact to cigarette smoke exposition on neoplasm development

Modelo in vivo de câncer colorretal: Impacto da exposição à fumaça de cigarro no desenvolvimento neoplásico

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Our proposal aims to investigate the impact that exposure to cigarette smoke has on the development of lesions in the colorectal mucosa of in vivo model of colorectal carcinogenesis. For that, 24 young male rats were induced to colorectal carcinogenesis and separated into two groups: exposure to cigarette smoke for 20 weeks (DMH+/Tobacco+) and control (DMH+/Tobacco-). In the group (DMH+/Tobacco+), 14.7% dysplasia and 85.29% malignant neoplasms were observed, including tubular adenocarcinoma (73%), carcinoma in situ (17%) and signet-ring cell adenocarcinoma (10%). Mononuclear inflammation and pleomorphisms were mild to moderate. In the control group, there was 7.69% dysplasia, 7.69% tubular adenoma and 84.62% malignant neoplasms, including tubular adenocarcinoma (82%), signet-ring cell adenocarcinoma (9%), carcinoma in situ (5%) and mucinous adenocarcinoma (4%). Mononuclear inflammation and pleomorphisms were moderate to severe. Thus, the experimental model showed that the lesions were predominantly malignant and with histopathological characteristics compatible with human colorectal cancer, including its possible applications for future studies.

**Keywords:** Experimental carcinogenesis; Histopathological findings; Rat; Tobacco smoking.

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**RESUMO**

Objetivamos investigar o impacto que a exposição à fumaça de cigarro tem no desenvolvimento de lesões na mucosa colorretal de modelo in vivo de carcinogênese colorretal. Para tanto, 24 ratos machos jovens foram induzidos à carcinogênese colorretal e separados em dois grupos: exposição à fumaça de cigarro por 20 semanas (DMH+/Tabaco+) e controle (DMH+/Tabaco-). No grupo (DMH+/Tabaco+) foram observadas 14,7% de displasia e 85,29% de neoplasias malignas, incluindo adenocarcinoma tubular (73%), carcinoma in situ (17%) e adenocarcinoma de células em anel de sinete (10%). A inflamação mononuclear e os pleomorfismos foram leves a moderados. No grupo controle, houve 7,69% de displasia, 7,69% de adenoma tubular e 84,62% de neoplasias malignas, incluindo adenocarcinoma tubular (82%), adenocarcinoma de células em anel de sinete (9%), carcinoma in situ (5%) e adenocarcinoma mucinoso (4%). A inflamação mononuclear e os pleomorfismos foram moderados a graves. Assim, o modelo experimental mostrou que as lesões eram predominantemente malignas e com características histopatológicas compatíveis com câncer colorretal humano, incluindo suas possíveis aplicações para estudos futuros.

**Palavras-chave:** Carcinogênese experimental; Achados histopatológicos; Ratos; Tabagismo.
INTRODUCTION

Smoking is a risk factor for the development of neoplasms in various compartment of the bodies such as: gallbladder (LUGO; PEVERI & GALLUS, 2019), kidney (LIU et al., 2019), pancreas (LUGO et al., 2018) and others. In the gastrointestinal tract, tobacco smoke-associated neoplasms are reported in the oral cavity, the oesophagus, the stomach, the ileum and the colon (GIOVANNUCCI, 2001). For colorectal neoplasms, it is estimated that a smoking habit of at least 20 years is significantly related to the appearance of small polyps; however, when this exposure exceeds 20 years, it is related to the appearance of large polyps, and when the exposure is over 35 years, it favours the appearance of colorectal carcinoma (GIOVANNUCCI, 2001).

Lifestyle is directly related to the development of colorectal cancer and smoking is a habit that predisposes to these neoplasms (CARR et al., 2018). In some developed countries, these neoplasms have occurred at an earlier age, affecting many young adults, and this may be related to smoking and others lifestyle habits that make contact with carcinogenic products earlier (SIEGEL et al., 2019).

Smokers have a higher predisposition to develop colorectal cancer isolated and synchronous compared to non-smokers. Another point that demonstrates the influence of smoking is that individuals who quit smoking after 10 years of smoking had a reduced chance of developing synchronous tumours, but were more likely to develop solitary neoplasms (DREW et al., 2017).

