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Antiinflammatory and antioxidant activity of aqueous and ethanolic extracts of spilanthes acmella

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ABSTRACT

Spilanthes acmella (Syn.Blainvella acmella) belonging to the family Compositae was extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, stimulant and sialagogue for stammering, tongue paralysis, stomatitis, toothache, throat and gum infections etc. The aqueous (SPA) and ethanolic extracts (SPE) of leaves were screened for its antinflammatory(both acute and chronic models)and antioxidant activity(Ferric thiocynate method and Thiobarbituric acid method). SPA and SPE exhibited significant *in vitro* antioxidant activity by inhibiting the oxidation of linoleic acid in both FTC and TBA methods with SPA having greater activity in TBA method and SPE having greater activity in FTC method.SPA and SPE exert their anti-inflammatory activity by retarding lipid peroxidation and causing a modulation in cellular defense antioxidant system.

KEYWORDS

Spilanthes acmella;
Antiinflammatory;
Antioxidant.

INTRODUCTION

Spilanthes acmella (Syn.Blainvella acmella) generally known, as toothache plant[1] is an indigenous herb belonging to the family Compositae[2]. It occurs in tropical and subtropical parts of India. It is an annual erect or ascending herb. It is used in indigenous medicines as a stimulant and sialagogue for stammering, tongue paralysis, stomatitis, toothache, throat and gum infections[3].The flowers are chewed to relieve toothache and the crushed plant used in rheumatism. In Brazilian folk medicine it is used in the treatment of infectious diseases and inflammatory disorders[4]. However no scientific data are available to validate the folklore claim. Therefore, the present study was carried out to evaluate the anti-inflammatory and antioxidant properties of aqueous (SPA) and ethanolic extracts (SPE) of leaves of *Spilanthes acmella* to substantiate their therapeutic claim.

MATERIALS AND METHODS

Plant material

The leaves were collected from Gandhi Krishi Vignan Kendhrya (G.K.V.K) Herbal, Bangalore, identified and authenticated. The voucher specimen (NV-102) has been retained in our laboratory for future reference. The leaves were cleaned, dried under shade and coarsely powdered before extraction.

Extraction method

The powdered drug was extracted with 95% etha-

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nol and distilled water using soxhlet apparatus. The extracts were concentrated to dryness by evaporating the solvents under reduced pressure and stored in amber colored storage vial until used for the experiment. The extracts were administered to the animals as a suspension in 2% gum acacia.

Animals

Wistar albino rats (140±20g) of either sex, procured from TANUVAS (Tamilnadu University of Veterinary and Animal Sciences) were used for the study. The animals were housed in large polypropylene cages in a temperature-controlled room (22±2°C) and provided with standardized pelleted feed (TANUVAS) and clean drinking water *ad libitum*. The study has got the clearance from the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Experimental protocol

The following experimental protocol was used to assess the anti-inflammatory activity.

The animals were divided into six groups of six animals each.

Group I	- Control
Group II	- Diclofenac sodium (5mg/kg/p.o)
Group III	- SPA-I (100 mg/kg/p.o)
Group IV	- SPA-II (200 mg/kg/p.o)
Group V	- SPE-I (100 mg/kg/p.o)
Group VI	- SPE-II (200 mg/kg/p.o)

Anti-inflammatory activity

Anti-inflammatory activity of the extracts was evaluated in both acute and chronic models of inflammation.

Acute model

Carrageenan induced hind paw edema

The carrageenan assay procedure was carried out according to the method[5]. Edema was induced by injecting 0.1ml of a 1% solution of carrageenan in saline into the plantar aponeurosis of the left hind paw of the rats. The extracts, reference drug and the control vehicle (distilled water) were administered 60min prior to the injection of the carrageenan. The volumes of edema of the injected and contra lateral paws were measured at +1,3 and 5 hrs after induction of inflammation using a plethysmometer[6] and percentage of anti-inflammatory activity was calculated.

Chronic model

Cotton pellet granuloma

Sterile cotton pellets (weighing 10±2mg) were implanted subcutaneously along the flanks of axillae and groins of wistar albino rats[7]. The extracts, reference drug and the control vehicle (distilled water) were administered as per protocol to rats everyday for a period of 7 days. On day + 8 the rats were sacrificed by cervical decapitation and cotton pellets were removed surgically, freed from extraneous tissue and weighed immediately for wet weight. One half of the pellets were dried in an incubator at 60ºC until a constant weight was obtained.

***In vitro* antioxidant activity**

Ferric thiocynate (FTC) method

The methods of Mitsuda et al.[8] and Osawa and Namiki[9] were slightly modified by Kikuzaki and Nakatani[10]. A mixture of 4 mg weight sample in 4ml absolute ethanol, 4.1ml of 2.52% linoleic acid in absolute ethanol, 8ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a test tube with a screw cap and then placed in an oven at 40°C in the dark. To 0.1ml of this solution 9.7ml of 75% ethanol and 0.1ml 30% ammonium thiocyanate was added. Precisely 3min after the addition of 0.1ml of 0.02M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500nm for every 24 hrs until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was added, and for the standard, 4mg sample was replaced with 4mg of vitamin C and vitamin E.

