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An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation

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Albuca setosa **is widely distributed in the Eastern Cape region of South Africa where its traditional usage is very extensive. This study was aimed to experimentally evaluate the effect of** *A. setosa* **water extract (ASWE) on inflammation events such as membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. This study was undertaken using hypotonicity and heat induced erythrocytes haemolysis, heat induced albumin denaturation, carrageenan and dextran induced peritonis and rat paw edema. The results showed that ASWE at a concentration range of 125 - 500 µg/ml significantly (p < 0.01) protects the erythrocyte membrane against lysis induced by heat and hypotonic medium solution. At the dose of 500 and 125 µg/ml, ASWE showed significant (p < 0.01) inhibition of 59 and 65% of protein denaturation of egg albumin. Oral administration of 150 and 300 mg/kg of ASWE significantly (p < 0.05) reduced the total WBC count in rat paw fluid after inflammation induced by carrageenin and in the peritoneal wash after acute inflammation induced by dextran and carrageenan, respectively. The present work contributes to the validation of the anti-inflammatory activity of the plant and may provide some evidence for its folk use and further exploitation.**

Key words: *Albuca setosa*, membrane stabilization, protein denaturation, white blood cell migration, anti-inflammatory activity.

INTRODUCTION

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. Several experimental protocols of inflammation are used for evaluating the potency of drugs. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants. *Albuca setosa* (AS) called *inqwebeba* in *Isixhosa* is a member of the Hyacinthaceae family. It is

widely distributed in the Eastern Cape region of South Africa where its traditional usage is very extensive. Xhosa people living in an rural area in the Eastern Cape, Province of South Africa use *A. setosa* for cultural purposes and often access it through commercial trade at around R32/kg depending on the availability of the plant (Dold et al., 2002). It is used as a ritual wash, an emetic and a facial steam treatment as protection against bad luck and sorcery (Cocks et al., 2006). Therapeutically*,* aqueous extract of *A. setosa* is traditionally used as a purgative, for spraying and steam treatment (Cocks et al., 2006). It is also used as anthelmintic, lotion for washing wounds in animal and to treat venereal diseases (Hutchings, 1996). This plant is also used to treat wound, articulation problems and arthritis rheumatoid (Hutchings, 1996). However, up to date, there is no scientific report or

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verification study of the use of this plant. The present work was planned to evaluate the effect of *A. setosa* water extract (ASWE) on inflammation events such as membrane stabilization, protein denaturation and white blood cell migration.

METHODS

Plant material and extract preparation

The plant material of *A. setosa* was collected in its natural environment approximately 5 km south west of Flagstaff in the OR Tambo municipality, Eastern Cape Province of South Africa. Identification of the plant was done at the Kei herbarium at Walter Sisulu University in Mthatha. Leaf samples of the *A. setosa* were chopped, air dried and ground to powder (pulverised). 40 g of dried powder was macerate in distilled water for 72 h on an orbital shaker. The extract was filtered using a Buchner funnel and Watman No. 1 filter paper and then concentrated to dryness under reduced pressure at a maximum of 55°C using a rotating evaporator (Bibby Sterilin rotator evaporator RE-100) and a brown powder yielding 5 g was obtained.

Animals

Male Wistar rats and male Swiss mice weighing 150 - 200 and 20 - 30 g, respectively were obtained from South African Vaccine Producers (SAVP). The animals were housed in the animal house of Walter Sisulu University (WSU). The animals were kept in groups of five in standard cages at room temperature in 12 h dark/12 h light control, with both food and water *ad libitum*.

Membrane stabilization activities

Preparation of erythrocyte suspension

Erythrocytes suspension was prepared by the method described by Shinde et al. (1999) with some modifications. Whole human blood was obtained from a healthy human volunteer and transferred to heparinized centrifuge tubes, centrifuged at 3000 rpm for 5 min and washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4, the composition of the buffer solution (g/l) was NaH_2PO_4 (0.2), Na_2HPO_4 (1.15) and NaCl (9.0).

