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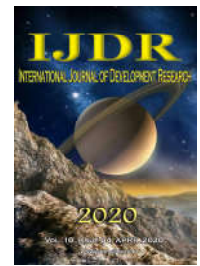
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RESEARCH ARTICLE

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## SPIRITIST “PASSE” (SPIRITUAL HEALING) HAS NO EFFECTS IN A CARCINOGENESIS *DROSOPHILA MELANOGASTER* ASSAY

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### ABSTRACT

**Background:** Spiritist “passe” is a type of Laying on of hands (LOH) commonly adopted in Latin America countries and there are no studies investigating this practice in animal models. **Objective:** Evaluate the effects of Spiritist “passe” (spiritual healing) in the animal model *Drosophila melanogaster* by using Epithelial Tumor Test. **Methods:** *Drosophila* larvae received treatment with mitomycin C (MMC) and they were randomly divided into four experimental groups: flies treated with MMC that received Spiritist “passe” (spiritual healing) group; that were submit a LOH laymen group; flies treated with MMC without LOH and that did not receive treatment. The Spiritist “passe” effects were evaluated for the ability to decrease the frequency and density of epithelial tumor in *D. melanogaster* treated with MMC. **Results:** The Spiritist “passe” was not able to decrease the total number of tumors in flies ( $P>0.05$ ) compared with positive control. No protector or/and inhibitor mechanism of MMC action was observed in any category analyzed. **Conclusion:** Spiritist “passe” was not able to decrease the tumor number in fruit fly carcinogenesis model and further studies comparing different animal models are necessary.

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### INTRODUCTION

Complementary and Alternative Medicine (CAM) refers to non-traditional practices and treatments that are not considered part of conventional medicine (Zollman, 2017). This type of practice is widely used in many countries, with an estimated 25-62% of the population utilizing at least one type of CAM (Singh, 2004; Hanyu, 2002; Falci, 2016) and in United States at least 13% use spiritual healing or prayer and 1% use energy replacement therapies (Hanyu, 2002). The use of CAM in the biofield modality category, which uses actions in the bioenergetic field, which supposedly involve and interpenetrate the human body (True, 2003) is very common in Spiritism, the third largest religion in Brazil (Lucchetti, 2016). Among the therapies proposed by the Spiritist Doctrine, the following stand out: prayer, fluid therapy (water treatment supposedly magnetized by the spirits), “disobsession” treatments, spiritual education and Spiritist “passe”. According to Spiritism, “passe” is defined as “a transfusion of energies

that alter the cellular field” and “transmission of psychic energy” (Lucchetti, 2011). Clinical studies conducted regarding the possible therapeutic effect of the Spiritist “passe” have shown a reduction in pain, muscle tension, anxiety and increased well-being (de Souza Cavalcante, 2016; Carneiro, 2019; Carneiro, 2017; Carneiro, 2018). Lucchetti *et al.* (2013) demonstrated that the Spiritist “passe” was able to inhibit the growth of bacterial cultures. There are no publications in animal models that investigate the therapeutic effects of this CAM modality. Although not as complex as human physiology, animal models are an excellent tool for broadening knowledge about the biological processes of human diseases. Among these models, the *Drosophila melanogaster* fly stands out for its short life cycle, low maintenance cost, simple genome and easy manipulation (Duffy, 2002). In addition, it is estimated that 75% of disease-related human genes have functional *D. melanogaster* orthologs, as well as 70% of cancer gene (Lloyd, 2010; Reiter, 2001).

Epithelial Tumor Test in flies is a fast tool for screening of novel compounds with carcinogenic and anti-carcinogenic activity (Orsolin, 2012). Among the reasons that population search complementary Spiritist therapies, the most commonly health problems related are depression and cancer (Lucchetti, 2016). In this context, the aim of this work was to evaluate the effects of Spiritist “passe” (spiritual healing) in the animal model *D. melanogaster* by using Epithelial Tumor Test.

## MATERIALS AND METHODS

**Religious involvement:** All the participants answered a scale of religiosity. The religiousness was assessed using the Duke University Religion Index (DUREL) (Koenig, 2010) in its validated Portuguese version (P-DUREL) for Lucchetti *et al.* (2012). P-DUREL is a five-item measure of religious involvement, which yields three subscales: (1) Organizational religious behavior (1 item), (2) Nonorganizational religious behavior (1 item), and (3) Intrinsic religious motivation (3 items). The study was evaluated and approved by Ethical Comitê (CAAE: 33014414.9.0000.5152) at UFU and all participants signed a consent form.

