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SYSTEMIC USE OF *Solidago microglossa* DC IN THE CICATRIZATION OF OPEN CUTANEOUS WOUNDS IN RATS

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ABSTRACT

Many popular medicinal plants have been used indiscriminately without a solid scientific basis for their efficacy, as is the case of *Solidago microglossa* DC, popularly known as arnica-do-Brasil or simply arnica. This plant is used to treat inflammation and to accelerate the healing of wounds. In this work we examined the effect of daily intraperitoneal applications of a crude aqueous extract of arnica on the cicatrization of open cutaneous wounds in Wistar rats. Male Wistar rats were randomly allocated to a control group that received no drug, a sham group that received only the drug vehicle, and a treated group that received daily intraperitoneal injections of a saline solution of arnica (16.1 $mg.kg^{-1}$), for 7 or 14 days after excision of a dorsal cutaneous flap. The area of the lesion and the amount of collagen fibers in the wound were monitored. By the 7^{th} day of observation, there were no significant changes in the area of the lesions or in the amount of collagen fibers in the wound. However, by the 14^{th} day of treatment, there was a small but significant reduction in the area of the wound with no significant change in the amount of collagen fiber deposition. The lack of significant changes in the serum levels of alanine aminotransferase and aspartate aminotransferase indicated that *Solidago microglossa* DC was not hepatotoxic at the dose used. But the LD_{50} and LD_{100} for arnica in rats were 54.7 and 86.2 $mg.kg^{-1}$, respectively, which were just 3.4 and 5.4 times higher than that therapeutic dose (16.1 $mg.kg^{-1}$), what means that the crude aqueous extract of arnica cannot be used systemically with enough safety to avoid intoxication. The wound-healing effect of arnica must be object of further investigation using the isolated constituents of the crude extract.

Keywords: Arnica-do-Brasil, cicatrization, hepatic toxicity, phytotherapy, *Solidago microglossa* DC

INTRODUCTION

Arnica montana Linnaeus, generally referred to as arnica, is a medicinal plant popularly used to treat inflammation and to promote wound healing [9,12]. Similar effects have also been attributed to *Solidago microglossa* DC, popularly known as arnica-do-Brasil [9]. Both plants belong to the family Asteraceae and have some constituents in common [14].

The anti-inflammatory properties of *A. montana* Linnaeus, *S. microglossa* DC, and other members of the family Asteraceae, have been extensively studied [1,3,5,6,12,13]. In contrast, little is known about the wound healing properties of these plants. The local application of arnica-do-Brasil has been reported not to affect the healing of open cutaneous wounds in rats [2], whereas the topical use of arnica may cause contact dermatitis [7,10].

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Since most arnica preparations are alcoholic extracts of the plant, the inefficacy of these solutions in wound healing after topical use may be attributed to effects of the alcohol.

In this paper, we evaluated the effect of the intraperitoneal administration of an aqueous preparation of *S. microglossa* DC on cutaneous wound healing and on the serum levels of markers of hepatotoxicity in rats. The lethality (LD_{50} and LD_{100}) of Arnica in rats was also determined.

MATERIAL AND METHODS

This study was approved by the institutional Ethics Committee.

The aerial parts of *S. microglossa* DC (voucher specimen deposited under accession number 13,495 at the Institute of Biosciences of the Federal University of Uberlândia) were collected, washed in running water, and dried naturally in the shade. An infusion of triturated aerial parts was prepared with deionized water by stirring for 24 h, followed by filtration, centrifugation and lyophilization [1].

To determine the LD_{50} and LD_{100}, and to establish a safe experimental dose, 11 groups of 6 male Wistar rats each (280-

360 g) received daily intraperitoneal injections of 6.3, 15.6, 18.8, 21.9, 25.0, 28.1, 31.3, 37.5, 46.9, 62.5 or 93.8 mg.kg^{-1}, respectively, of an aqueous solution of the crude lyophilized extract of *S. microglossa* DC in sterile 0.9% NaCl, for 14 days [4,17]. Each group of rats received always the same dose during that period.

To evaluate the effect on cicatrization, 90 male Wistar rats (284-340 g), were wounded and randomly allocated to one of three groups of 30 rats each: control (group I), to evaluate the spontaneous cicatrization, sham (group II), to evaluate the effect of the arnica diluent, and treated (group III), to evaluate the effect of arnica. Each group was divided into two subgroups for observation on the 7th day (subgroup A) and 14th day (subgroup B) after wound generation.