Heat induced haemolysis

This test was carried out as described by Okoli et al. (2008). The isotonic buffer solution (5 ml) containing 500, 250 and 125 µg/ml of the ASWE were put in 5 sets (per concentration) of centrifuge tubes. Control tubes contained 5 ml of the vehicle or 5 ml of 100 µg/ml of indomethacin. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. A pair of the tubes was incubated at 54°C for 20 min in a regulated water bath. At the end of the incubation, the reaction mixture was centrifuged at 1300 g for 3 min and the absorbance (OD) of the supernatant measured at 540 nm using Spectronic 20 Genesys[TM] spectrophotometer. The level of inhibition of hemolysis was calculated using the following relation:

% inhibition of haemolysis = 100 × (1-A2/A1)

Where, A1 = Absorption of the control sample, A2 = Absorption of test sample solution.

Hypotonicity-induced haemolysis

The hypotonic solution (distilled water) containing 500, 250 or 125 µg/ml of ASWE was put in 2 pairs (per dose) of centrifuge tubes (5 ml). Control tubes contained 5 ml of the vehicle (distilled water) or indomethacin 100 µg/ml. Erythrocyte suspension (0.05 ml) was added to each tube and after gentle mixing, the mixtures were incubated for 1 h at room temperature (31°C). After incubation, the reaction mixture was centrifuged for 3 min at 1300 g and the absorbance (OD) of the supernatant measured at 540 nm using Spectronic 20 Genesys[TM] spectrophotometer. The inhibition (%) of haemolysis was calculated using the following relation:

% inhibition of haemolysis = 100 × (1-A2/A1)

where A1 = Absorption of the control sample
A2 = Absorption of test sample solution.

Effect on protein denaturation

The test was performed following the method decribed by Gambhire et al. (2009) with some modifications. Test solution containing 500, 250 or 125 µg/ml of ASWE or indomethacin 100 µg/ml were put in 5 sets (per concentration) and Albumin 25% (5 ml) was added to each tube and after gentle mixing, the mixtures were incubated for 15 min in ambient temperature. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the turbidity was measured using a spectrophotometer at 660 nm. Percentage of inhibition of denaturation was calculated from control where no drug was added using the following equation:

% inhibition of denaturation = 100 × (1-A2/A1)

where, A1 = Absorption of control sample and A2 = Absorption of test sample

Carrageenan induced peritonitis

Peritonitis was induced by modification of the technique described by Gupta et al. (2006). Twenty five mice (20 - 30 g) of both sexes were randomly assigned into 5 groups of rats each (cohort), and they received, *per os,* four different treatments: one group was given distilled water (control); another group received indomethacin 100 mg/kg, the other two groups received two dose levels of ASWE 150 and 300 mg/kg and the last group was not treated. Carrageenan (0.25 ml, 1% in saline) was injected intraperitoneally 1 h later and after 4 h, the animals were sacrificed under anaesthesia and the peritoneal cavity washed with 3 ml of phosphate buffer saline containing 0.5 ml of 10% EDTA. The total WBC count was determined in a Neubauer chamber and the differential cell count was determined by microscopic counting (Anam et al., 1997). The percentage of the leukocyte inhibition was calculated by the following equation:

% inhibition of WBC migration = 100 × (1-T/C)

where, T represents the treated group's WBC counts and C represents the control group's WBC counts.

Dextran induced peritonitis

Peritonitis induced with dextran was performed by modification of the technique as previously described by Ribeiro et al. (1991). Twenty five mice (20 - 30 g) of both sexes were randomly assigned into 5 groups of 5 rats each (cohort), and they received *per os*, four different treatments: one group was given distilled water (control); another group received indomethacin 100 mg/kg, the other two groups received two dose levels of ASWE 150 and 300 mg/kg and the last group was not treated. One hour after treatment, inflammation was induced by injecting 1% (w/v) dextran in normal saline, 0.5 ml, into the peritoneal cavity. The last group was injected with normal saline following the same procedure. Four hours later, the animals were sacrificed under anaesthesia and the peritoneal cavity washed with 3 ml of phosphate buffer saline containing 0.5 ml of 10% EDTA. The total number of white blood cells (WBC) in the peritoneal wash was counted under a microscope (x40). The Inhibition of migration of WBC was evaluated as percent reduction/inhibition in the treated animals relative to control animals using the relation:

% inhibition of WBC migration = 100 × (1-T/C)

Where, C is the total WBC in control group and T is the total WBC in treated group.

