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Absence of Mutagenic Effect of *Mikania glomerata* Hydroalcoholic Extract on Adult Wistar Rats *in vivo*

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ABSTRACT

This work makes an assessment of the dominant lethality of *Mikania glomerata* in male Wistar rats. Adult male received 1 mL of *M. glomerata* hydroalcoholic extract at a dose level of 3.3 g/kg body weight for 52 days and were mated with untreated females for seven weeks (group 1) or one week prior to the beginning of treatment and on the week following the end of treatment (group 2). The parameters analyzed were: number of implanted embryos, resorptions and corpora lutea; mating, gestation, preimplantation loss, implantation and resorption indexes (group 1); number of offspring and weaning animals (group 2). The administration of *M. glomerata* did not show any impairment of fertility and no significant difference in the parameters analyzed, suggesting an absence of mutagenic effect on Wistar rats.

Key words: *Mikania glomerata*, mutagenicity, Wistar rat

INTRODUCTION

Mikania glomerata (Sprengel – Asteraceae), popularly known in Brazil as "guaco", is a plant employed in folk medicine for treating respiratory tract diseases (Neves and Sá, 1991) and for its anti-snake venom, anti-inflammatory and analgesic activities (Ruppelt et al., 1990). Phytochemical studies have revealed the presence of several substances in *M. glomerata*, including kaurenoic acid, cinnamoylgrandifloric acid, stigmasterol, flavonoids and coumarin (Cabral et al., 2001; Martins et al., 2000; Oliveira et al., 1993; Vilegas et al., 1997a; Vilegas et al., 1997b) which is the main active compound from the leaves of this species. Coumarin is a well-known liver toxicant (Born et al., 2000) with reported antifertility activity in mature female rats (Ulubelen et al., 1994) whereas flavonoids have been shown to produce antiandrogenic activity and affect male fertility in dogs (Bhargava, 1989).

There are a number of agents that can have an adverse effect on the male reproductive system, for example by interfering with sexual behavior and fertility (Kimmel et al., 1995). Since *M. glomerata* contained substances with antifertility activity, this study was designed to make an assessment of the dominant lethality of *M. glomerata* in order to verify the occurrence of mutagenic effects on male Wistar rats.

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MATERIALS AND METHODS

Animals and housing

Adult male Wistar rats (*Rattus rattus norvegicus* Berkenhout, 1769) (90 days old and weighing around 230 g) were obtained from the vivarium of Universidade Federal de Juiz de Fora (UFJF), where they were born and bred. The animals were housed individually under standard laboratory conditions, with a 12h light/12h dark photoperiod. They were fed on rat chow pellets and received water *ad libitum*. The experimental protocol was approved by the Ethical Committee of the Centro de Biologia da Reprodução (UFJF).

Plant material

M. glomerata (Sprengel) was collected in the botanical garden of the Pharmacy and Biochemistry Faculty (UFJF) and authenticated in the Herbarium Leopoldo Krieger, Department of Botany (UFJF), where a voucher specimen registered under the number CESJ 34456 is deposited. The hydroalcoholic extract was prepared using aerial parts of this plant. The aerial parts were powdered and extracted with 70 % ethanol. The solvent was evaporated in a rotavapor and the residue was dissolved in distilled water.

Treatment and mating procedure

Wistar rats were randomly divided into four groups: treatment **T1** and its control **C1** of 12 animals each, treatment **T2** and its control **C2** of 11 animals each. The rats of the treatment group received, by gavage and once daily, 1 mL of *M. glomerata* extract at a dose level of 3.3 g/kg of body weight, administered for 52 days. This period of time corresponded to the duration of the spermatogenic cycle of this species (Hilscher, 1964). The control group received 1 mL of distilled water following the same protocol as the treatment group. Each animal of groups **T1** and **C1** was mated with 2 untreated virgin females in estrus from the second to the eighth week of treatment (Green et al., 1985; Zenick et al., 1994) whereas each animal of groups **T2** and **C2** was mated with 2 untreated virgin females in estrus one week before the beginning of treatment and on the week following the end of treatment. The presence of spermatozoa in the vaginal smear indicated successful mating and was considered as day one of gestation (Gleich and Frohberg, 1977; Kato et al., 1979).

