Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 6, Issue 9, 2014

Original Article

ANTI-INFLAMMATORY AND PROTECTIVE PROPERTIES OF ALOE VERA LEAF CRUDE GEL IN CARRAGEENAN INDUCED ACUTE INFLAMMATORY RAT MODELS

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Received: 29 Jul 2014 Revised and Accepted: 26 Aug 2014

ABSTRACT

Objectives: Current clinical treatment regimes for inflammatory diseases have different drawbacks including side effects of the drugs and the high cost of long term treatment. In the last few decades different promising herbal medicines have been explored for their anti-inflammatory and anti-rheumatic effects, but conclusive evidences are not available in the case of crude *Aloe vera* gel for its anti-inflammatory effects. The objective of the study was to document the protective and curative roles of orally administered and peritoneally injected crude wild *Aloe vera* gel in carrageenan-induced inflammation in a rat model.

Methods: Inflammation was induced by injecting 1% carrageenan in the left hind paw of Wistar albino rat. Crude, unprocessed *Aloe vera* gel was peritoneally injected and orally fed to experimental and control rat groups to investigate its effect on paw joint edema by measuring the paw circumference with vernier caliper. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cell viability assay was performed to investigate the cytotoxic effect of the gel.

Results: Paw edema was brought to near normal levels in the experimental groups after the treatment with crude *Aloe vera* gel. Orally fed gel showed no cytotoxicity on macrophages and spleenocytes. Protective property of crude *Aloe* gel was also evident in both the experiments.

Conclusion: *Aloe vera* crude gel has both protective and curative properties against inflammation.

Keywords: *Aloe vera*, Carrageenan, Indomethacin, Medicinal plant, Paw edema, Pharmacology.

INTRODUCTION

Carrageenan induced inflammatory rat model is a standard model system for experimentation on acute inflammatory conditions. Generally non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immuno-suppressive drugs used in the relief of inflammatory diseases worldwide are often associated with severe adverse side effects like peptic ulcer and gastrointestinal bleeding [1]. In traditional medicine and Ayurveda, many plant products are used as anti-inflammatory agents to cure the inflammatory pain and swelling which still lack a proper screening process. In the northern region of West Bengal, traditional Ayurveda practice uses *Aloe vera* as a potent anti-inflammatory agent. The gel-like layer under the leaf of the plant, actually the parenchyma cells, is traditionally known to decrease inflammatory pains. *Aloe vera* (Family Xanthorrhoeaceae) is a stem-less or short-stemmed succulent plant growing upto 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up [2]. *Aloe vera* contains different phytochemical agents which are able to cure different disease symptoms [3]. The plant is used widely in dermal diseases and is a good laxative agent [4]. It also represents compounds responsible for anti diabetic [5], anti-oxidant [6], antimicrobial [7] and wound healing activities [8]. However, the experiment-based proof is still lacking regarding the anti-inflammatory properties of the crude unprocessed gel of this plant. Our study was aimed to evaluate the anti-inflammatory properties of different doses of aqueous crude extract of *Aloe vera* leaf gel to give a scientific base to the traditional claim.

MATERIALS AND METHODS

Collection of Plant Specimens

Wild *Aloe vera* (Class Magnoliopsida, Order Asparagales, Family Xanthorrhoeaceae) plants were collected from the sub-Himalayan Terai areas of northern West Bengal and were identified by Prof. A. P. Das, plant taxonomist in the Department of Botany, University of North Bengal [Accession no. 09884 (NBU)].

Preparation of Extract

Crude gel was collected by peeling out the outer cuticle and cutting out the gel aseptically into small pieces. The gel was weighed, mixed with distilled water (1:5 w/v) and then homogenized to create a homogenate. The sample was freshly prepared every time before use. It contained all the ingredients of the crude gel in the same proportion as it appears in the leaf. To know the dry weight of the gel (weight without water parts), each piece was dried separately in an air oven at 37°C for 48 hours and was then weighed.