**Chemical Agents:** Mitomycin C (CAS 50-07-7) as lyophilized powder was produced by Kyowa Hakko Kirin Co. Ltda. Shizuoka (Japan), packaged by Squibb S.r.l Sermoneta-Latina-Itália and imported by Bristol-Myers Squibb Farmacêutica S.A.

***Drosophila* stocks, crossing and mitomycin C treatment:** One mutant strain was used in this test: *warts* (*wts*, 3-100) from Bloomington *Drosophila* Stock Center of the University of Indiana, USA: (BL# 7052). The *warts* stock has the marker *wts* gene at chromosome 3, maintained in hemizygous in the presence of balancer chromosome named *TM3, Sb<sup>1</sup>*. The wild condition of the *wts* gene acts as tumor suppressor gene. The deletion of this gene, or expression of the recessive allele, leads to cellular clones formation highly invasive, that causes epithelial tumor in the fly body and appendices. The wild type Canton S strain was used in crossing to remove the balancer chromosome *TM3*. Flies used in this experiment were maintained in the incubator at 25°C with a light:dark cycle of 12:12 h in standard Bloomington's medium. The crossing was performed between *CantonS* males and virgin *wts* females *wts* [3-17]/*TM3, S B* (Zollman, 2017). Two different progenies are obtained from this crossing. The progeny with *Tm3, Sb<sup>1</sup>* was not analyzed, which is identified by expression of *Sb<sup>1</sup>* dominant gene in the balancer chromosome *TM3*, showing stubby hairs phenotype in the fly body. Egg collection was done during 8 hours in bottles with agar-based medium (4%) and sucrose-supplement yeast. After 72 ± 4 hours, third stage larvae were washed with ultrapure water Milli-Q (Millipore) and collected using fine mesh sieve. The larvae were transferred to vials (2.5 cm diameter by 8 cm length) containing medium with 1.5g of mashed potatoes (Yoki® Alimentos S.A) and 0.1 mM of mitomycin C dissolved in 5 mL of ultrapure water. The larvae L3 were exposed to mitomycin C for 8 hours. Then they were washed with ultrapure water and transferred for new vials with fresh medium. Toxicity was measured according to rate of flies that completed metamorphosis process (Figure 1). Ultrapure water was used as negative control and 0,1 mM mitomycin C (MMC) as positive control. The employed mitomycin C concentration was based in studies of mitotic recombination in *Drosophila*

*melanogaster* (Tsuda, 1987) and carcinogenicity assays (Orsolin, 2012; Orsolin, 2009).

## Experimental groups and Spiritist “passe” (spiritual healing) treatment

### Four different groups were employed to our study:

- Flies treated with MMC that received Spiritist “passe” (spiritual healing) group: According to Spiritism, “passe” is defined as a “transfusion of energy which changes the cellular field” and “a transfusion of psychic energy”. Spiritist healers undergo a course of two years in which they learn the fundamentals of the application of “passes” with the aim of curing individuals through practical classes. Having completed this course, they are authorized to act in the capacity of healers within Spiritist Center. For the present study, healers affiliated to the Spiritist Federation of Uberlândia were recruited. Inclusion criteria for healers was having at least two years of experience, aged older than 18 years and having taken part in courses accredited by the institution.
- Flies treated with MMC that were submit a Laying on of hands (LOH) laymen group: The group was randomly selected from students of Federal University of Uberlândia. Inclusion criteria for this group was adults (older than 18 years of age), not belonging to the Spiritism religion, and no prior knowledge of LOH techniques such as Reiki, Johrei or Therapeutic Touch.
- Flies treated with MMC without LOH or Spiritist “passe” (Positive control).
- Flies that did not receive treatment (Negative control).

After the *Drosophila* larvae treatment with mitomycin C and transference to new vials with fresh food, the vials were randomly divided into the four experimental groups: flies treated with MMC that received Spiritist “passe” (spiritual healing) group; flies treated with MMC that were submit a Laying on of hands (LOH) laymen group; flies treated with MMC without LOH (Positive control); flies that did not receive treatment (Negative control). Spiritist “passe” (spiritual healing) and Laying on of hands (LOH) treatments occurred for five consecutive days (Figure 1). They took place for 5 to 10 minutes with a distance of 10-15 cm from the vials containing the *Drosophilas*. Spiritist healers carried out a 5-minute preparation of prayer and “Gospel according to Spiritism” reading, before applying the treatment. Both groups applied the Spiritist “passe” (spiritual healing) or Laying on of hands (LOH) with the intention of curing and reducing tumors.