Previous studies have shown the influence of tobacco components on the large intestine, explaining their actions on this organ, especially regarding inflammation and its contribution to colorectal carcinogenesis (VERSCHUERE et al., 2012). In addition, in the last 40 years, experimentally induced intestinal carcinogenesis in rodents has been studied to elucidate the aetiology and mechanisms involved and the histopathological similarities between rodent neoplasia and human intestinal neoplasms, along with their molecular and genetic similarities (WARD & TREUTING, 2014).

Animal models are increasingly important in understanding the relationship between CRC and smoking, and as already mentioned, several studies have been carried out in experimental models to better understand the biology of CRC (ALAMO et al., 2014). For example, a study of the interaction between smoking and CRC in an in vivo model revealed that HIF-1α protein expression and AgNOR count are modified according
to the degree of tumor differentiation, in the presence of exposure to cigarette smoke, highlighting the importance and relevance of animal models in research on the relationship between CRC and tobacco (TRIVILIN et al., 2017).

Given the importance of tobacco components in the development of pathological processes of the gastrointestinal tract, and the possibility of studying smoking and colorectal cancer in an *in vivo* model of colorectal carcinogenesis, our objective was investigate the impact that exposure to cigarette smoke has on the development of lesions in the colorectal mucosa of *in vivo* model of colorectal carcinogenesis, describing the main histopathological findings related to tumor progression, as well as demonstrating the potential that the experimental model of colorectal carcinogenesis to be applied in cigarette studies has involvement in smoking and colorectal cancer, subsidizing studies of survival, responses to treatments, and biology of colorectal cancer.

**MATERIAL AND METHODS**

The animals used in this study were maintained according to the Brazilian Law on Procedures for the Scientific Use of Animals (#11794/2008), and the experimental procedures were reviewed and approved by the Ethics and Animal Use Committee of the Federal University of Espírito Santo (Federal University of Espírito Santo – UFES), Protocol 003/2014.

Twenty-four male young Wistar rats (Rattus norvegicus), weighing 181.35 g (± 18.7 g), were kept in a room at a controlled temperature between 21 °C and 24 °C, relative humidity between 45 and 55% and a light/dark cycle of 12 hours. The animals received commercial feed and water ad libitum.

The induction of colorectal carcinogenesis with the chemical agent 1,2-dimethylhydrazine (DMH) was conducted in all animals and occurred according to the methodology modified by Laranjeira et al. (1998) based on the study of Nauss et al. (1984). In this protocol, the DMH was dissolved in 0.9% NaCl containing 1.5% EDTA as vehicle at a final pH of 6.5, which was reached with the use of 1 N NaOH solution when necessary. The chemical agent was given subcutaneously once a week for a period of five weeks at a dose of 65 mg/kg/week.

During the process of induction and promotion of colorectal cancer, the animals induced to carcinogenesis were divided into two experimental groups: exposure to
cigarette smoke (DMH+/Tobacco+) and control (DMH+/Tobacco-), each composed of 12 animals.

The exposure of the group (DMH+/Tobacco+) to cigarette smoke occurred in an inhalation chamber equipped with a smoke puff and corresponded to 12 cigarettes per day, divided into two shifts (morning and afternoon) of exposure of 60 minutes each, over 20 weeks. The exposure methodology followed modifications of the one used by Paiva et al. (2003) where the exposure occurred over 30 days; in the first week, the smoke was released at a rate of 5 cigarettes/30 minutes, twice daily in the afternoon, with rest intervals of 10 minutes, changing to 10 cigarettes/30 minutes, twice in the morning and twice in the afternoon, until the end of the experiment.

At the 21st week of the experiment, all animals were euthanized and had their entire large intestines opened by insertion into the mesentery to remove lesions over 0.1 centimetres in size at their largest point. The collected tissues were fixed in 10% buffered formalin and subjected to a routine paraffin inclusion process, followed by histological section staining with haematoxylin and eosin.

The following histopathological features were evaluated: inflammatory process (type and intensity), cell pleomorphism (intensity), mitosis figures (absent, ≤ 3/field, > 3/field), submucosal invasion (absence or presence), vascular invasion absence or presence), perineural invasion (absence or presence), lymphatic invasion (absence or presence), desmoplasia (absence or presence), glandular debris (absence or presence) and necrosis (absence or presence).