Chemicals

Carrageenan was purchased from Hi-Media Laboratories Pvt. Ltd., (Mumbai, India). Indometacin or Indomethacin (Jagsonpal Pharmaceutical Ltd., New Delhi, India), the non-steroidal anti-inflammatory drug was purchased from local drug suppliers and used as the control drug of inflammation. RPMI-1640, fetal bovine serum (FBS), antibiotics and EZcount™ MTT assay kits were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

Experimental design

Studies were carried out using Wistar albino rats of either sex weighing 60 ± 15 g. They were maintained in the animal house of the Department of Zoology, University of North Bengal. The animals were clustered in six groups; each contained six rats for anti-inflammatory activity study (PC, NC, 125D, 250D, STDG, and PROG). Rats were maintained under standard laboratory conditions (temperature 25 ± 2°C) with normal daily cycle (12/12h). The rats were acclimatized to laboratory condition for 10 days before commencement of experiments. The study was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of

Experiments on Animals) of University of North Bengal, West Bengal, India. PC refers to Positive Control i. e. Normal rats, NC refers to Negative Control where inflammation was induced by 1% carrageenan but no treatment was done. In the anti-inflammatory test, the group 125D represents the group injected with 1% carrageenan and treated orally with 125 µl aqueous extract of *A. vera* corresponding to approximately 25 gm wet gel/kg body weight (20 mg dry weight/kg body weight), 250D represents 1% carrageenan injected rat treated with 250 µl aqueous extract of *Aloe vera* gel orally corresponding to approximately 50 gm wet gel/kg body weight (40 mg dry weight/kg body weight). PROG or protection group was treated with *A. vera* 125 µl doses orally once a day for 7 days prior from the day of injection. STDG or Standard group represents carrageenan-induced inflammatory rats treated with 60 µl of 10mg/ml Indomethacin dose equivalent to 10mg/kg b. w., known to be a standard drug of inflammatory conditions [9].

Determination of the cytotoxic effect of the extract on peritoneal macrophages and spleenocytes were performed by MTT assay. Same amounts of crude gel, present in the 125 µl and 250 µl homogenized solution of 1 gm/5 ml (w/v) in dH_2O were applied to experimental rat groups for MTT assay and designated as dose groups M1 and M2 respectively. M1 contained 32.4 mg of wet gel homogenate (corresponding to 125 µl aqueous homogenate) and M2 contained 64.8 mg of wet gel homogenate (corresponding to 250 µl aqueous homogenate), each prepared in 50 µl of dH_2O. The PROG group rats were tested for the protective activity of the gel when fed orally 125 µl of gel solution (corresponding to 1 gm/5 ml in dH_2O) continuously for 7 days prior to the experiment. No gel was further used in the culture medium for this group directly. A control group with untreated cells (C) was included to compare the data of the test and protection groups.

The doses of *A. vera* gel was calculated on the basis of the optimum dose taken by human, which is 50 grams per day for 60kg body weight.

Carrageenan induced paw edema

The anti-inflammatory activities of the aqueous extracts were determined using the methods described by others [10, 11, 12]. In 125D, 250D and STDG dose groups, the extracts were injected peritoneally 30 min prior to induction of oedema by administering 0.1 ml of 1% w/v carrageenan in the sub-plantar region of rat left hind paw. Protection group (PROG) received carrageenan injection 30 minutes after the oral feeding of *Aloe vera* on the experimental day. The degree of paw circumference of all the groups was measured (in millimeters) using a vernier calliper after 30, 90, 150, 210 minutes (0.5h, 1.5h, 2.5h, 3.5 h) of carrageenan injection. The Percentage of Inhibition (PI) was calculated using the following equation: PI (%) = [(V_t – V_0) Negative Control - (V_t – V_0) Treatment Group / (V_t – V_0) Negative Control] X 100, where V_t = final reading of paw circumference, V_0 = Initial reading of paw circumference [13].

MTT cytotoxicity assay

Rats were sacrificed under proper anesthesia and spleens were collected. Macrophages were collected from the peritoneal exudates by flushing the region with cold RPMI-1640. Cell suspensions were prepared at a concentration of 2 X 10^6 cells/ml following kit manufacturer's instructions. One hundred microlitre (100 µl) cell suspension was added to 12 µl of nutrient supplement containing 50 U/ml penicillin, 50 U/ml streptomycin, 50U/ml nystatin and 10% FBS. In 96 well plates, all four groups were columned with 6 replicates. Fifty microlitre (50 µl) of *Aloe* extract was added in each well along with 112 µl of such suspension and 10 µl of MTT, and then incubated for 4 hours. Absorbance was measured at 570 nm using BioRad I Mark microplate reader, BioRad, USA [14], [15].

