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Effects of melatonin in rats in the initial third stage of pregnancy exposed to sub-lethal doses of herbicides

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ABSTRACT

Exposure to the herbicides Paraquat (PQ) and Roundup® may cause cell lesions due to an increase in oxidative stress levels in different biological systems, even in the reproductive system.
Objective: Evaluate the possible changes in reproductive parameters and hepatic, as well as its prevention by simultaneous application of melatonin.
Methods: Thirty-five female rats at the age of 3 months were divided into seven groups: three groups exposed to sub-lethal doses of the herbicides PQ (50 mg/kg) and Roundup® (500 mg/kg) (n = 5, G2, G3 and G4); three groups exposed to herbicides and simultaneous treatment with 10 mg/kg of Melatonin (n = 5, G5, G6 and G7) and control group (n = 5, G1) from the first to the seventh day of pregnancy. On the seventh day of pregnancy, the rats were anesthetized and euthanized, followed by laparotomy to remove their reproductive tissues and liver. Body and ovary weights were taken and the number of implantation sites, corpora lutea, preimplantation losses, implantation rates were counted and histopathology of the implantation sites, morphometry of the surface and glandular epithelia of endometrium and hepatic oxidative stress were undertaken.
Results: The present study shows the decrease in body and ovary weight, decrease in the number of implantation sites, implantation rate, in the total number of corpora lutea and increase of preimplantation percentages were observed when compared to the G1: Fig. 1 and Table 1, ($p > 0.001$ ANOVA/Tukey). The histopathological analysis of the implantation sites showed a disorder of the cytotrophoblast and cell degeneration within the blastocyst cavity in Fig. 4. Morphometry revealed a reduction in surface and glandular epithelia and in the diameter of the endometrial glands (Table 2; $p > 0.05$ ANOVA/Tukey), whereas in liver, serum levels of thiobarbituric acid reactive substances (TBARS) were found to be significantly elevated (Fig. 2; $p > 0.001$; $p > 0.05$ ANOVA/Tukey), and serum level of reduced glutathione (GSH) was significantly lower (Fig. 3; $p > 0.001$ ANOVA/Tukey). However, treatments with melatonin exhibited improvements in reproductive parameters, as well as reduced lesions in the implantation sites (Fig. 4.) and in serum levels TBARS (Fig. 2; $p > 0.001$ ANOVA/Tukey), serum levels GSH (Fig. 3; $p > 0.001$; $p > 0.05$ ANOVA/Tukey).
Conclusions: These results reveal that melatonin is a protective agent against experimentally induced maternal/embryo toxicity with herbicides and favoring normalization of reproductive parameters and hepatic.

1. Introduction

Exposure to herbicides is a serious public health problem in developing countries, especially those with economies based on agribusiness. The use of these products has grown rapidly in emerging countries, but in most cases there is no effective control over its

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sale and use. In addition, incorrect and discontinuous use of personal protective equipment, the limitations in the monitoring of exposure and the flaws in the diagnosis and treatment of cases of toxicity further aggravate the problem (Forget, 1989; IBGE, 2012).

Among some of the consequences of exposure observed in animals and humans, they are the endocrine imbalance associated with the onset of cancers, infertility, congenital malformations in the genital tract and changes in semen quality (Koifman and Hatagima, 2003), injuries in the embryo-fetal development, in the maturation of physiological systems, anatomic deficiencies, among others (Dent, 2007; Oliveira et al., 2014). Thus, changes during sensitive periods or critical development as the embryonic period can generate major changes, which can manifest itself in later stages of the life cycle or even be translocated to later generations. (Reis Filho et al., 2007). Moreover, herbicides such as Paraquat (PQ) and Glyphosate-Roundup® can promote dysfunction in the pineal gland and can reduce the production of melatonin (MLT) (Bartlett et al., 2011; Seneff et al., 2015) and thus compromise the process of embryo implantation, as well as interfere with pregnancy (Fernando and Rombauts, 2015), and produces reactive oxygen species (ROS) which triggers the lipid perioxidation of the cellular membranes (Peter et al., 1992),

The great interest in the effective treatment for humans and animals intoxicated by herbicides has focused in the impairment or minimizing of cell lesions caused in the several biological systems (Melchiorri et al., 1995; Xu et al., 2007; Serra et al., 2003) including the development of preimplantation embryos *in vivo* (Hausburg et al., 2005). The use of several compounds, especially those with antioxidant effects, such as MLT, has been underscored (Soares et al., 2008; Maganhin et al., 2008; Reiter et al., 2009). Several studies report that MLT stimulates the production of glutathione peroxidase for cell-level defense against oxidative stress (Reiter et al., 1995; Pablos et al., 1996) with the promotion of the stabilization of the cell membrane by making it stronger against oxidative attacks (Garcia et al., 1998), beneficial effect on the processes associated with the development of oocytes, ovulation and early embryonic development (Manjunatha et al., 2009; Vázqueza et al., 2010) on endometrial morphology and maintenance of embryo implantation procedure (Dair et al., 2008).