Total WBC count in paw fluid after acute inflammation

Collection of paw fluid from treated rats

Twenty five wistar rats (150 – 200 g) of both sexes were randomly assigned to 5 groups of 5 rats each (cohort). The rats received, *per os,* four different treatments: one group was given distilled water (control); another group received indomethacin 100 mg/kg, the other two groups received two dose levels of ASWE 150 and 300 mg/kg and the last group was not treated. One hour after treatment, inflammation was induced by injecting 1% (w/v) carrageenan in normal saline, 0.1 ml, into the subplantar surface of the hind paw of the control and the treated groups of rats, but the last group was injected with normal saline following the same procedure. Three hours after the administration of the inflammatory agent, the plantar aponeurosis of the inflamed paw was injected with 2% xylocaine, incised and the paw fluid of each rat aspirated (using 26G hypodermic needle) and slowly squirted into a test tube. The residual fluid was gently squeezed out, ensuring that blood did not mix with the fluid. Any fluid that had blood in it was discarded. The fluid was examined under microscope (x40) for any sign of breakages of the blood cells.

Total WBC count in paw fluid

Total white blood cell count in paw fluid was performed as described by Prempeh et al. (2008). 0.02 ml of paw fluid was mixed with 0.38 ml of WBC fluid (3% acetic acid with crystal violet dye), in a test tube. The mixture was transferred into a counting chamber, and the total number of WBC counted under a microscope (×40). The inhibition of migration of WBC was evaluated as percent reduction/inhibition in the treated animals relative to control animals using the relation:

% inhibition of WBC migration = 100 × (1 – T/C)

where, C is the total WBC in control group and T is the total WBC in treated group.

Statistical analysis

Data was reported as mean ± S.E.M. and were analyzed statistically by analysis of variance (ANOVA) followed by Dunnett's test. Results with $p < 0.05$ were considered significant.

RESULTS

Membrane stabilization activities

Heat induced haemolysis

The results showed that ASWE at concentration range of 125 - 500 µg/ml protect significantly ($p < 0.01$) the erythrocyte membrane against lysis induced by heat solution (Table 1). Indomethacin 100 µg/ml offered a significant ($p < 0.05$) protection against damaging effect of heat solution. At the concentration of 500 and 250 µg/ml, ASWE showed 38 and 34% protection, respectively, where as indomethacin at 100 µg/ml showed 24% inhibition of RBC heamolysis when compared with control (Table 1).

Hypotonicity-induced haemolysis

The results showed that ASWE at concentration range of 125 - 500 µg/ml protect significantly ($p < 0.01$) the erythrocyte membrane against lysis induced by hypotonic solution (Table 2). Indomethacin 100 µg/ml offered a significant ($p < 0.01$) protection against damaging effect of hypotonic solution. At the concentration of 500 and 250 µg/ml, ASWE showed 30% protection where as indomethacin at 100 µg/ml showed 34% inhibition of RBC heamolysis when compared with control (Table2).

Protein denaturation test

The inhibitory effect of different concentration of ASWE on protein denaturation is showed in Table 3. ASWE at the dose of 500 and 125 µg/ml showed significant ($p < 0.01$) inhibition of protein denaturation of egg albumin (59 and 65% respectively). Indomethacin showed 48% inhibition compared with control (Table 3).

Carrageenan induced peritonitis

The results showed that WBC population in the inflammatory exudates increased significantly after inflammation induced by carrageenan as indicated by higher WBC count in carrageenan treated group than normal saline group (Table 4). Oral administration of ASWE significantly ($p < 0.05$) reduced the total WBC count in the peritoneal fluid after inflammation induced with carrageenan by 57 and 60% for the dose of 150 and 300 mg/kg, respectively. Indomethacin also showed 64%

Table 1. Effect of ASWE on heat induced haemolysis of erythrocyte.

Treatment (s)	Concentration (µg/ml)	Absorbance at 540 nm	% inhibition of haemolysis
Control	--	0.29 ± 0.02	--
ASWE	500	0.18 ± 0.006**	38
ASWE	250	0.19 ± 0.005**	34
ASWE	125	0.25 ± 0.02	14
Indomethacin	100	0.22 ± 0.005*	24

Each value represents the mean ± SEM. N = 5. Experimental group were compared to control *P < 0.05, **P < 0.01. ASWE: *Albuca setosa* water extract.

