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Evaluation of Effects *Baccharis Dracunculifolia* (Green Propolis) in Embryonic Development

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ABSTRACT

Propolis is a resinous substance made by bees from various plant fragments. Bees add salivary enzymes to their composition and this partially digested material is mixed with wax and used to make the beehive (MARCUCCI, 1995; BANKOVA et al., 2000). She may exhibit varied coloration, such as dark brown, greenish tones and reddish brown.

A recently discovered type in Brazil is the red propolis, originating from the mangrove plant, Dalbergia ecastophyllum (SILVA et al., 2008). However, the one that has gained international prominence is the one produced from the plant Baccharis dracunculifolia (Asteraceae), the green propolis (MARCUCCI, BANKOVA, 1999). Although many studies have been carried out to evaluate the characteristics of several propolis samples, none of them confirmed or allowed any relationship between the chemical composition of propolis and its therapeutic efficacy (BANKOVA, 2005a).

Due to the importance of the biological properties of green propolis, in addition to its wide use. This study aimed to add existing data on the use of propolis during pregnancy, especially warning about the possible risks of using green propolis (Baccharis dracunculifolia) during this period.

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METHODOLOGY

Embryonic hatchlings of the Gallus gallus domesticus species of the Ross lineage (n = 120), of medium standard size (approximately 55g), fertilized and free of specific pathogens, were given within a period of up to 12 hours after laying. After storage in a cold room (19-20 ° C), the eggs were incubated in an oven at 37.5 ° C equipped with forced ventilation and digitized moisture control (33 %). After 48 hours of incubation (HAMBURGER; HAMILTON, 1951). The experiments were carried out at the Laboratory of Toxicity and Cellular Communication (CB - UFPE).

Vasculogenesis assay in the chicken embryo vitelline vesicle was performed from the green propolis extract (GPE), Baccharis dracunculifolia, with Registration without Ministry of Agriculture SIF / DIPOA under nº 0029/3733 produced by Apis Flora® - lot 0490111 / to evaluate the activity of blood vessel formation through the yolk vesicle method of the chicken embryo, adapted from the chorioallantoic membrane assay, which has been used to evaluate angiogenic activity.

The embryos were divided into two groups equally (n = 10): treated with EPV at the concentration 1: 5, and the control that received 0.09 % saline solution. Afterwards, the embryos were re-incubated in the controlled oven for 120 hours and then dissected for vasculogenesis assays.

An evaluation of the response was based on counting, macroscopic analysis and morphometry of the blood vessels after seven days of incubation corresponding to the total period of experimentation (MARIK et al., 2007, STOLZMANN et al., 2011).

After incubation procedures, the embryo length was measured, expressed in millimeters and collected through body length measurements in the definitive segments, such as axes and cephalic - cervical - caudal flexions.

The angiogenesis assay on the chorioallantoic membrane of the chicken embryo was performed for the evaluation of the angiogenesis process using the in vivo test of the chorioallantoic membrane and performed according to the procedures described. The eggs were incubated at 37.5 °C, equipped with forced ventilation and digitized moisture control (33 %) during the seven days of experimentation. Angiogenic response was determined as a function of the number of blood vessels relative to the control (CRUM et al., 1985; TUFAN et al., 2005; SAHNI et al., 2006; MURUGESAN, 2007).

Statistical analyze were performed using means ± standard error (S.E) and obtained from independent experimental replicates. Statistical analyzes were performed using ANOVA (univariate variance analysis) using the Tukey method. The results were essential for p ≥ 0.05 values.

RESULTS AND DISCUSSION

The aim of this study was to evaluate the relationship between the group treated with green propolis extract (GPE) and the control group in the following areas: flexural (cephalic and cervical (CCV), cervical and caudal (CVC), cephalic and caudal (CCC) axes), body mass, upper and lower limb size, optical vesicle size Experimental data showed that there was interference of the green propolis extract (GPE) in the development of the embryos studied (Figure 1).