Statistical Analysis

All statistical analyses were done using the softwares MS-Excel and Kyplot ver 2.0 beta. In Kyplot analysis, the data represented Mean ± S. E. M which was analyzed by one way ANOVA. The results were considered significant when $p <= 0.05$.

RESULTS

It is evident from the graphical presentation that indomethacin showed the best PI of 92.7% after 3.5 hours (210 min) of carrageenan injection,. Treatment groups 125D and 250D also effectively repressed paw swellings by 58.69% and 74.09% respectively. The PROG group also showed a substantial effect by reducing paw swelling by 82.6% which was even better than the 125D and 250D dose groups (Table 1 and Figure 1).

Table 1: Table showing paw circumference (mm) of different groups at different time intervals (h). All data represent Mean ± S. D Percentage of inhibition (PI) is mentioned in the brackets.

Groups	0 h	0.5 h	1.5 h	2.5 h	3.5 h
Positive Control (PC)	32.35±0.114	32.73±0.275	32.34±0.232	32.33±0.270	32.33±0.173
Negative Control (NC)	32.36± 0.709	37.94±0.344	38.82±0.553	38.22±0.448	38.15±0.287
Standard Group (STDG)	32.06±0.697	36.37±1.00	35.03±0.239	33.19±0.681	32.46±0.540 (92.7%)
Protection Group (PROG)	32.15±0.430	34.85±0.718	33.62±0.534	33.52±0.726	33.11±0.558 (82.6 %)
125 µL dose Group (125D)	31.77±0.923	35.36±0.779	35.38±0.637	34.51±0.785	34.05±0.711 (58.69%)
250 µL dose Group (250D)	31.93±0.776	37.74±0.315	36.48±0.369	34.79±0.645	33.36±0.774 (74.09%)

In the MTT assay, both peritoneal macrophage and spleenocyte cultures of M2 groups showed higher cell cytotoxicity than that of the M1 groups. Here again, the PROG group showed the best result with no cytotoxicity or some protective properties (Figure 2, A and B respectively).

DISCUSSION

Carrageenan-induced paw edema is an established model for *in vivo* study of anti-inflammatory activities. The early phase of carrageenan induction is mediated by the histamines and serotonins; the surrounding damaged tissues also showed an increased prostaglandin synthesis [9]. The later stage is mediated by bradykinins, polymorphonuclear cells and macrophage-secreted prostaglandins [9]. Prostaglandins elevate the temperature of paw and causes inflammation and pain. NSAID drugs like aspirin, ibuprofen or indomethacin inhibits the enzyme prostaglandin H_2 synthase (also known as cycloxygenase or COX) which catalyzes an early step of prostaglandin synthesis. There are evidences that high polysaccharides and anthroquinones present in *A. vera* can act as a pro-oxidant and pro-inflammatory product.

Polysaccharides between 5- and 400-kDa were found to exhibit the most potent macrophage-activating activity, as determined by increased cytokine production, nitric oxide (NO) release, expression of surface markers, and phagocytic activities. Talmadge *et al* (2004) purified a high-molecular-weight fraction of *A. vera* and showed the increased haematological and the hematopoietic activity compared to the gel starting material [16].

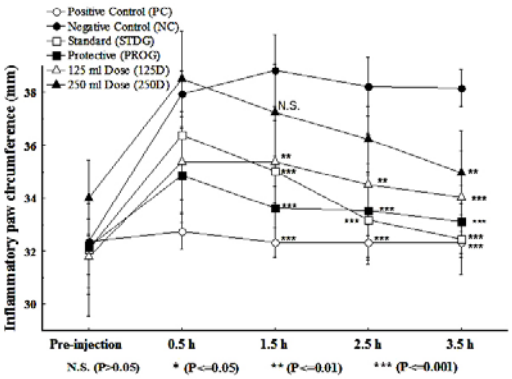

Fig. 1: Graphical presentation of paw circumference (mm) with respect to time in different rat groups. * showing the level of significance. * refers to most significant value.**