Table 2. Effect of ASWE on hypotonicity induced haemolysis of erythrocyte.

Treatment (s)	Concentration (µg/ml)	Absorbance at 540 nm	% inhibition of haemolysis
Control	--	0.38 ± 0.03	
ASWE	500	0.27 ± 0.005**	30
ASWE	250	0.27 ± 0.008**	30
ASWE	125	0.28 ± 0.007**	28
Indomethacin	100	0.25 ± 0.009**	34

Each value represents the mean ± SEM. N = 5, Experimental group were compared to control **P < 0.01. ASWE: *Albuca setosa* water extract.

Table 3. Effect of ASWE on heat induced protein denaturation

Treatment (s)	Concentration (µg/ml)	Absorbance at 660 nm	% inhibition of protein denaturation
Control	--	0.38 ± 0.09	--
ASWE	500	0.16 ± 0.03**	59
ASWE	250	0.56 ± 0.02	-45
ASWE	125	0.13 ± 0.01**	65
Indomethacin	100	0.20 ± 0.02*	48

Each value represents the mean ± SEM. N = 5, Experimental group were compared to control *P < 0.05, **P < 0.01. ASWE: *Albuca setosa* water extract.

inhibition (Table 4). Concerning the proportion of lymphocytes and neutrophils in the peritoneal fluid after inflammation induced by carrageenan, the results showed no significant difference for control, ASWE 150 and 300 mg/kg. Indomethacin 100 mg/kg showed that the proportion of lymphocytes was significantly greater than neutrophils (Figure 1).

Dextran induced peritonitis

The result in this experiment showed that the total number of WBC in the peritoneal wash increased significantly after inflammation induced by dextran (Table 5). Oral administration of ASWE significantly (p < 0.05) reduced the total white blood cell count in the peritoneal wash after inflammation induced by dextran by 60 and 38% for the dose of 150 and 300 mg/kg, respectively. Indomethacin also showed 18% inhibition (Table 5).

Total WBC count in paw fluid

The results showed that WBC population in the inflammatory exudates increased significantly after inflammation induced by carrageenan as indicated by higher WBC count in carrageenan treated group than normal saline group (Table 6). Oral administration of ASWE significantly (p < 0.05) reduced the total white blood cell count in paw fluid after inflammation induced by carrageenan by 55 and 45% for the dose of 300 and 150 mg/kg, respectively. Indomethacin also showed 40% inhibition (Table 6).

Table 4. Effect of ASWE on the peritoneal level of WBC during peritonis induced by carrageenan.

Phlogistic agent	Treatment (s)	Concentration (mg/kg)	TLC $\times 10^3/mm^3$	Inhibition (%)
Normal saline	Control 1	No administration	9.2 ± 0.86	--
	Control 2	Vehicle	50.4 ± 5.4	--
Carrageenan 1%	ASWE	150	21.6 ± 4.5**	57
	ASWE	300	20.0 ± 4.7**	60
	indo	100	18.0 ± 3.2**	64

