

# Ultrastructure of platelets and fibrin networks of asthmatic mice exposed to selenium and *Withania somnifera*

Etheresia Pretorius · Hester Magdalena Oberholzer ·  
Warren A. Vieira · Eureka Smit

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**Abstract** Platelets and fibrin play an important role in allergic processes, including allergic asthma. The asthmatic BALB/c mouse model was used to induce asthma, and asthmatic mice were treated with the anti-inflammatory plant *Withania somnifera*, separately and in combination with the antioxidant selenium. Selenium is an important supplement in asthma, because asthmatics may have a selenium deficiency. Hydrocortisone was used as positive control. Results indicate control mice possess major thick fibers, minor thin fibers, and tight round activated platelets with typical pseudopodia formation. Minor fibers of asthmatic mice have a netlike appearance covering the major fibers whereas the platelets form loosely connected, granular aggregates. Hydrocortisone made the fibrin more fragile and platelet morphology changes from a tight activated platelet to a more granular activated platelet, not closely fused to each other. The plant extracts, separately and in combination with selenium did not affect the fragility of the fibrin and reversed the formation of the dense minor netlike layer over the major fibers, and the platelets formed a dense aggregate. Asthmatic mice treated with selenium showed a dense minor fibrin layer; however, the platelets formed a dense aggregate. We conclude that the anti-inflammatory products affect the stability of fibrin networks but not platelet stability (seen with hydrocortisone). Selenium does not affect the stability of the fibrin networks, but affects platelet stability. These results suggest that asthmatic patients should indeed use an antioxidant supplement, e.g. selenium, because it stabilizes

activated platelets, together with anti-inflammatory products.

**Keywords** *Withania somnifera* · Selenium · Platelets · BALB/c asthmatic model

## Introduction

Platelets and fibrin play an important physiological role in allergic processes and immunological mechanisms. In conditions such as allergic asthma, platelets participate by acting as inflammatory cells, by releasing mediators, spasmogens, and/or by interacting with other inflammatory cell types. Platelets are activated by a number of stimuli resulting in the expression and/or activation of surface receptors, secretion of vaso-active substances, adhesion, aggregation, and finally thrombus formation (Lazarus et al. 2003). The activation may be because of, among others, inflammatory processes (Camera et al. 1999; Butenas and Mann 2002). In previous research Pretorius et al. (2007) showed that the murine asthma BALB/c model can be used successfully to study platelets and fibrin. The effects of the plant *Euphorbia hirta* (a phytomedicine traditionally used to treat asthma) on platelet and fibrin formation and morphology was studied to determine how these products affect the coagulation system.

In our current research we investigated the effect of the phytomedicine *Withania somnifera* and the well-known anti-oxidant selenium in the treatment of asthma. Selenium is an important anti-oxidant in the treatment of asthma (Anbalagan and Sadique 1982; Chu et al. 1988; Davis and Kuttan 2000).

Because of the importance of investigating the effect of anti-inflammatory herbal medicine and anti-oxidants, for

E. Pretorius (✉) · H. M. Oberholzer · W. A. Vieira · E. Smit  
Department of Anatomy, University of Pretoria,  
PO Box 2034, Pretoria 0001, Gauteng, South Africa  
e-mail: resia.pretorius@up.ac.za

example selenium, in the treatment of asthma, the BALB/c murine asthma model was used. Previous research indicated that *W. somnifera* alone and in combination with selenium lowered inflammatory white blood cell counts in bronchial lavage and blood smears (Oberholzer et al. 2009). Therefore, the current research investigated the effect of *W. somnifera* and selenium separately and in combination on platelet and fibrin ultrastructure.

## Methods

### Preparing water extracts of *W. somnifera*

Plant material was collected in the Pretoria region of South Africa. A herbarium specimen was prepared and compared with an authentic specimen in the HGJW Sweikerdt herbarium at the University of Pretoria.

The aboveground parts of the plant were allowed to dry at room temperature for one week. The material was ground to a fine powder and subdivided into four 50-g samples. The samples were subsequently extracted with 500 ml water, after which it was filtered and dried on a rotary evaporator at 40°C. The water extract was freeze-dried. This procedure was repeated three times for each sample. A stock solution of 330 mg/ml was prepared, and administered orally to the mice, with each animal (average weight 20 g) receiving 45 µl of plant material. This dose was decided after study of literature that mentioned physiological doses suggested by herbalists. Rasool and Varalakshmi (2006) performed an animal study in which they used *W. somnifera* root powder at a dosage of 500/1,000 mg/kg of body weight. In the current study this dosage was used and converted back to the average weight of a BALB/c mouse (Rasool and Varalakshmi 2006).

### Implementing the BALB/c asthma model

Six-week-old (female) BALB/c mice each of average weight 20 g maintained in the University of Pretoria Biomedical Research Centre (UPBRC) and provided OVA-free food and water ad libitum, were used. All experimental protocols complied with the requirements of the University of Pretoria's Animal Use and Care Committee.