On day zero of the experiments, each rat was anesthetized at a dose of 0.2 mL/100 g of body weight intramuscularly with a mixture of xylazine (20 mg.mL^{-1}) and ketamine (50 mg.mL^{-1}) and then positioned in ventral decubitus and immobilized on a surgical board. After dorsal trichotomy, a fragment of dorsal skin 2 cm in diameter was removed with a circular metallic punch to expose the muscular fascia and to limit the wound to the skin. The dorsal region was chosen so as to avoid possible trauma produced by the rat itself. The rats were housed individually to prevent other rats from interfering with cicatrization.

The rats in group III received daily intraperitoneal injections of the crude lyophilized extract of *S. microglossa* DC diluted in 0.9% NaCl (16.1 mg.kg^{-1}), while those in group II received daily intraperitoneal injections of 0.9% NaCl and were used as controls for the stress conditions. The rats in group I received no intraperitoneal injection and were used as controls to assess the spontaneous healing of the wound. In all cases, the rats were sacrificed after 7 days (subgroup A) and 14 days (subgroup B).

A regression line of mass *versus* the area of square pieces of paper was used to determine the area of the wound from the corresponding mass of paper cut to the exact shape of the wound.

On the 7th and 14th day after surgery, small fragments of skin were collected, fixed in 10% formaldehyde, embedded in paraffin, and sectioned (5 μm thick) with hematoxylin-eosin and Gomori trichrome to visualize collagen fibers by light microscopy. A single slide was prepared for each rat, and five microscopic fields chosen at random used to assess the presence of collagen fibers, scored as weak (+), moderate (++) and strong (+++).

Blood was collected from the orbital venous plexus using micro-hematocrit capillaries before and 7 and 14 days after surgery. The blood was allowed to clot in Eppendorf tubes for 1 h at room temperature and the serum was then obtained by centrifugation. The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercial kits and an automated analyzer (Cobas Integra 700, Roche Diagnostic Systems). Both reactions were monitored by the decrease in absorbance (A) at 340 nm as NADH + H$^+$ was consumed. Values of ΔA.min^{-1} (x) were substituted in the linear regression equations y = 1.003x - 2 and y = 1.03x - 0.6 to calculate the ALT and AST activities (y, in IU.L^{-1}), respectively.

All statistical analyses were done using the software Origin 6.0 (Microcal Software Inc.), with values of p≤0.05 indicating significance. The LD_{50} and LD_{100} values were determined by interpolation from a sigmoidal curve (Boltzmann function) of the mortality rate *versus* the dose of Arnica. The relative changes (%) in the wound areas and enzyme levels were compared using analysis of variance (ANOVA). The intensities (+, ++ and +++) of the collagen fibers in the tissue sections of the different groups were compared using the χ^2-test.

RESULTS

Figure 1 shows the mortality associated with different doses of *S. microglossa* DC. Using this dose-response curve, the LD_{50} and LD_{100} were calculated to be 54.7 ± 0.3 and 86.2 mg.kg^{-1}, respectively. No deaths were seen with doses up to 40 mg.kg^{-1}.

Table 1 shows the decrease in the wound areas in all groups of rats on the 7th and 14th day of observation compared to the day before surgery. There was a small but significant decrease in the area of the lesion in group III compared to groups I and II on the 14th day of observation, but not on the 7th day. There were no significant changes in the serum levels of ALT and AST between any of the groups.

Table 2 shows the occurrence of collagen fibers in the cicatrix fragments analyzed.

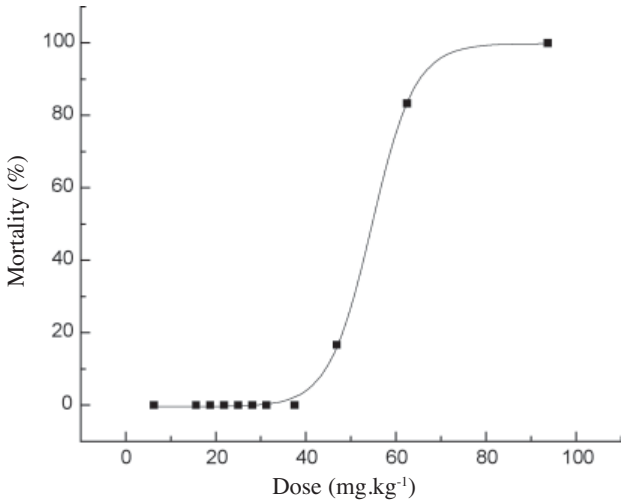

Figure 1. Mortality of rats treated with an aqueous extract of *S. microglossa* DC. The LD_{50} and LD_{100} for rats, determined by interpolation using the Boltzmann function, were 54.7 and 86.2 mg.kg^{-1}, respectively.