Females mated with males of groups **T1** and **C1** were killed by an overdose inhalation of anesthetic on the 15^{th} day of gestation. The following parameters were analyzed: total number of implanted embryos, number of resorptions, number of corpora lutea per pregnant female, mating index [(inseminated females/females mated) X 100], gestation index [(females with implants/inseminated females) X 100], preimplantation loss index [(corpora lutea – implantations/corpora lutea) X 100], implantation index [(implantations/ corpora lutea) X 100], resorption index [(resorptions/implantations) X 100]. Females mated with males of groups **T2** and **C2** completed the gestational period and the following parameters were analyzed: total number of offsprings, number of males and females, total number of weaning animals, number of weaning males and females. The data were analyzed using the Fisher Exact Test and the Mann-Whitney Test ($\alpha = 0.05$) (Sokal and Rohlf, 1996).

RESULTS

The administration of *M. glomerata* hydroalcoholic extract at the dose level of 3.3 g/kg of body weight for 52 days did not interfere with mating of the treated males. The mating, gestation, preimplantation loss, implantation and resorption indexes as well as the number of corpora lutea, implants and resorptions obtained from the females mated with treated males were not significantly different from the control values, with the exception of the mating index on the 6^{th} week which was significantly reduced in the treated group (Tables 1, 2 and 3). The numbers of offspring born to females mated with control and treated males one week before the beginning of treatment and on the week following the last treatment were not statistically different between the groups. The numbers of weaning females and males were also not statistically different between these groups (Table 4).

DISCUSSION

The dominant lethal test is an important method for assessment of mutagenic substances (Shively et al., 1984). In this context, mating experiments are needed to evaluate the effect of substances on

fertility, its duration of action and whether the antifertility effect is reversible (Parveen et al., 2002). The search for an effective and reversible male antifertility agent with minimum side effects remains a challenge because although a number of compounds having antifertility effects have been isolated from higher plants most of them are metabolically toxic. In a mating experiment, the presence of implantation sites in females mated with treated males is taken as a criterion of successful insemination and the fertility test is considered to be positive. If no implants are observed the test is considered to be negative (Sakar et al., 2000). Recently, many studies have showed the antifertility effects of plant extracts in rats. For instance, the administration of *Eugenia jambolana* extract (Myrtaceae) reduced the number of pregnant rats and the number of implantations (Rajasekaran et al., 1988). Similar effects were observed after treatment with *Abrus precatorius* (Leguminosae) ethanolic extract (Sinha, 1990).

M. glomerata is a plant currently used in folk medicine for its therapeutic properties. It contains active compounds, namely flavonoids and coumarin, which have been reported to affect male dog and female rat fertility, respectively, in experiments carried out using other plant genera (Bhargava, 1989; Ulubelen et al., 1994). The antifertility effect of the substances present in the *M. glomerata* extract suggested a possible role of this plant as a potential agent in the field of male fertility regulation. The administration of *M. glomerata* extract for 52 consecutive days at a dose level (3.3 g/kg of body weight) that was 600 times higher than the human dose did not reduce the libido and, consequently, did not alter the mating behavior of the treated rats. This datum was confirmed by the presence of spermatozoa in the vaginal smear and by the number of inseminated females and with implantations, which were not reduced due to treatment. The only significant alteration was observed in the mating index of females mated with treated males on the 6th week of treatment (**C1** = 87.5 %, **T1** = 33.3 %). From 2nd to 5th week and on the 7th and 8th weeks, the mating index as well as the other parameters analyzed, including those of the 6th week, did not differ between the control and treatment groups. The reduced mating index on the 6th week could be considered an isolated event, which did not result in a biologically significant adverse effect.

The treatment with *M. glomerata* extract did not show any antifertility activity as evidenced by the number of implantations and resorptions observed in the females mated with treated males, which were not significantly different from the values obtained from the females mated with untreated males. Furthermore, the numbers of female and male pups born to females mated with treated males were also similar to the control values.

Table 1 - Parameters analyzed in the dominant lethality of *Mikania glomerata* hydroalcoholic extract in females mated with male Wistar rats submitted to chronic treatment from the 2nd to the 4th week.

Parameters	Week					
	2nd		3rd		4th	
	C1	T1	C1	T1	C1	T1
Females mated	21	21	17	19	16	16
Inseminated females	8	10	9	11	5	9
Females with implants	8	7	8	11	5	9
Corpora lutea	90	74	86	121	55	108
Implantations	74	60	80	99	51	102
Resorptions	3	7	2	3	2	4
Mating index (%)	38.1	47.6	52.9	57.9	31.2	56.2 [1]
Gestation index (%)	100	70	88.9	100	100	100 [2]
Preimplantation loss index (%)	17.8	18.9	7	18.2	7.3	5.5 [3]
Implantation index (%)	82.2	81.1	93	81.8	92.7	94.4 [4]
Resorption index (%)	4	11.7	2.5	3	3.9	3.9 [5]

C1 = control, T1 = treatment. 1 – mating index = (inseminated females/females mated) X 100. 2 – gestation index = (females with implants/inseminated females) X 100. 3 – preimplantation loss index = (corpora lutea – implants/corpora lutea) X 100. 4 – implantation index = (implants/corpora lutea) X 100. 5 – resorption index = (resorptions/implants) X 100.