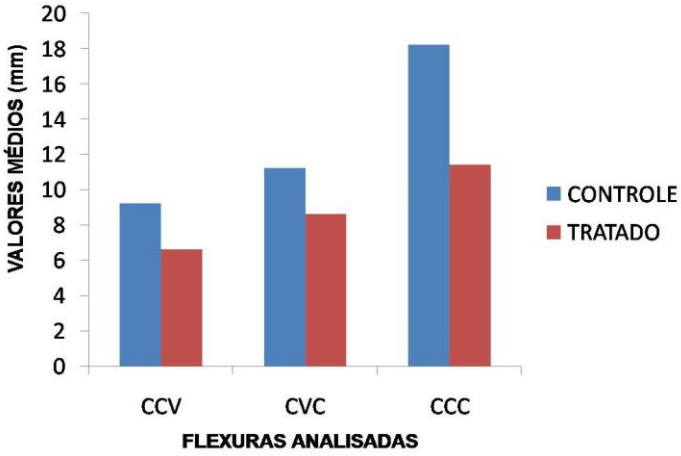

Figure 1 - Mean of the cephalic and cervical flexural measurements (CCV); cervical and caudal (CVC); cephalic and caudal (CCC) of

Gallus gallus domesticus embryos from the control and treated groups.

According to Gray, Ostby (1998), determination of age and fetal growth is important for weight size, for example. For them, how multiple measures can improve the ability to determine the degree of fetal growth (Figure 2). Embryos treated with GPE showed a significant decrease of 65 % of their body mass.

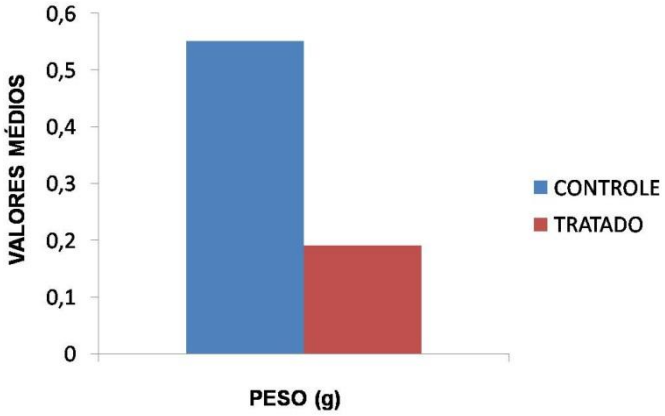

Figure 2 - Arithmetic mean of weights of *Gallus gallus domesticus* embryos from the control and treated groups.

In our experiments, it was demonstrated that the growth of the members of the treated group suffered interference of the GPE with reduction of 10 % in the upper limbs and 18 % for the lower limbs, when compared to the control (Figure 3).

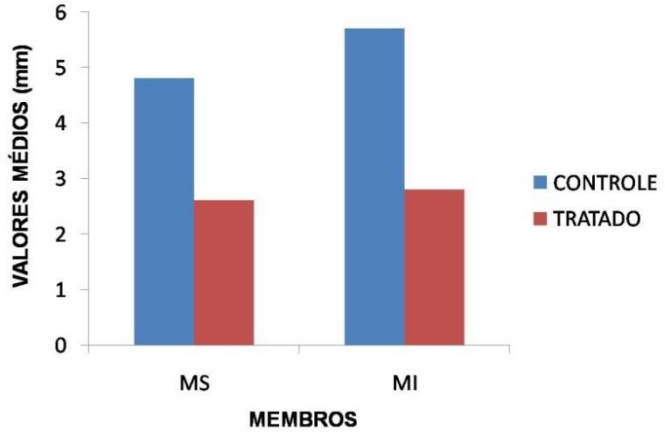

Figure 3 - Mean of the measurements of upper limbs (MS) and lower limbs (MI) of *Gallus gallus domesticus* embryos from the control and treated groups.

The initial development of the eyes show results of a series of inducing signals with tissue transformations (CARLSON, 2001). The GPE-treated embryos presented a significant reduction of the optical vesicle (OV), about 47 %, which possibly provoked, functional.

In relation to the study of the midbrain (cephalic vesicle), it was shown that the embryos treated with GPE did not present a significant difference (2 %) in the diameter of the midbrain. Already the height of this structure, a significant reduction of approximately 35 % in relation to the control.

Behrman et al. (1996) report that exposure to a potent teratogen before the 33rd day can cause serious anomalies, such as absence of limbs. Levinsohn et al. (1991) report that limb defects are caused by genetic factors. For them non-lower limb related anomalies would be associated with the aberrant arterial pattern similar to a pathogenesis of these defects. Developmental disorders occur during the formation of structures, still in the embryonic period. The disorganization caused by the morphological changes of this order is due to destructive processes (CUNNINGHAM et al., 2001).

The initial development of the eye results from a series of inducing signals with tissue transformations (CARLSON, 2001). The EPV-treated embryos showed a significant difference in the otic vesicle (VO), which may have caused anatomic and functional alterations. The formation of the optic vesicles is induced by the mesenchyme adjacent to the developing brain, probably through chemical mediators (MOORE, PERSAUD, 2008). The mechanism of action of plant drugs, due to its composition, has action on cells, tissues or organs, fighting or canceling morbid manifestations (FERRO, 2006). The EPV may interact and promote this type of response in the body.