DISCUSSION

Arnica is widely used in oral and topical pharmaceutical formulations [1,12, 15]. In this study, we used an aqueous extract of arnica to avoid the interference of ethanol in cicatrization. The topic use of arnica was avoided here because of its ability to cause contact dermatitis [7,10]. Intraperitoneal administration was used to guarantee the absorption

Table 1. The decrease in wound area and the change in serum ALT and AST levels, on the 7th and 14th day of observation compared to day zero of the experiment.

Parameter	7th day			14th day		
	I	II	III	I	II	III
Decrease in area (%)*	73.5 ± 6.4(n = 15)	77.8 ± 6.7(n = 15)	76.1 ± 6.0(n = 15)	95.7 ± 2.0(n = 15)	94.1 ± 7.0(n = 15)	98.1 ± 0.9(n = 15)
Change in ALT (%)**	12.27 ± 24.15	19.28 ± 19.93	-0.82 ± 26.33	3.98 ± 20.87	14.93 ± 59.06	-10.16 ± 17.53
Change in AST (%)**	-0.10 ± 35.18	1.90 ± 20.98	-13.05 ± 53.96	11.73 ± 32.63	6.23 ± 26.69	18.98 ± 35.69

The values are the mean ± SD of n rats. *Differences among groups were significant ($p<0.05$) on the 14th but not on the 7th day of observation. **Differences among groups were not significant ($p>0.05$) on the 7th and 14th day of observation.

Table 2. Densities of the collagen fibers in cicatrix fragments of rats. The values indicate the numbers of rats in each category of density.

Intensity	Group I		Group II		Group III	
	7th day	14th day	7th day	14th day	7th day	14th day
+	15	0	15	1	15	0
++	0	11	0	8	0	7
+++	0	4	0	4	0	8

There were no significant differences among the groups ($p>0.05$, χ^2 test). +: weak; ++: moderate; +++: strong.

of the drug, since rats reject chow containing arnica.

As shown by Figure 1, the dose of arnica used here was considerably below the LD_{50} for the extract.

The area of a lesion during cicatrization is often estimated mathematically using the expression $\pi.r_L.r_S$, which requires measurement of the largest (r_L) and smallest (r_S) radii of the lesion. As the borders of the lesion become very irregular during cicatrization, those measurements will not represent the true area of the lesion. Estimation of the area using the approach described here yields more reliable data.

The slight but significant decrease in lesion area in group III rats compared to group I and II rats, by the 14th day of observation (Table 1), was real and indicated that *S. microglossa* DC may contain a chemical constituent with wound-healing property. However, this conclusion was not supported by the levels of collagen fibers in cicatrix fragments of group III compared to the other groups (Table 2). In addition, an earlier report showed that the topical use of an alcoholic crude extract of *S. microglossa* DC [2] had no effect on cicatrization. This lack of effect could be attributable to the antagonistic actions of other components of the preparation. Thus, an accelerator effect of arnica in cicatrization could be antagonized by the local irritation of the wound by ethanol.

The lack of significant changes in ALT and AST activities among the groups indicated that arnica was not hepatotoxic at the concentration used here. This finding agrees with the protective effect of arnica against the hepatotoxicity caused by carbon tetrachloride [8].

The LD_{50} and LD_{100} were 3.4 and 5.4 times higher, respectively, than the therapeutic dose known to be effective in wound healing (16.1 $mg.kg^{-1}$). This lethality may be related to the cytotoxicity [16] and genotoxicity [11] reported for sesquiterpene lactones present in several species of the family Asteraceae. The close proximity between the lethal and therapeutic doses indicates that the crude plant extract cannot be used systemically for cicatrization or to combat inflammation without further purification of its active components and detailed characterization of their actions.