All samples from the experimental groups were diagnosed according to Perše and Cerar (2010) absence of benign neoplasms in the (DMH+/Tobacco+) group did not allow comparison to the control group and independence analysis by the G-Test. The comparison of the number of macroscopic lesions, as well as the number of malignant neoplasms was performed by Student's t-test. To evaluate the effect of exposure to cigarette smoke on the histopathological characteristics of malignant neoplasms and dysplasia, the independence G-test of was used. For all analyses, statistical significance was considered when p<0.05, using GraphPad prism 7.0 DEMO software.
RESULTS

In the group (DMH+/Tobacco+), 34 lesions were found, with a mean of 2.83 lesions per animal, with a minimum number of one lesion and a maximum of nine lesions. In the control group, 26 lesions were found, with a mean of 2.17 lesions per animal. The smallest number of lesions found was one, and the largest number was eight. Statistical analysis showed no significant difference in the number of lesions in the two groups evaluated (p=0.309).

Regarding the histopathological characteristics evaluated in the two groups, Table 1 summarizes the numbers and percentages found, considering only the classifications of benign lesions, malignant lesions and dysplasia, and the main microscopic findings are evidenced in Figure 1 (DMH+/Tabaco+ group) and Figure 2 (Control group). No benign neoplasia was found in group (DMH+/Tobacco+); however, 85.29% (29/34) were classified as malignant and 14.70% (5/34) as dysplasias. Among the malignant lesions, 73% (21/29) corresponded to tubular adenocarcinoma, 17% (5/29) to carcinoma in situ and 10% (3/29) to signet-ring cell adenocarcinoma. In the control group, 7.69% (2/26) of the lesions were dysplasia, 7.69% (2/26) tubular adenoma and 84.62% (22/26) malignant neoplasms, representing tubular adenocarcinoma (82%), signet-ring cell adenocarcinoma (9%), carcinoma in situ (5%) and mucinous adenocarcinoma (4%). Malignant lesions did not differ between experimental groups (p=0.810).

Table 1 - Numbers and percentages of histopathological characteristics found in the colorectal mucosa of carcinogenesis induced rats with 1,2-dimethylhydrazine and exposed to cigarette smoke (DMH+/Tobacco+) or control conditions (DMH+/Tobacco).
<table>
<thead>
<tr>
<th>Category</th>
<th>Absent Count</th>
<th>Present Count</th>
<th>Absent Percentage</th>
<th>Present Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SUBMUCOSAL INVASION</strong></td>
<td>2 (100%)</td>
<td>19 (86.34%)</td>
<td>26 (89.66%)</td>
<td>23 (88.24%)</td>
</tr>
<tr>
<td><strong>PERINEURAL INVASION</strong></td>
<td>2 (100%)</td>
<td>22 (100%)</td>
<td>26 (100%)</td>
<td>29 (100%)</td>
</tr>
<tr>
<td><strong>LYMPHATIC INVASION</strong></td>
<td>2 (100%)</td>
<td>6 (27.27%)</td>
<td>24 (92.31%)</td>
<td>26 (89.66%)</td>
</tr>
<tr>
<td><strong>BLOOD INVASION</strong></td>
<td>2 (100%)</td>
<td>21 (95.46%)</td>
<td>25 (96.15%)</td>
<td>29 (100%)</td>
</tr>
<tr>
<td><strong>DESMOPLASIA</strong></td>
<td>2 (100%)</td>
<td>16 (72.73%)</td>
<td>16 (61.54%)</td>
<td>16 (55.17%)</td>
</tr>
<tr>
<td><strong>GLANDULAR DEBRIS</strong></td>
<td>-</td>
<td>2 (100%)</td>
<td>24 (92.31%)</td>
<td>26 (89.66%)</td>
</tr>
<tr>
<td><strong>NECROSIS</strong></td>
<td>2 (100%)</td>
<td>21 (95.46%)</td>
<td>25 (96.15%)</td>
<td>21 (61.76%)</td>
</tr>
<tr>
<td><strong>PERCENTAGE OF GLANDS</strong></td>
<td>-</td>
<td>16 (72.73%)</td>
<td>16 (61.54%)</td>
<td>16 (55.17%)</td>
</tr>
<tr>
<td>&gt; 95%</td>
<td>2 (100%)</td>
<td>4 (18.18%)</td>
<td>8 (30.77%)</td>
<td>4 (13.79%)</td>
</tr>
<tr>
<td>50-95%</td>
<td>10 (45.45%)</td>
<td>10 (41.67%)</td>
<td>12 (41.38%)</td>
<td>12 (41.38%)</td>
</tr>
<tr>
<td>&lt; 50%</td>
<td>8 (36.37%)</td>
<td>8 (30.77%)</td>
<td>10 (41.67%)</td>
<td>10 (41.67%)</td>
</tr>
<tr>
<td><strong>DEGREE OF DIFFERENTIATION</strong></td>
<td>2 (100%)</td>
<td>4 (18.18%)</td>
<td>6 (25%)</td>
<td>4 (13.79%)</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>-</td>
<td>10 (45.45%)</td>
<td>12 (41.38%)</td>
<td>12 (41.38%)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>10 (45.45%)</td>
<td>10 (41.67%)</td>
<td>12 (41.38%)</td>
<td>12 (41.38%)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>-</td>
<td>8 (36.37%)</td>
<td>8 (33.33%)</td>
<td>8 (29.26%)</td>
</tr>
</tbody>
</table>