**Fly fixation and epithelial tumor analysis:** To epithelial tumor analysis, adult flies emerged from the crossing *Canton Sx wts+/TM3, Sb<sup>1</sup>* were fixed in 70% (v/v) ethanol and analyzed under stereo microscope (Bel® Photonics) in petri dishes with glycerin (Figure 1). Analysis was based on tumor count according with the description of Justice (1995). Results were registered in a standard diagram expressing the number of tumors observed in each part of flies' body: eyes, head, body, wings, legs and halteres. This was a blind analysis; researchers were not aware of which treatment the analyzed flies had received.

**Statistical analysis:** The frequency of fly with tumors and the frequency of tumors per appendix of fly in each treatment

series were compared with the negative control to evaluate tumor induction effects. Statistical differences between epithelial tumors frequency at tested concentrations and negative and positive controls were determined by Mann-Whitney U test, nonparametric, using  $P < 0.05$  as significance threshold. Statistical comparisons referring to fly survival test exposed to Spiritist "passe" (spiritual healing) and controls were performed by Chi-square test for independent samples.

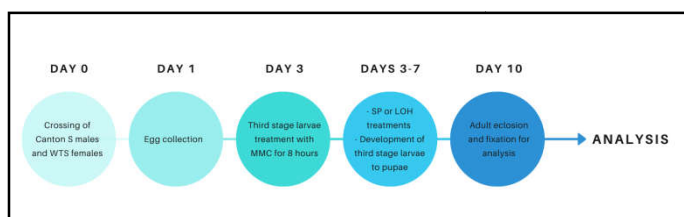
## RESULTS

**Characteristic of participants:** A total of five Spiritist healers and five individuals for the LOH group were selected. The mean age of subjects in the Spiritist healer group was 53.20 (SD: 17.08) years. In LOH subject group the mean age was 23.00 (SD: 3.00) years ( $P = 0.0159$  compared to Spiritist healer). Related to gender, the subjects were three female and two males either in LOH and Spiritist healer group.

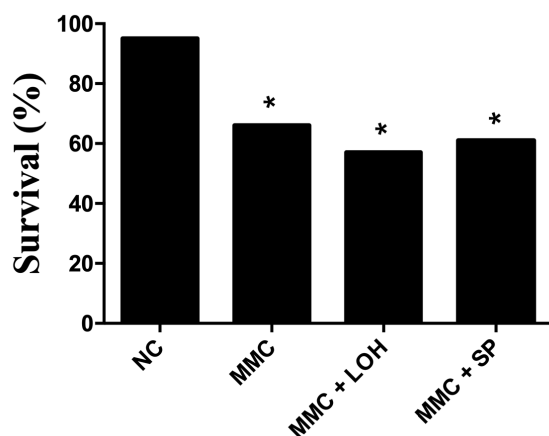
**Religious involvement of participants:** The Duke Religious Index scales was applied to all subjects to measure of religious involvement. Although the score was higher in Spiritist healer group there was no significant difference compared to LOH (Table 1).

**Table 1. Means, SD for Duke Religious Index scales (OR Organizational religiosity, NOR No organizational religiosity, LOH laying on of hands).**

Scale	LOH		Spiritist "passe"		P
	Mean	SD	Mean	SD	
OR	4.6	2.074	6	0	NS
NOR	4.4	2.074	5.8	0.447	NS
Intrinsic	11	4.743	15	0	NS
TOTAL	20	8.775	26.8	0.447	NS



**Figure 1. Timeline of the treatment steps and developmental phase of the *Drosophila melanogaster* progenies**



\*Statistically significant difference ( $P < 0.05$ ) according to the chi-square test for independent sample ratios compared to negative control.

**Figure 2. Survival rate of the *Drosophila melanogaster* progenies resulting from the CantonS x *wts/TM3, sb1* crosses. NC: Ultrapure water (negative control); MMC: 0.1 mM mitomycin C (positive control); LOH: Laying on of hands; SP: Spiritist "passe"**

## Epithelial Tumor Test (ETT)

**Effects of Spiritist "passe" (spiritual healing) in toxicity induced by mitomycin C:** The MMC treatment induced toxicity in all sample tested ( $P < 0.05$  compared to negative control). The Spiritist "passe" was not able to modulate toxicity of MMC in flies ( $P > 0.05$  compared to positive control). These data indicated the absence of protector effects of the Spiritist "passe" in toxicity promoted by MMC (Figure 2).

**Effects of Spiritist "passe" in tumor number in *D. melanogaster*:** Firstly, the capacity of MMC to induce epithelial tumor was measured. Flies treated with this compound had significant higher number of tumor ( $P < 0.05$ ) compared to negative control (water). This data showed MMC was efficient to induce tumor in this carcinogenesis model (Table 2). After validation of the model, the Spiritist "passe" effects were evaluated for the ability to decrease the frequency and density of epithelial tumor in *D. melanogaster* treated with MMC. The Spiritist "passe" was not able to decrease the total number of tumors in flies ( $P > 0.05$ ) compared with positive control. No protector or/and inhibitor mechanism of MMC action was observed in any category analyzed (flies with 3-5 or more than 5 tumors) when compared to positive control (MMC), except in 1-2 tumors category which Spiritist "passe" showed significant difference ( $P < 0.05$ ) compared to MCC, and no significant difference ( $P > 0.05$ ) compared to negative control (water) (Table 2).