In conclusion, daily intraperitoneal injections with a crude aqueous extract of *S. microglossa* DC (16.1 $mg.kg^{-1}$) for 14 days produced a small but significant decrease in the area of open cutaneous wounds in rats;

there was no significant change in the amount of collagen fibers in the cicatrix. At this dose, there were no significant changes in the blood levels of the hepatic enzymes AST and ALT, but the therapeutic dose of the extract is too close to the lethal doses to be used systemically with enough safety to avoid intoxication. These results indicate that the wound-healing effect of arnica must be object of further investigation using the isolated constituents of the crude extract.

REFERENCES

1. Cerqueira MBS, Souza JT, Amado Jr. R, Peixoto ABF (1987) Ação analgésica do extrato bruto aqüoso liofilizado do caule e folhas da *Lychnophora ericoides Mart* (arnica). *Cienc. Cult.* **39**, 551-553.
2. Contrera MGD, Lopes RA, Pozetti GL, Bernardi AC, Cabrera A (1985) Ação da tintura mãe de *Lichnophora ericoides*, *Aristolochia esperanzae* e *Solidago microglossa*, em feridas cutâneas do rato. *Rev. Esc. Farm. Odont.* **8**, 13-17.
3. Gertsch J, Sticher O, Schmidt T, Heilmann J (2003) Influence of helenanolide-type sesquiterpene lactones on gene transcription profiles in Jurkat T cells and human peripheral blood cells: anti-inflammatory and cytotoxic effects. *Biochem. Pharmacol.* **66**, 2141-2153.
4. Lorke D (1983) A new approach to practical acute toxicity testing. *Arch. Toxicol.* **54**, 275-287.
5. Lyss G, Knorre A, Schmidt TJ, Pahl HL, Merfort I (1998) The anti-inflammatory sesquiterpene lactone helenalin inhibits the transcription factor NF-kappaB by directly targeting p65. *J. Biol. Chem.* **273**, 33508-33516.
6. Lyss G, Schmidt TJ, Merfort I, Pahl HL (1997) Helenalin, an anti-inflammatory sesquiterpene lactone from arnica, selectively inhibits transcription factor NF-κB. *Biol. Chem.* **378**, 951-961.
7. Machet L, Vaillant, L, Callens A, Demasure M, Barruet K, Lorette G (1993) Allergic contact dermatitis from sunflower (*Helianthus annuus*) with cross-sensitivity to Arnica. *Contact Dermatitis* **28**, 184-185.
8. Marchishin SM (1983) Efficacy of the phenol compounds of Arnica in toxic lesion of liver. *Farmakol. Toksikol.* **46**, 102-106.
9. Pio Corrêa M, Penna LA (1984) *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. Ministério da Agricultura: Rio de Janeiro.
10. Pirker C, Moslinger T, Koller DY, Gotz M, Jarisch R (1992) Cross-reactivity with *Tagetes* in Arnica contact eczema. *Contact Dermatitis* **26**, 217-219.
11. Rivera IG, Martins MT, Sanchez PS, Sato MIZ, Coelho MCL, Akisue M, Akisue G (1994) Genotoxicity assessment through the Ames test of medicinal plants commonly used in Brazil. *Environ. Toxicol. Water Qual.* **9**, 87-93.
12. Sancin P, Lombard A, Rossetti V, Buffa M, Borgarello E (1981) Evaluation of tinctures of *Arnica montana* L. roots. *Acta Pharm. Iugosl.* **31**, 177-183.
13. Schröder H, Lösche W, Strobach H, Leven W, Willuhn G, Till U, Schör K (1990) Helenalin and 11α,13-dihydrohelenalin, two constituents from *Arnica montana L.*, inhibit human platelet function via thiol-dependent pathways. *Thromb. Res.* **57**, 839-845.
14. Torres LMB (1985) *Estudo químico da espécie Solidago microglossa* DC. Instituto de Química da Universidade de São Paulo: São Paulo.
15. Wagner S, Suter A, Merfort I (2004) Skin penetration studies of arnica preparations and of their sesquiterpene lactones. *Planta Med.* **70**, 897-903.
16. Woerdenbag HJ, Merfort I, Paβreiter CM, Schmidt TJ, Willuhn G, van Uden W, Pras N, Kampinga HH, Konings AW (1994) Cytotoxicity of flavonoids and sesquiterpene lactones from arnica species against the GLC_4 and the COLO 320 cell lines. *Planta Med.* **60**, 434-437.
17. Zbinden G, Flury-Roversi M (1981) Significance of LD_{50} - test for the toxicological evaluation of chemical substances. *Arch. Toxicol.* **47**, 77-99.

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