Source: Authors (2024).

**Figure 1** - Photomicrography of colorectal neoplasias of rats subjected to carcinogenesis with 1,2-dimethylhydrazine belonging to the group (DMH+/Tobacco+) at the 21st week of the experiment. A – Mononuclear inflammation with the presence of numerous eosinophils in tubular adenocarcinoma (asterisks), B – Lymphatic invasion in a sample of
Figure 2 - Photomicrography of colorectal neoplasias of rats subjected to carcinogenesis with 1,2-dimethylhydrazine belonging to the control group at the 21st week of the experiment. A – Note the mononuclear inflammation with the presence of neutrophils and eosinophils in a tubular adenocarcinoma (asterisk), B – Blood invasion in signet-ring cell adenocarcinoma (arrow), C – Well-differentiated tubular adenocarcinoma, D – Moderately differentiated tubular adenocarcinoma, E – Signet-ring cell adenocarcinoma representing poorly differentiated neoplasia and F – Moderate desmoplasia (arrowhead). Haematoxylin and eosin.
The inflammatory process intensity in malignant neoplasms and dysplasia was not influenced by exposure to cigarette smoke (p=0.2504 and p=0.6916, respectively). However, mononuclear inflammation with the presence of eosinophils was the most predominant finding in the (DMH+/Tobacco+) group, and mononuclear inflammation with the presence of neutrophils and eosinophils was predominant in control group (DMH+/Tobacco-).

As for the pleomorphism intensity, both in malignant neoplasms and dysplasia there was no influence by the cigarette smoke (p=0.5589 and p=0.4392, respectively). For mitosis figures in malignant neoplasms, exposure to cigarette smoke influenced the absence of these (p=0.0342), while in the samples classified as dysplasia there was no influence of cigarette smoke on the number of mitosis figures (p=0.6916). The absence
of mitosis figures occurred in one sample of tubular adenocarcinoma, one of signet-ring cell adenocarcinoma and one carcinoma in situ.

In relation to malignant neoplasms, perineural invasion was absent in the experimental groups, and there was no influence of exposure to cigarette smoke on submucosal invasion \((p=0.5085)\) (occurring in all adenocarcinomas in both experimental groups), as well as the lymphatic invasion \((p=0.2848)\) and blood invasion \((p=0.2880)\). But in \((\text{DMH+/Tobacco+})\) group one sample classified as signet-ring cell adenocarcinoma presented lymphatic invasion, while in control group only one sample classified as signet-ring cell adenocarcinoma presented blood invasion.

In dysplasia samples, the submucosal invasion, perineural invasion, lymphatic invasion, blood invasion, desmoplasia, glandular debris and necrosis was absent in both experimental groups, while percentage of glands was major that 95%.

In malignant neoplasms, the presence of desmoplasia \((p=0.2026)\), as well the presence of glandular debris \((p=0.0603)\) is not influenced by exposure to cigarette smoke. However, three samples of carcinoma in situ in the group \((\text{DMH+/Tobacco+})\) showed absence of glandular debris. As for necrosis, exposure to cigarette smoke significantly influenced the presence of this lesion \((p=0.0007)\), occurring in 10 samples of tubular adenocarcinoma, two signet-ring cell adenocarcinoma and one carcinoma in situ.