Each value represents the mean ± SEM. N = 5, Experimental group were compared to control *P < 0.05, **P < 0.01. ASWE: *Albuca setosa* water extract.
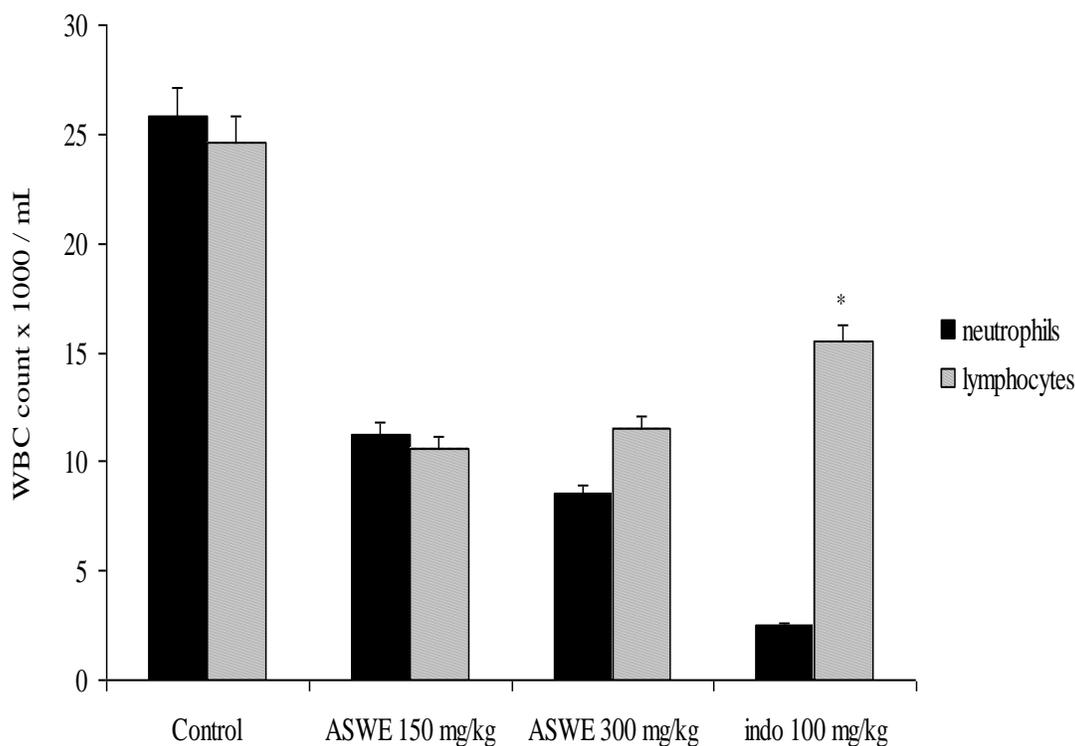

Figure 1. Lymphocytes and neutrophils proportion in mice peritoneal cavity fluid after inflammation induced by carrageenan. Each value represents the mean ± SEM. N = 5, Experimental group were compared to control *P < 0.05, ASWE: *Albuca setosa* water extract, indo: Indomethacin.

Table 5. Effect of ASWE on the peritoneal level of WBC during peritonis induced by dextran.

Phlogistic agent	Treatment (s)	Concentration (mg/kg)	TLC $\times 10^3/mm^3$	Inhibition (%)
Normal saline	Control 1	No administration	7.6 ± 0.7	--
	Control 2	Vehicle	49.2 ± 13.8	--
Carrageenan 1%	ASWE	150	19.8 ± 3.7**	60
	ASWE	300	30.6 ± 9.3*	38
	indo	100	40.2 ± 4.1	18

Each value represents the mean ± SEM. N = 5, Experimental group were compared to control *P < 0.05, **P < 0.01. ASWE: *Albuca setosa* water extract.

Table 6. Effect of water extract of *A. setosa* leaves on rat paw fluid level of WBC during acute inflammation induced by carrageenan.

Phlogistic agent	Treatment(s)	Concentration (mg/kg)	TLC ×10^3/mm^3	Inhibition (%)
Normal saline	Control 1	No administration	126 ± 36	--
Carrageenan 1%	Control 2	Vehicle	325 ± 37	--
	ASWE	150	146 ± 7*	45
	ASWE	300	179 ± 32*	55
	indo	100	194 ± 78*	60

Each value represents the mean ± SEM. N = 5, Experimental group were compared to control *P < 0.05, **P < 0.01. ASWE: *Albuca setosa* water extract.

DISCUSSION

In the present study, the evaluation of anti-inflammatory effects was undertaken using the effect of ASWE on membrane stabilization, protein denaturation and migration of WBC during acute inflammation. The results of the study revealed that ASWE possess membrane stabilizing property, as it offered significant protection of human RBC membrane against lysis induced by heat or hypotonic medium. ASWE also decreases protein denaturation and the migration of WBC to the site of acute inflammation induced by carrageenin and dextran significantly.

