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TOXICOLOGICAL EFFECTS OF *Erythrina mulungu* Mart. ON THE REPRODUCTIVE PERFORMANCE OF PREGNANT RATS

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Summary

The objective of the present study was to examine the possible toxicological effects of *Erythrina mulungu* Mart hydroalcoholic extract (10%w/v) in rats using reproductive toxicological models. The extract was orally administered at a dose of 1,5 g/kg of body weight, from day-1 to day-19 of gestation. The intact rat fetuses were isolated on day-19 of gestation. Results suggest that *Erythrina mulungu* Mart hydroalcoholic extract induced general changes in reproductive performance of female rats.

Key words: *Erythrina mulungu* Mart; Reproductive performance; Toxicological assays

Introduction

Erythrina mulungu Mart (Leguminosae-Papilionaceae) is a thorny plant, 10-14 m tall, with trunk coated with a thick and grooved layer of red color cork[1]. *Erythrina mulungu* popularly known as mulungu, is a medium-sized well-branched tree native to Southern and Northeastern Brazil[1]. In Brazil, it has been used in popular medicine due to its hypnotic and analgesic effects and properties and it is considered to calm agitation and other disorders of the nervous system, including insomnia and nervous cough[2,3]. The bark presents some curarizing and indolic alkaloids. Other species can be cited such as *Erytrina americana, Erytrina lysistemon, Erytrina glauca* which have been reported to hold antiviral and antibacterial activity[4]. Some studies have shown that all extracts and fractions from *Erytrina mulungu* possess significant antinociceptive and anti-inflammatory effects on animals and that the effects were independent of the opioid system[5]. The objective of the present study was to examine the possible toxicological effects of *Erythrina mulungu* Mart (*Em*) hydroalcoholic extract on rats using reproductive toxicological models.

Materials and methods

Extract preparations and phytochemical screening

The *Erythrina mulungu* Mart specimens were collected in Sorocaba city (State of São Paulo, Brazil). A voucher specimen has been deposited on the herbarium, at the University of Sorocaba (Uniso), after identification was carried out by the Botanic Institute of São Paulo (Brazil), as authenticated by Dr. Sérgio Romaniuc Neto (PQC IV, specialized in floristics/ Atlantic Forest/ Moraceae taxonomic family). The bark without petiole was dried, powdered and a hydroalcoholic (70%) extract was obtained by percolation. The drug was analyzed by thin layer chromatography (TLC), using silica gel G plate to characterize the main phytochemical compounds and chromatographic profile of alkaloidal fractions. The levels of volatile substances, total ash, dried residues and fluid extract density were determined. The extract was concentrated under reduced pressure and lyophilized. It was stored at room temperature without light and humidity until the toxicological assays were performed. The hydroalcoholic extract was freshly prepared in distilled/deionized water by oral route (p.o).

Experimental animals

Adult Swiss mice (male and female) weighing 25g to 30g and male Wistar rats weighing 160g to 200g, of both genders, were obtained and kept in the UNISO/Pharmacy School facilities according to "*The Guide for the Care and Use of Laboratory Animal*" (National Research Council 1996) and "*European Community Guidelines*" (EEC Directive of 1986; 86/609/EEC). All animals were maintained in groups (10 mice or 5 rats per cage) with food and water *ad libitum*. A twelve hour light/dark cycle and constant temperature (23 ± 1°C) were maintained. All animals were previously adapted to laboratory conditions during one week before the experiments. The study design was previously approved by the Research Ethics Committee, at the University of Sorocaba.

Doses screening

Acute Toxicity Assay (LD_{50}) and Subcronic Toxicity Assay were used to determine the dose for the reproductive assay. Acute toxicity assay was carried out according to previous studies[6]. Fifty mice (50% of each gender) were distributed into five groups (one control and four experimental, n=10 mice; five animals of each gender). Experimental groups received 0.5, 1.0, 2.5 and 5.0 g/kg/p.o. of *Erythrina mulungu* Mart hydroalcoholic extract (w/w) (n=10 mice). Control group received the vehicle (deionized water). Subcronic toxicity assay was performed with eight rats (50% of each gender), distributed into four groups (one control and three experimental, n=10). Experimental groups received 0.5, 1.0 and 2.5 g/kg/p.o. of *Erythrina mulungu* Mart hydroalcoholic extract (w/w) daily, for a period of 30 days. Control group received the

vehicle (deionized water). The following parameters were observed: weigth gain, open field assay, plus maze assay and pentobarbital sleep time assay[7,8,9,10].