The percentage of glands, and consequently degree of differentiation is not influenced by exposure to cigarette smoke \((\text{both, } p=0.8896)\). In \((\text{DMH+/Tobacco+})\) group, samples considered well differentiated included three tubular adenocarcinomas \((75\%)\) and one signet-ring cell adenocarcinoma \((25\%)\). Eleven tubular adenocarcinomas \((84.62\%)\) and two carcinomas in situ \((15.38\%)\) were classified as moderately differentiated, whereas samples considered as poorly differentiated included seven tubular adenocarcinomas \((58.33\%)\), two signet-ring cell adenocarcinomas \((16.67\%)\) and three carcinomas in situ \((25\%)\). In control group, lesions considered well differentiated were classified as tubular adenoma \((33.33\% \text{ - two samples})\), while the remaining four \((66.67\%)\) were classified as tubular adenocarcinoma. Moderately differentiated lesions consisted of nine samples of tubular adenocarcinoma \((90\%)\) and one of carcinoma in situ \((10\%)\). Of the samples classified as poorly differentiated, five were tubular adenocarcinoma \((62.5\%)\), one was mucinous adenocarcinoma \((12.5\%)\) and two were signet-ring cell adenocarcinoma \((25\%)\).
DISCUSSION

The results showed a good number of lesions in the group exposure to smoke of the direct burning of cigarettes, with lesions presented mild to moderate pleomorphism and inflammation, and the cigarette smoke is associated to few mitosis figure and presence of the necrosis; furthermore, the histopathological characteristics were compatible with lesions in humans. In addition, as expected, the protocol using 1,2-dimethylhydrazine (DMH) associated to exposure to cigarette smoke induced macroscopic lesions in the (DMH+/Tobacco+) group, which were possible to diagnose by histopathology.

Studying the association of a single dose of DMH as a pro-carcinogen followed by chronic ulcerative colitis, Wang et al. (2004) found high incidence rates of dysplasia and colorectal cancer. In addition, the association between a smoking habit and colitis has been well studied and has shown that important molecular changes are found and favour the appearance of dysplastic lesions and adenomatous polyp lesions (LIU et al., 2003), along with their involvement in the co-regulation of 5-lipoxygenase and cyclooxygenase-2 in the promotion of colorectal cancer (SHEN et al., 2016). In this context, our experimental model of the carcinogenesis colorectal using DMH associated to cigarette smoke exposure allowed us to find lesions with histopathological characteristics similar to those found in colorectal neoplasms in humans and that may also favour molecular and protein expression studies.

Colorectal carcinogenesis protocols using DMH are well established and allow the study of pre-neoplastic changes until to malignant lesions (BALMAIN & HARRIS, 2000), whether for morphological, molecular or therapeutic purposes (SUMAN; FORNACE & DATTA, 2012). In addition, the experimental model using DMH/Azoxymethane (AOM) is useful in the study of gene-gene and gene-environment interactions, which significantly influence the pathogenesis of colorectal cancer (JOHNSON & FLEET, 2013), as is the case with a tobacco smoking habit.

The time of exposure to tobacco influences the development of colorectal mucosal lesions (CARR et al., 2018). In this sense, the choice of protocol used by Laranjeira et al. (1998) was because he found macroscopic lesions from the 15th week after DMH administration, which represents sufficient time to allow the action of the elements
present in cigarette smoke for the development of colorectal neoplastic lesions, mimicking a considerably long smoking habit when compared to the lifetime of a human.

The numbers of lesions found in this study were variable in the two groups studied. Using DMH as an inducer of colorectal carcinogenesis, Laranjeira et al. (1998) found nine lesions at most in the colorectal mucosa, similar of the results found in our study, which makes feasible the experimental model applied to studies aimed at smokers who develop sporadic colorectal cancer, with respect to pathogenesis, morphology and biomolecular changes, along with survival and prognostic studies and treatment strategies.

The present study showed that malignant lesions were predominant in both the group exposed to cigarette smoke and the non-exposed group. However, in the group of animals exposed to cigarette smoke, no benign neoplasms were observed, suggesting a possible influence of cigarette components on neoplastic differentiation during carcinogenesis. As the first lesions observed after application of DMH are aberrant crypt foci (PARK; GOODLAD & WRIGTH, 1997), in addition to the abundant source of carcinogenic and genotoxic compounds found in cigarettes (HECHT, 2013), it is likely that exposure to cigarette smoke has also led to the induction of DNA changes and the failure of mucosal cell repair systems (CHEN et al., 2010), causing the development of dysplasias or hyperplasias and resulting in the formation of malignant neoplasia.