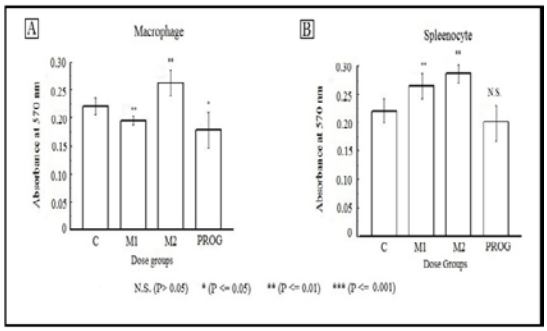

Fig. 2: Peritoneal macrophage (A) and spleenocyte (B) MTT assay of different dose groups. M1 and M2 represent 125 and 250 µl of Aloe dose equivalents.

We have recorded an increased level of inflammatory responses at the beginning of anti-inflammatory test (0.5 h interval) in the high dose group (250D). The high concentration of anthroquinones, like aloe-emodin and high amount of polysaccharides present in the crude *Aloe*-gel may be responsible for the flaring up of immune system in case of 250D groups [3] (see Figure 1). It appears that a synergistic role is played by the polysaccharides and anthroquinone derivatives that may be instrumental in the initial trigger of the immune response which then subsequently inhibits prostaglandin synthesis as the concentration goes down. A sharp decline of paw circumference during 0.5 to 3.5 h period in the 250D group supports the hypothesis. On the other hand, a lower amount of injected polysaccharides and anthroquinone in the 125D group do not induce such flaring up of immune system but protects the initial swelling and subsequently act significantly in decreasing the inflammatory symptoms in the experimental rats.

MTT cell viability tests were performed to investigate the cytotoxic effects of *Aloe* crude gel on peritoneal macrophages and spleenocytes collected from the experimental groups. In the MTT assay, it is evident that addition of higher dose of plant extract increased the death rate of the cells. The exact reason behind it is to be studied in detail to elucidate the molecular pathways of such cytotoxicity. However interestingly, we have observed that the animals (PROG group) that were fed orally before the experiment showed no toxic effects in the culture, rather the Aloe-gel showed some protective property on spleenocytes (Figure 2B). This finding confirms that the gel in low dose may act in a synergistic way by maintaining the normal immune status in one hand and by suppressing the inflammatory activity on the other hand. Therefore, our results suggest that the initial immunostimulatory activity of the gel is probably due to the presence of polysaccharides like acemannan, which can initiate macrophage activation and subsequently cytokine production when the gel is in high amount [17]. The later inhibitory effect of the aqueous extracts of *A. vera* on scarrageenan-induced paw edema may occur due to the suppression of the release of mediators including histamine, serotonin, bradykinin and prostaglandins that are responsible for the first and the second phase of acute inflammation by other bioactive compounds present in the gel, especially when the gel is present in low dose. The inhibitory effect of *Aloe vera* extract on carrageenan-induced inflammation could also be mediated via inhibition of cyclooxygenases (COX) [18].

Phytochemical analysis of *Aloe vera* has revealed the presence of flavonoids, anthraquinones, saponins. Saponins and flavonoids have previously been reported to have anti-inflammatory activities [3]. Such compounds may be responsible in part for the described anti-inflammatory activity of *A. vera* extract. However, a high dose of *A. vera* may elicit a different role in organism's body by inducing some serious inflammatory and pro-oxidant response as evidenced in the MTT assay. Low dose of the plant gel is suggested to be better for consumption; however the dosage has to be standardized accordingly for application in human.

CONCLUSIONS

Our data documents that *Aloe vera* crude unprocessed gel can reduce the inflammatory pain very efficiently if consumed daily. However, high dose may have some cytotoxic activity. It is also suggested that the consumption of low dose of *A. vera* orally in a regular fashion is better in case of human as repeated peritoneal injection may become inhuman or unethical.

CONFLICT OF INTERESTS

The authors declare no conflict of interest in the outcome of the study.

ACKNOWLEDGEMENT

Authors want to acknowledge Prof. A. P. Das, Department of Botany, University of North Bengal for the identification of the plant. The authors also thank the Department of Zoology for providing the animal house and instrument facilities for the research purpose.

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