In relation to the study of the midbrain (cephalic vesicle), it was evidenced that the embryos treated with EPV did not present a significant difference in the diameter of the midbrain (DM),

different from the height of this structure (AM). Congenital anomalies of the brain can be caused by changes in Organogenesis or Histogenesis of nerve tissue, or may result from developmental failures that occur in associated structures, such as abnormal histogenesis of the cerebral cortex causing seizures and various types of mental retardation (BEHRMAN et al., 1996).

Most developmental processes depend on the coordinated and precise interaction of genetic and environmental factors (ENGLAND, 1990; WOLPERT, 1991). Possibly the EPV, which is an environmental agent, is favoring these responses, which does not seem to alter the geometry of the midbrain. However, the height of this vesicle may have caused lesion in the histology, favoring the compromise of this system.

Embryonic development is, in essence, a process of growth and increasing structural complexity and function (MOORE; PERSUAD, 2008). In the liver of the treated animals, it was evidenced that the EPV promoted a decrease in its size (Figure 4).

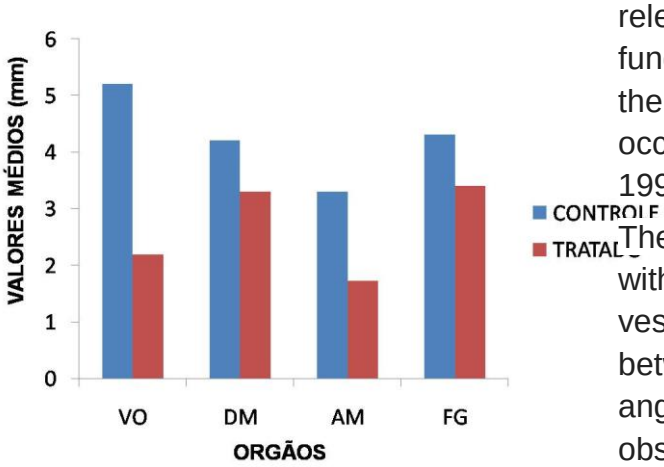

Figure 4 - Mean of the measurements of the analyzed organs: optic vesicle (VO); diameter of the midbrain (DM); height of midbrain (AM); liver (FG) of *Gallus gallus domesticus* embryos from the control and treated groups.

According to Champetier et al (1989), the liver grows rapidly and, from the 5th to the 10th week, fills a large part of the abdominal cavity. These authors also refer to the fact that the amount of oxygenated blood that has been from the umbilical vein to the liver determines liver development and functional segmentation. In view of this, probably some component of EPV acted during incubation triggering this type of response.

The analysis of vasculogenesis and angiogenesis among the studied groups showed that there was no significant difference (Table 1).

Table 1 - Results of counts of embryos with 120 hours of age, by visual analysis, between the control and treated groups with EPV at a concentration of 1: 5.

AMOSTRA	VASCULOGÊNESE	ANGIOGÊNESE
CONTROLE (veículo)	**4,3**	**22,6**
TREATMENT (1:5)	**3,6**	**15,6**

The biological properties of vessels that are relevant in embryogenesis are also fundamental for the growth of tumors, since in their vessels, most of the neovascularization occurs through angiogenesis (FOLKMAN, 1995, HENDRIX et al., 2003).

The embryos of the control group and treated with EPV present the apparently preserved vessel morphology. However, variation between 1 and 4 mm in the diameter of the angiogenic vessels in the treated group was observed in relation to the control (2 mm), which remained uniform.

The formation of blood vessels in the embryo and extra-embryonic membranes occurs during the 3rd week (HANAHAN, 1997). In this phase the cardiovascular system is established, and the response to specific growth factors or signals determines the differentiation of endothelial cells and smooth muscle cells and

the organization of these cells into tubes (DIAS et al, 2003).

In physiological angiogenesis, new vessels undergo rapid maturation and become stable, while in tumors growth is kept constant and unstable (DVORAK, 1986). Vasculogenesis is essential in the context of the development of new cellular therapies, due to the induction of vascularization, an interesting condition in the control of pathologies (HALLIDAY et al., 2002).

CONCLUSION

Therefore, in view of the present, bioactive compounds of the green propolis extract act interfering in the metabolism, functionality and morphology of the embryos, causing developmental disturbance whose mechanisms of action need to be elucidated in later studies.

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