Reproductive performance of female rats

Male and female (*Rattus norvegicus albinus*, Wistar) rats, age 3 to 4 months, weighting 160 to 200g, were used. In order to allow cohabitation, 2 males and 5 females were kept together overnight in plastic cages. The indicative of the first day of pregnancy was the presence of spermatozoids in the vaginal-washing smear observed at optical microscopy[11] (Vickery and Bennett 1970). Twenty pregnant females were divided into two groups (n=10). One group was exposed to 1.5 mg/Kg/po *Erythrina mulungu Mart*. The second group received deionized water (control group). Water and food were supplied ad libitum during all the experiment. The body-weight of all females was recorded daily. On the twentieth day of pregnancy, all animals were sacrificed and the ovaries were removed to count the corpora lutea. The uterus, placenta, and fetuses were also removed and the vitality of fetuses was recorded[12]. A pachymeter was used to take measurements of the following: antero-posterior and latero-lateral skull lengths; antero-posterior and latero-lateral thorax lengths, cranio-caudal length and tail length[13,14].

The fertility rate of females was calculated by using the following formulas:

$$\text{Pre-implantation losses} = \frac{\text{number of corpora lutea} - \text{number of implantations}}{\text{number of corpora lutea}}$$

$$\text{Post-implantation losses} = \frac{\text{number of implantations} - \text{number of alive fetuses}}{\text{number of implantations}}$$

Statistical analysis

The results were submitted to statistical analysis, considering a significance level of 5%. Tukey-Kramer test was used to compare experimental and control groups, considering body-weight gain, the reproductive performance of female rats (placenta and fetal weights) and all offspring morphological parameters. Chi-square test was used to evaluate changes (in percentage) of pre-implantation losses, post-implantation losses and fetuses vitality. The software used for data analysis was the GraphPad Prism 5.01.

Results

Phytochemical screening

The results of phytochemical screening of the *Erythrina mulungu* Mart indicated the presence of alkaloids, flavonoids, tannins and triterpenes and steroids. The average content of volatile substances was 6.65% and 6.66 g% total ash. The fluid extract showed an average density of 0.969 g/mL and 17.57g% dry weight.

For the alkaloidal fraction of the drug in TLC, the silica gel G adsorbent was saturated for 30 minutes, 10 cm ascendent trail and mobile phase: ethyl acetate, formic acid, glacial acetic acid and water (100:11:11:26; Merck®). Spots were visualized under UV light, at 365nm with 5% (v/v) ethanolic NP (diphenylboric acid 2-aminoethyl ester, Sigma®, USA) followed by 5% (v/v) ethanolic PEG 4000 (polyethylene glycol 4000, Synth, Brazil). A 1% rutin solution in methanol was used as standard.

Doses screening

The results of the acute toxicity assay showed that the general activity of mice was slightly reduced with a 0.5, 1.0, 2.5, 5.0 g/Kg doses of hydroalcoholic extract of *Erythrina mulungu* Mart. Piloerection of small proportion was observed with the same dose. It has not been observed any convulsions, contortions, straub tail, trembling and ataxia among other acute toxicity parameters under the dosages being studied, as well as no death was observed with any of the tested doses. No toxic effect was observed after 14 days. Based on that, 0.5, 1.0 and 2.5 g/Kg doses were selected for subcronic studies.

The results of subcronic toxicity assay showed no alterations regarding body weight gain in animals. However, our results indicated that the general activity of rats was slightly reduced with 2.5 g/Kg doses of hydroalcoholic extract of *Erythrina mulungu* Mart, in the open field and plus maze tests; the 2.5 g/Kg dose also increased the duration of pentobarbital-induced hypnosis (sleep-time). Based on that, the 1.5 g/Kg/day /po dose was selected for the reproductive studies.

Reproductive performance of female rats

Results in table 1 indicate that administration of *Erythrina mulungu* Mart during pregnancy, 1.5g/kg dose, reduced body weight gain in female, significantly increased the percentage of post-implantation losses and induced changes in placenta wet weight and body weight. However, *Erythrina mulungu* Mart did not induce statistically significant differences in the vitality of fetuses.

see Table 1.

Table 2 shows *Erythrina mulungu* Mart extract, at doses 1.5 g/Kg, caused statistically significant reductions in the external measure of morphological parameters of the fetuses. Other common anomalies such as syndactyly, cleft palate, and abnormal eyes/ears implantation were not observed.

see Table 2.