Thiobarbituric acid (TBA) method

The method of Ottolenghi[11] was used to determine the TBA values of the samples. Two ml of 20% trichloroacetic acid and 2ml of TBA aqueous solution were added to 1ml of sample solution prepared as in FTC procedure, and incubated in a similar manner. The mixture was placed in boiling water bath for 10min. After cooling, it was centrifuged, at 3,000 rpm for 20min

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and the absorbance of the supernatant was measured at 532nm. Antioxidant activity was based on the absorbance on the final day. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

Percent inhibition = [Ao-A_1/Ao]×100

where Ao is the absorbance of control and A_1 is the absorbance of sample[12].

Statistical analysis

The data were expressed as mean ± SEM. Results were analysed statistically by One-way ANOVA followed by Tukey's multiple comparison using SPSS software student's version. The difference was considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Anti-inflammatory activity

The inflammatory process involves a series of events that can be elicited by numerous stimuli, for example, infectious agents, antigen-antibody interactions, chemical, thermal or mechanical injury. The inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms: an acute one characterized by local vasodilatation and increased capillary permeability, a sub acute phase characterized by infiltration of leucocyte and phagocyte cells, and chronic proliferative phase, in which tissue degeneration and fibrosis occur[13].

Carrageenan induced hind paw edema

Carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has frequently been used to assess the anti-edematous effect of natural products[14]. As shown in figure 1 oral pretreatment of animals with SPA and SPE resulted in a significant ($p<0.001$) and a dose-related inhibition of carrageenan induced hind paw edema. The percentage inhibition of inflammation by SPA, SPE at the doses of 100 and 200 mg/kg and Diclofenac (5mg/kg) was 25.26%, 45.26%, 23.15% 42.10% and 44.21% respectively, when compared with the control.

Both extracts exerted maximal anti-inflammatory effects at 3hr after carrageenan administration. The time course of edema development in carrageenan induced paw edema model in rats is generally represented by a biphasic curve[5]. The first phase occurs within an hour and is partly due to the trauma of injection and also to the serotonin component[15]. Prostaglandins play a major role in the development of the second phase of reaction, which is measured around 3 hr time. The presence of PGE_2 in the inflammatory exudates from the injected foot paw can be demonstrated at 3 hr and period thereafter[16]. The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors [17]. Based on the results it could be inferred that the inhibitory effect of the extracts on carrageenan induced paw edema in rats could be mediated through the inhibition of enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

Cotton pellet granuloma

The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation. The inflammatory granuloma is a typical feature of established chronic inflammatory reaction[18]. The repair phase of inflammation starts as proliferation of fibroblast as well as multiplication of small blood vessels. Such proliferating cells penetrate the exudates producing a highly vascularised reddened mass known as granulation tissue[19]. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed[7]. SPA and SPE administration at two different doses significantly ($p<0.001$) inhibited both wet and dry weight of the granuloma (Figure 2).

During the inflammatory process lipid peroxidation

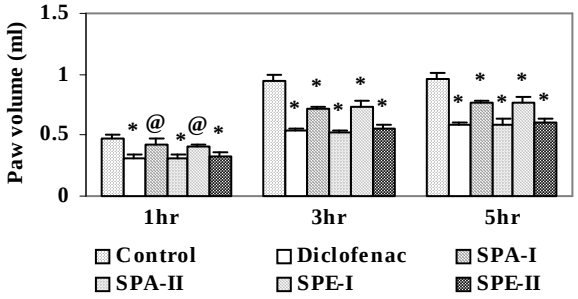

Figure 1: Effect of SPA and SPE on rat paw edema induced by carrageenan. Data represent mean ± SEM of 6 animals. *p<0.001, @ p<0.05 compared to control (One way ANOVA followed by Tukey's multiple comparison test)

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TABLE 1: Effects of SPA and SPE on various biochemical parameters in the exudates, serum and liver of rats exposed to cotton pellet

Groups	Exudate lipid peroxide	Liver lipid peroxide
Group I	100	100
Group II	45.28 ± 2.43a*	40.21 ± 1.70a*
Group III	35.26 ± 2.77a*	33.45 ± 1.76a*
Group IV	42.46 ± 4.34a*	38.12 ± 2.51a*
Group V	32.17 ± 2.50a*	32.64 ± 3.50a*
Group VI	44.23 ± 3.39a*	39.48 ± 2.38a*

Values are expressed as mean ± SEM of 6 animals : Enzyme units: Lipid peroxide (% Inhibition); Comparisons were made between : a-Group I vs II, III, IV, V and VI, b-Group II vs III, IV, V and VI; Symbols represent Statistical significance; *-p<0.001, #-p<0.01, @-p <0.05