The inflammation observed in the experimental groups ranged from mild to severe. Regarding the effects of tobacco smoking and nicotine on inflammation of the large intestine, Cabral and Barbosa (2014) showed in their review that animal models used to analyse the effect of tobacco on the level of healthy intestinal mucosa reached a consensus that the use of tobacco and nicotinic exposure does not cause histological or macroscopic damage or inflammation in the intestinal mucosa; therefore, in general, tobacco has favourable effects on the colon, with most of the effects being of "tolerogenic" nature.

Inflammation is considered a critical point in the development of colorectal cancer since ulcerative colitis and inflammatory bowel disease are associated with the development of this neoplastic type, as there are correlations between the length and duration of inflammation and the risk of cancer (ULLMAN & ITZKOWITZ, 2011). Thus, some studies have been developed to control inflammation, and despite the need for more
research, it is now known that macrophage depletion can be sought as a therapy in the case of smoke-induced intestinal inflammation (LIM et al., 2018).

However, in relation to tobacco, inflammation and cancer, published data suggest that there is no consensus on the role of cigarette smoke in the development of colorectal neoplasia associated with inflammation (POHL; HOMBACH & KRUIS, 2000). Thus, in our study, we observed that tumours found in the group exposed to cigarette smoke were mainly related to the action of DMH and that a smoking habit itself did not significantly alter tumour induction.

The results showed that exposure to cigarette smoke was associated with absence and fewer mitosis figures per field, while the control group tended to have a higher number of mitosis figures. The mitotic activity in the final stage of the adenoma-carcinoma sequence remained elevated in human colorectal cancer samples, amplifying the production of transformed cells for further progression and development of invasive colorectal cancer (KOHOUTOVA; PEJCHAL & BURES, 2018). However, exposure to cigarette smoke was expected to increase the mitotic rate, as occurred in experimentally induced lung cancer (HUSARI et al., 2017). Nevertheless, it is inferable that tobacco has tolerogenic effects on the colon (CABRAL & BARBOSA, 2014), which could explain the tendency to the absence of mitosis figures in malignant neoplasms in this study.

The exposure of the animals in this study to cigarette smoke reflected in the high frequency of necrosis in the samples of malignant neoplasms, compared to the control group. Morphologically necrosis is characterized by membranous swelling of the organelles and degradation of DNA (KOHOUTOVA; PEJCHAL & BURES, 2018). Moreover, in colorectal cancer necroptosis, programmed necrosis induced by deficiency or inhibition of caspase 8 and stimulation of TNFα (VERCAMMEN et al., 1998), seems to favor the elimination of colorectal neoplasm, because the negative regulation of the necroptotic signaling pathway is related to a poor prognosis in colorectal cancer (LI et al., 2017). Thus, it is inferable that exposure to cigarette smoke favors cell death in colorectal malignant neoplasms. In addition, the percentage of necrosis in pulmonary metastases from colorectal cancer in humans was significantly higher in patients with positive smoking status (SUZUKI et al., 2019), corroborating the results found in this study.

In our study, malignant neoplasms of animals exposed and not exposed to cigarette smoke were moderately to poorly differentiated. According to Fleming et al. (2012), in human colorectal tumours, approximately 70% of colorectal adenocarcinomas
are diagnosed as moderately differentiated, followed by poorly differentiated (20%) and well differentiated (10%). We observed that the tumours found in this study followed this dynamic, contributing even more to the application of this model in studies related to tobacco smoking and colorectal cancer.

The histopathological characteristics evaluated in this experiment were important for the diagnosis of the lesions produced by the carcinogenic induction with DMH. In addition, the histopathological characteristics can be used to stratify the samples in prognostic and survival studies. It is important to emphasize that these characteristics were based on histopathological presentations of human colorectal cancer, such as those described in the review by Fleming et al. (2012), which favours the application and comparison of experimental versus human models. However, as we worked with the experimental model of colorectal cancer, we sought to limit our classification according to the descriptions of lesions in colorectal mucosa reported by Perse and Cerar (2010) in their review on the histopathological features found in these models.

**CONCLUSION**

We conclude that the experimental model of colorectal cancer exposed to cigarette smoking used in this study showed advantages, such as a considerable number of lesions, which were predominantly malignant, along with the possibility of studying the modulation of inflammation, the mitotic and necrosis aspects and the presence of histopathological characteristics compatible with human colorectal cancer, including its possible applications in prognostic and survival studies. Also noteworthy is the possibility of molecular and therapeutic response studies. In addition, we did not observe noteworthy clinical changes or the loss of animals due to neoplasia or smoking habit that would make such a study impossible.

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