Discussion and Conclusion

Doses screening results indicated that subchronic administration of *Erythrina mulungu* Mart hydroalcoholic extract (2.5 g/kg/po) in the open field test reduced general activity in rats. Previous studies performed with *E. mulungu* Mart indicated ansiolitic activity in rats and suggest this activity might be related to the presence of alkaloids[15]. Flausino *et al*[16] isolated (+)-α-hydroxy-erysotrine, erythravine and (+)-11-α-hydroxy-erythravine from flowers of *E. mulungu* and investigated two animal models of anxiety in mice - the light-dark transition model (LDTM) and the elevated plus-maze (EPM). The results also suggested the alkaloids are responsible for the anxiolytic effects of the crude extract of *E. mulungu*. On the other side, Ribeiro *et al.*[17] observed, in models of anxiety and depression, that exposition to *Erythrina mulungu* reduced activity in the open field, however it is not related to reduction of motor activity in the animals. Results found in our studies corroborate with these authors indicating that subchronic exposition to *E. mulungu* hydroalcoholic extract, 2.5 g/kg, promotes a reduction in the general activity.

E. mulungu Mart hydroalcoholic extract (2.5 g/Kg) significantly increased sleeping time assessed with the loss of righting reflex. Vasconcelos *et al.*[18] suggested that the hydroalcoholic extract of *E. mulungu* has anticonvulsant effects only on the strychnine-induced seizure model, mentioning their possible action in glycine system and a potentiation of pentobarbital sleeping time, that might set a depressant action in the central nervous system. Rosa *et al*[19] study of the alkaloid erysothrine from the hydroalcoholic extract of flowers from *E. mulungu*, which screened for its anticonvulsant and anxiolytic actions based on neuroethological and neurochemical experiments, showed that erysothrine (0.001-10µg/mL) did not alter the GABA or glutamate synaptossomal uptake and binding. Altogether, the results described an alkaloid with anticonvulsant activity and mild anxiolytic activity that might be considered well tolerated as it does not alter the general behavior of the animals in the used doses.

Health governmental agencies and guidelines usually recommend developmental and reproductive toxicology (DART) tests for drugs destined for human use. DART studies require at least one of three segments of reproductive cycle[20]: 1) premating and mating through implantation (reproduction and fertility studies); 2) from implantation through major organogenesis (teratology and/or development toxicological studies); 3) late pregnancy and post-natal development (the perinatal/postnatal

studies). In the present study, segment 1, segment 2 and late pregnancy were observed.

In our study, *Erythrina mulungu* Mart hydroalcoholic extract (1.5 g/kg/po) performed alterations on the reproductive performance of female rats, with body weight gain reduction, increase of pre-implantation losses and reduction of fetuses and placenta weights. However, it seems these effects were not associated with maternal toxicity, once the chosen dose for the reproductive toxicology assay was below the subchronic dose administered (2.5 g/kg), what resulted in effects as reduction in the general activity (open field and plus maze) and increase of sleep time. Moreover, the administration of *E. mulungu* Mart hydroalcoholic extract promoted reduction in fetal external morphological measurements suggesting toxic activity of this drug to the conceptus when it is used during all the gestation period.

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Parameters		Control	*Erythrina mulungu* Mart 1,5 g/kg
Weight gain of pregnant rats (grams)	1 - 5 day	13.5 ± 0.07	13.6 ± 0.50
	6 - 10 day	19.0 ± 0.24	12.4 ± 0.35*
	11 - 15 day	27.5 ± 0.94	21.4 ± 0.43*
	16 – 20 day	29.5 ± 0.43	31,0 ± 0.14
Pre-implantation loss (%)		0	0
Post-implantation loss (%)		0,01	0,16*
Placenta weight (grams)		0.50 ± 0.01 (n = 59)	0.41 ± 0.02* (n = 51)
Fetuses' weight (grams)		1.95 ± 0.02 (n = 59)	1.62 ± 0.07* (n = 51)
Fetuses' vitality (%)		99.5	98

Table 1: Effects of Erythrina mulungu Mart on the reproductive performance of female rats.

N=10 female rats per group. Data are reported in percentage and mean ± S.E.M. * - p<0.05 (Chi-square test or Tukey-Kramer test).

Parameters (cm)	Control (n: 59)	*Erythrina mulungu* Mart 1,5 g/kg (n: 51)
Skull - antero-posterior	1.31 ± 0.07	1.19 ± 0.15*
Skull - latero-lateral	0.72 ± 0.05	0.67 ± 0.09
Thorax - antero-posterior	0.87 ± 0.07	0.79 ± 0.11
Thorax - latero-lateral	0.81 ± 0.06	0.72 ± 0.01*
Cranio-caudal length	2.51± 0.16	2.22 ± 0.03*
Tail length	0.89 ± 0.01	0.83 ± 0.02*

Table 2: Effects of Erythrina mulungu Mart on fetuses' external morphological parameters.

Data are reported in mean ± S.E.M. *p<0.05 (Tukey-Kramer test).