Table 2 - Parameters analyzed in the dominant lethality of *Mikania glomerata* hydroalcoholic extract in females mated with male Wistar rats submitted to chronic treatment from the 5th to the 8th week.

Parameters	Week							
	5th		6th		7th		8th	
	C1	T1	C1	T1	C1	T1	C1	T1
Females mated	10	12	8	12	12	12	12	12
Inseminated females	7	4	7	7	5	2	7	9
Females with implants	7	3	7	4	5	2	6	8
Corpora lutea	74	36	85	43	55	23	59	82
Implantations	73	33	83	41	50	22	55	77
Resorptions	4	1	5	2	2	0	3	4
Mating index (%)	70	33.3	87.5	33.3*	41.7	16.7	58.3	75[1]
Gestation index (%)	100	75	100	100	100	100	85.7	88.9[2]
Preimplantation loss index (%)	1.3	8.3	2.3	4.6	9.1	4.3	6.8	6.1[3]
Implantation index (%)	98.6	91.7	97.7	95.3	90.9	95.7	93.2	93.9[4]
Resorption index (%)	5.5	3	6	4.9	4	0	5.4	5.2[5]

C1 = control, T1 = treatment. 1 – mating index = (inseminated females/females mated) X 100. 2 – gestation index = (females with implants/inseminated females) X 100. 3 – preimplantation loss index = (corpora lutea–implants/corpora lutea) X 100. 4 – implantation index = (implants/corpora lutea) X 100. 5 – resorption index = (resorptions/implants) X 100. * p = 0,0281.

Table 3 - Total values obtained during the seven weeks of mating of control and *Mikania glomerata*-treated animals.

Parameters	Control	Treated
Females mated	96	104
Inseminated females	48	49
Females with implants	46	44
Corpora lutea	504	487
Implants	466	434
Resorptions	21	21

Table 4 - Males and females born and weaned of females mated with control and treated males one week before the beginning of treatment and on the week following the last treatment with *Mikania glomerata* hydroalcoholic extract.

Parameters	Before		After	
	Control	Treated	Control	Treated
Born males	43	32	66	53
Born females	50	46	55	45
Total	93	78	121	98
Weaning males	42	32	64	52
Weaning females	49	45	55	45
Total	91	77	119	97

In rats, the number of embryonic deaths can be measured by the resorption index. This correlates the total number of implants with live embryos, dead embryos, embryo remains or just the residue of an implantation. In this work, the resorption index, a reliable measure of dominant lethal mutations (Aravindakshan et al., 1985), was not significantly altered in females mated with treated males. In conclusion, despite the presence of potential mutagenic substances in its aerial parts, the *M. glomerata* extract was not genotoxic to the germinal cells of Wistar rats at the dose level used during treatment. It did not cause the death of embryos nor did it affect their development and fixation on the maternal uterus.

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RESUMO

Mikania glomerata é uma planta utilizada na medicina popular, cujas folhas possuem flavonóides e cumarina. Essas substâncias, segundo a literatura, interferem na fertilidade de cães e ratas, respectivamente. O presente trabalho faz um estudo do teste do letal dominante com *M. glomerata* em ratos Wistar. Animals adultos foram tratados com 1 mL de extrato hidroalcoólico de *M. glomerata* na dose de 3.3 g/kg de peso corporal durante 52 dias. Os animais foram acasalados com fêmeas não tratadas por sete semanas (grupo 1) ou uma semana antes do início do tratamento e na semana seguinte ao término do mesmo (grupo 2). As variáveis analisadas foram: números de embriões implantados, reabsorções e corpos lúteos, índices de acasalamento, gestação, perda pré-implantação, implantação e reabsorção (grupo 1); número de filhotes nascidos e de animais desmamados (grupo 2). A administração de M. glomerata não interferiu com a fertilidade dos animais e não foram observadas alterações significativas das variáveis analisadas, o que sugere a ausência de efeito mutagênico em ratos Wistar por parte dessa planta.

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