However, the literature does not register MLT effects in female rats submitted to acute toxicity by sub-lethal doses of the associated herbicides PQ and Roundup® and its protective effect on the embryo implantation process.The hypothesis tested was that MLT might act as a protective agent in the mother/embryo interface during the third stage of pregnancy, with an improvement of important pregnancy parameters. The histopathology of the implantation sites, the morphometry of surface and glandular epithelia of the endometrium, the weight of females, ovaries, the number of implantation sites, corpus luteum, implantation rates and pre-implantation losses were assessed.

2. Materials and methods

2.1. Reagents and chemical products

Commercial formulation of glyphosate (Roundup®) made of 360 g/L of glyphosate (N-phosphonomethyl glycine) and 16% (w/v) polyoxyethylene amine (surfactant), Gramoxone® with 200 g/L of PQ (1,1′-dimethyl-4.4′-bipiridinium) and MLT from Sigma-Aldrich (St. Louis, MO, USA), Dopalem® (ketamine chloral hydrate), Rompum® (xylazin) and Thionembutal® (thiopental) were employed during the experiments.

2.2. Ethical aspects

Procedures involving animals followed recommendations by the *Guidelines for the Testing of Chemicals* (OECD, 2008), and were approved by the Committee for Ethics in the use of animals of the Universidade Federal Rural de Pernambuco, Brazil, by protocol 063/2013.

2.3. Animals

The experiments were conducted in the Laboratory of Histology of the Department of Animal Morphology and Physiology (DMFA) and at the Research Center (Cenapesq) of the Universidade Federal Rural de Pernambuco. Thirty-five female rats (*Rattus norvegicus albinus*, Wistar) from the DMFA vivarium were used.

90-day-old females, weight 200 ± 20 g, were kept in a controlled cage (22 ± 2 °C, humidity 60 ± 10% and photoperiod of 12 h light/dark) with food and water *ad libitum*. After 10 days of acclimatization, the females underwent standard vaginal smear to determine the regularity of the estrous cycle. Females with 3 regular estrous cycles were separated randomly and mated to form seven experimental groups ($n = 5$ each). They were then submitted to treatments and monitored daily with regard to body weight and survival.

2.4. Mating system and verification of copula

The mating system was temporary polygamous, in which a male was maintained in a cage with two females until each mating was verified and was removed afterwards. After mating, a vaginal smear was made every day. The presence of a vaginal plug or sperm cells in the vaginal smear was taken as an indication of effective copulation. Cytological detection of sperm cells was performed daily by the same collector. The collection of vaginal smears was performed using swabs of sterile cotton (Absorve®) and subsequent deposit of biological material in histological slides, which were stained with Harris-Shorr method (Shorr, 1941), then the slides were preserved under glass cover slips using Entellan® mounting medium (EMS, Hatfield, PA, USA). All stained slides were examined using a Leica® DM500 light microscope (Leica®, Wetzlar, Germany).

2.5. Experimental groups

The experimental groups comprised of: G1 control (treatment with saline solution 0.9% NaCl); G2 exposure to 50 mg/kg dose of PQ; G3 exposure to 500 mg/kg dose of Roundup®; G4 exposure to an associated dose of PQ and Roundup®; G5 exposure to 50 mg/kg dose of PQ plus treatment with 10 mg/kg MLT; G6 exposure to 500 mg/kg dose of Roundup® and treatment with 10 mg/kg MLT; G7 exposure to an associated dose of PQ and Roundup® plus treatment with 10 mg/kg MLT. After exposure to herbicides and treatments with MLT, the rats in their seventh day of pregnancy were anesthetized by intramuscular method with ketamine hydrochloride (80 mg/kg) and xylazine (6 mg/kg) between 9 and 10 h in the morning. They were immediately euthanized with thiopental (40 mg/kg), followed by laparotomy to remove the uterine horns with the implantation sites and the ovaries (Damasceno et al., 2002; Camargo et al., 2009). Histopathological analysis of the implantation sites was performed, coupled to morphometry of the surface and glandular epithelia of the endometrium. The weight of the female rats, ovaries, number of implantation sites, corpus luteum, implantation rate and pre-implantation loss were calculated.

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