## DISCUSSION

The use of alternative therapies in medical field has been increasing worldwide. Among these therapies, LOH therapies such as Reiki and Spiritist "passe" are the most spread. Lucchetti *et al.* (2013) published a work showing the effects of Spiritist "passe" on *Escherichia coli* bacteria growth. Nevertheless, to our knowledge there is no studies evaluating this healing treatment in a nonhuman animal. Because of this, the conception of this work was to analyse the effects of Spiritist "passe" in an animal model. This can be considered a strength of this study regarding the minimized placebo effect bias. Mitomycin C is a potent DNA intercalant and it can promote breaks in single- and double-strand during the replication process, increasing the frequency of mutations above the basal tolerable limits in cells (Iyer and Szybalski, 1963). At 0.1 mM this compound triggers recombinogenic events and it shows toxicity in *D. melanogaster* after 6 hours of exposition (Tsuda, 1987). Therefore, here we use this compound to induce epithelial tumor in flies. In this work we observed that this compound was able to increase the frequency of epithelial tumors in adults *wts/TM3, sb1* x Canton S, which larvae were treated with 0.1mM mitomycin C for 8 hours. This result showed this carcinogenesis model is running well. After validation of the model we evaluated the effects of "Spiritist" passe in reducing the frequency and density of tumors in the flies. This treatment was not able to increase the frequency of flies with no tumors. Moreover, we could observe that most of the flies treated with Spiritist "passe" were in the higher category tumor density (5 or more tumors). Based on the carcinogenesis model chosen, the treatment with Spiritist "passe" was ineffective. However, this result must be evaluated with caution. Cancer is a complex disease with multiple physical symptoms and impacts on mental health that were not accessed in this work.

**Table 2. Frequency of Tumor clones observed (density) in *Drosophila melanogaster*, heterozygous for the tumor suppressor gene *wts* treated with mitomycin C and submitted to Laying on of hands (LOH) and Spiritist “passe” (SP).**

Groups	N° of flies	Frequency of fly with tumor (number of flies with tumor)				Total of flies with tumor
		0 tumor	1-2 tumors	3-5 tumors	+ 5 tumors	
Water (Negative control)	161	0.882(142)	0.118(19) <sup>a</sup>	0.000(00)	0.000(00)	0.118(19)
MMC (positive control)	161	0.024(04) <sup>a</sup>	0.142(23) <sup>b</sup>	0.205(33) <sup>a</sup>	0.627(101) <sup>a</sup>	0.981(157) <sup>a</sup>
MMC + LOH	161	0.037(06) <sup>a</sup>	0.099(16) <sup>b</sup>	0.329(53) <sup>a</sup>	0.534(86) <sup>a</sup>	0.962(155) <sup>a</sup>
MMC + SP	161	0.024(04) <sup>a</sup>	0.049(08) <sup>a</sup>	0.217(35) <sup>a</sup>	0.708(114) <sup>a</sup>	0.981(157) <sup>a</sup>

Statistical diagnostics according to the Mann-Whitney test. Significance level ( $P \leq 0.05$ )

<sup>a</sup> Values different from the negative control.

<sup>b</sup> Values different from the positive control (MMC).

MMC, Mitomycin C (0.1 mM); Laying on of hands; SP: Spiritist “passe”

A systematic review of biofield therapies indicates moderate evidence for positive effects on acute pain in cancer patients and conflicting evidence for longer term pain, fatigue, quality of life and physiologic indicator of the relaxation response for these populations (Jain, 2010). However, there is no works evaluating specifically the impacts of Spiritist “passe” in cancer patients. Several clinical studies indicated that Spiritist “passe” had shown positive effects in reducing pain, muscle tension and anxiety and in improving the well-being in patients with other diseases. These findings added to our results indicate that future research would be necessary to evaluate Spiritist “passe” as a palliative care intervention instead of focus as a treatment for the carcinogenesis per se. It is also important to remember that in our work the treatment was conducted by healers belonging to one Brazilian Spiritist institution and we could not generalize our findings to all Spiritist “passe” treatments. We concluded that LOH by Spiritist “passe” was not able to decrease the tumor number in fruit fly carcinogenesis model. Further studies comparing different animal models are necessary.

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