Another key aspect of inflammatory response is cellular infiltration due to the pivotal role played by leukocytes. As part of their defensive roles during inflammation, these cells release their lysosomal contents such as bactericidal enzymes and proteases causing further tissue damage and inflammation (Okoli et al., 2008; Chou, 1997). Such injury to cell membrane will further render the cell more susceptible to secondary damage through free radical induced by lipid peroxidation. Membrane proteins are largely responsible for the physical properties of the cell membrane and may contribute to the regulation of the volume and water content of cells by controlling the movement of sodium and potassium ions (Okoli et al., 2008; Rowman, 1996). Since the RBC membrane is similar to that of lysosomal membrane, inhibition of RBC heamolysis will therefore, provide good insights into the inflammatory process especially as both events are also consequent of injury. Injury to lysosome membrane usually triggers the release of phospholipase A2 that mediates the hydrolysis of phospholipids to produce inflammatory mediators (Umukoro et al., 2006; Aitadafoun et al., 1996). Stabilization of the membranes of these cells inhibits lysis and subsequent release of the cytoplasmic contents which in turn limits the tissue damage and exacerbation of the inflammatory response (Okoli et al., 2008). It is therefore expected that compounds with membrane stabilisation activity should offer significant protection of cell membrane against injurious substances. *In vitro*

assessment of the effect of *A. setosa* on membrane stabilization showed that it inhibited heat and hypotonicity-induced lysis of red blood cells. Extracts with membrane stabilizing properties are well known for their interring activity with the early phase of the inflammatory mediators release, namely, the prevention of phospholipases release that trigger the formation of inflammatory mediators (Aitadafoun et al., 1996). Although, we do not know the precise mechanism of membrane stabilization in this case, direct interaction of constituents of the extract with membrane components such as proteins seems most probable. Membrane proteins are largely responsible for the physical properties of the cell membrane and may contribute to the regulation of the volume and water content of cells by controlling the movement of sodium and potassium ions through the protein channels which make up ion channels in the cell membrane. To verify this suggestion, we evaluated the effect of ASWE on protein denaturation activities.

Denaturation of proteins is well documented and is caused by inflammation process, mostly in conditions like arthritis. Thus, protection against protein denaturation, which was the main mechanism of action of NSAIDs postuled by Mizushima (1964) before the discovery of their inhibitory effect on cyclooxygenase (Vane, 1971), may play an important role in the antirheumatic activity of NSAIDs. The ability of ASWE to inhibit thermal and hypotonic protein denaturation may contribute to its anti-inflammatory properties. Under normal conditions, WBC circulates in the blood stream waiting to be called by damaged tissues to the site of injury or infection. Movement of the leukocytes into the damaged tissue from blood requires the cells to squeeze between endothelial cells that line the blood vessels wall. This rapid process known as transendothelial cell migration (TECM) or diapedesis leads to the normal inflammation process of the tissue. Furthermore, inhibition of cell migration or infiltration is associated with antiinflam-matory effect (Okoli et al., 2008). Cellular migration involves a sequence of well-documented events such as expression of chemoattractant factors and response of

these cells to chemotactic signals is central to their migration (Okoli et al., 2008). Carrageenan and dextran induced increase in total WBC count in the inflammatory exudates of the rat paw and the mice peritoneal fluid, respectively. It is consistent with the fact that increase in WBC population occurs at the site of inflammation. The increase in the total WBC count could mean that either there was migration of WBCs to the site or increase in the population of the white cells pre-existing at the site of inflammation. Leukocyte aggregation at the site of inflammation is a fundamental event in the inflammatory process. Cell migration occurs as a result of much different process including adhesion and cell mobility. The decrease in the WBC count caused by the ASWE and indomethacin suggests that the two agents inhibited migration of WBCs to the site of inflammation. It is well established that NSAIDs inhibit migration of inflammatory cells by inhibiting the release of chemical mediators (Almieda et al., 1980; Prempeh et al., 2008), inhibiting the expression of cell adhesion molecules (Gonzalez et al., 1998; Endemann et al., 1997) and inhibiting cell motility (Phelps et al., 1971). Considering our finding, it is possible that ASWE inhibited migration of the WBCs by one or more of these mechanisms.

In summary, ASWE has been confirmed to possess membrane stabilization properties, limit protein denaturation process and white blood cell anti-migration properties. This can contribute to the validation of the anti-inflammatory activity of the plant and may provide some evidence for its folk use and further exploitation. On the other hand, it is also suggested that identifying the potent fractions or components is essential and remains to be clarified in further studies.

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