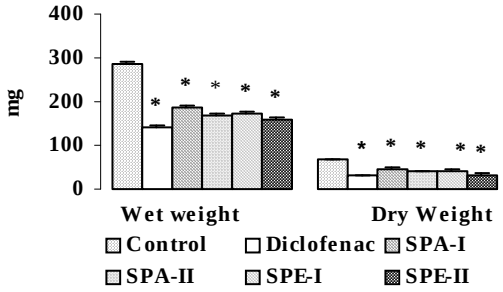

Figure 2 : Effect of SPA and SPE on wet and dry weights of implanted cotton pellets. Data represent mean ± SEM of 6 animals. *p<0.001 compared to control (One way ANOVA followed by Tukey's multiple comparison test)

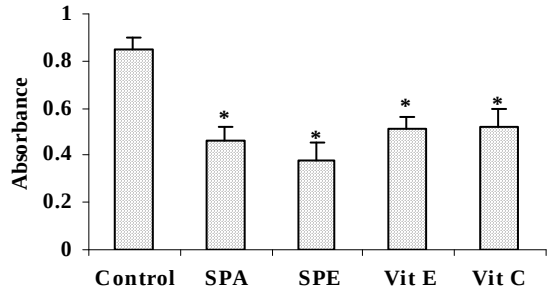

Figure 3 : *In vitro* antioxidant activity of SPA and SPE by FTC method *p<0.001 compared to control (One way ANOVA followed by Tukey's multiple comparison test)

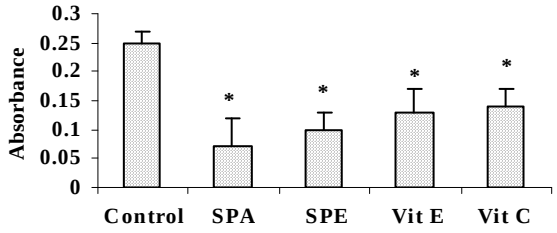

Figure 4 : *In vitro* antioxidant activity of SPA and SPE by TBA method *p<0.001 compared to control (One way ANOVA followed by Tukey's multiple comparison test)

is initiated when reactive oxygen species produce reversible or irreversible damage to molecules of all biochemical classes[20]. The treatment with SPA (I and II) and SPE (I and II) significantly (p<0.001) reduced the elevated levels of lipid peroxide in liver and exudates (TABLE 1).

Lysosomes become activated in inflammation and their membranes are altered, so that they become so permeable that their contents leak out or they excrete their products by exocytosis[21]. The activity of lysosomal enzymes (acid and alkaline phosphatases) was markedly increased in serum and liver during inflammation[22]. The elevated levels of these lysosomal enzymes in serum and liver were significantly (p<0.001) decreased after treatment with SPA (I and II) and SPE (I and II), which may be due to their ability to stabilize the lysosomal membrane and thereby preventing the release of lysosomal enzymes (TABLE 1).

The extracts exert their anti-inflammatory activity probably by inhibiting lipid peroxidation and suppressing the infiltration of neutrophils into the granuloma by inhibiting either the formation of chemotactic mediators or by suppressing the ability of inflammatory cells to respond to a chemotactic stimulus mediated through oxygen free radicals.

The antioxidant activity exhibited by SPA and SPE in FTC and TBA methods were represented in figures 3 and 4.

FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. During the linoleic acid oxidation, peroxides formed and these compounds oxidize Fe^{2+} to Fe^{3+}. The Fe^{3+} ions form complex with SCN−, which has a maximum absorbance at 500nm. In this method, the concentration of peroxide decreases as the antioxidant activity increases. Both SPA and SPE exhibited effective antioxidant activities at a concentration of 4mg compared to standards vitamin E and C. Lower the absorbance values exhibited higher the antioxidant activities of the samples. The control had the highest absorbance value (0.85), followed by SPA (0.38), SPE (0.46), Vitamin E (0.51) and Vitamin C (0.61). Based on the results SPE had the highest percentage inhibition of 55.29% followed SPA (45.88%), Vitamin E(40%) and Vitamin C (38.83%).

In TBA method formation of malonaldehyde is the

basis for evaluating the extent of lipid peroxidation. At low pH and high temperature (100°C) malonaldehyde binds TBA to form a red complex that can be measured at 532nm. The increase of the amount of red pigment formed correlates with the oxidative rancidity of the lipid. The control had the highest absorbance value (0.25), followed by SPA(0.07), SPE(0.10), Vitamin E(0.13) and Vitamin C(0.15). Based on the results SPA had the highest percentage inhibition of 72% followed SPE(60%), Vitamin E(48%) and Vitamin C(44%).

SPA and SPE exhibited significant ($p<0.001$) *in vitro* antioxidant activity by inhibiting the oxidation of linoleic acid in both FTC and TBA methods with SPA having greater activity in TBA method and SPE having greater activity in FTC method. Incidentally the activities of both SPA and SPE were found to be better than the standards Vitamin E and C in both the methods.

The above results establish the fact that SPA and SPE have good antioxidant property and they exert their anti-inflammatory activity by retarding lipid peroxidation and causing a modulation in cellular defense antioxidant system.

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