Mice were divided in the following groups (six animals per group):

- Control mice
- Asthmatic mice
- Mice exposed to physiologically comparable levels of *W. somnifera* (45 µl/kg of body mass)
- Mice exposed to anti-oxidant (selenium) (0.02 µg/100 µl)

- Mice exposed to combination *W. somnifera* and anti-oxidant (45 µl + 100 µl)
- Mice exposed to a low dose of hydrocortisone (100 mg/kg)

Sensitization (on day 0 and day 5) of mice was by intraperitoneal injection of 25 mg OVA (grade V; Sigma–Aldrich) and 2 mg Al(OH)<sub>3</sub> dissolved in 0.5 ml 0.9% saline solution. All mice except the control mice (which were left untreated) were sensitized.

Nebulization with 1% OVA in PBS (1 mg OVA in 100 ml PBS) was performed twice daily, for 1 h on days 13, 14 and 15. *W. Somnifera*, selenium, the combination of *W. somnifera* and selenium, and hydrocortisone was administered on days 15 to 18, and again on days 21, 22, 25, and 28. Animals were again nebulized on days 34, 35, and 36 and treated daily from days 39 to 42 and the animals were terminated on day 43.

### White blood cell counts

Blood samples of mice from each group were collected by cardiac puncture on the day of termination, pooled, citrate was added (11 µl citrate per 100 µl blood), and histological blood smears were prepared and stained with Giemsa Wright stain. A total of hundred leucocytes were counted in each blood smear.

### Preparation of fibrin clots

On day 43 during termination, 100–500 µl of blood was drawn from the heart of each of the BALB/c mice in each of the groups and 11 µl citrate for every 100 µl of blood was added. Blood was then centrifuged at 1,000 rpm for 2 min to obtain platelet-rich plasma (PRP).

Human thrombin (provided by The South African National Blood Services) was used to prepare fibrin clots (Pretorius et al. 2006). The thrombin is 20 U/ml and is made up in biological buffer (PBS) containing 0.2% human serum albumin. When thrombin is added to PRP, fibrinogen is converted to fibrin and intracellular platelet components, e.g. transforming growth factor, platelet-derived growth factor, and fibroblastic growth factor are released into the coagulum.

A total of 10 µl mouse PRP was mixed with 10 µl human thrombin. The PRP and thrombin mix was immediately transferred with a pipette tip to a 0.2-µm Millipore membrane to form the coagulum (fibrin clot) on the membrane. This Millipore membrane was then placed in a Petri dish on filter paper dampened with PBS to create a humid environment and placed at 37°C for 10 min. A washing process in which the Millipore membranes with the coagula were placed in PBS and magnetically stirred

for 120 min followed this. This was done to remove any blood proteins trapped within the fibrin network (Pretorius et al. 2006).

#### Preparation of washed fibrin clot for SEM

Washed fibrin clots were fixed in 2.5% glutaraldehyde in Dulbecco's phosphate-buffered saline (DPBS) buffer of pH 7.4 for 1 h. Each fibrin clot was rinsed thrice in phosphate buffer for 5 min before being fixed for 1 h with 1% osmium tetroxide ( $\text{OsO}_4$ ). The samples were rinsed thrice with distilled water for 5 min and were dehydrated serially in 30, 50, 70, 90% ethanol and three times with 100% ethanol. The SEM procedures were completed by critical point drying of the material. The material was mounted and coated using ruthenium ( $\text{RuO}_4$ ) (van der Merwe and Peacock 1999). Material was exposed to  $\text{RuO}_4$  for 1 h, yielding the material completely conductive and stable, even at very high magnifications. Material was examined with a Zeiss Ultra 55 FEG.SEM with an accelerating voltage of 3.00 kV (EHT = 3.00 kV).

Preparation of the fibrin clots for SEM was performed in triplicate. Therefore, three filters from each experimental group were investigated using SEM. All three samples from each group showed the same trend and only one micrograph was chosen to include in this article.

#### Fiber thickness measurement

Fiber thickness was assessed using SEM micrographs of the fibrin clots from each of the specified experimental mice groups. Approximately 100 fibrin fiber diameters, at random within each of the visualized fibrin networks, were marked by use of Photoshop version 6.0.1. The marked diameters were subsequently quantified, at the specified positions, with the aid of UTHSCSA Image tool version 3.00. The recorded diameters were then tested for normality in their distribution, with the aid of the Shapiro–Wilk  $W$  test for normality in NCSS software, because the notion of specific thick and thin fibers constituting the fibrin network would be associated with a bi-variant distribution in fibrin diameter and not a classical “bell-shaped” distribution curve. The data for each experimental group were plotted as a histogram by the NCSS software, to enable the distributions of fiber diameters to be visualized and to subsequently allow for any fibrin size classes to be quantified and the recorded diameters to be classified according to the above quantified fibrin size classes. The recorded diameters were then classified according to the above quantified fibrin size classes. The classified fibrin size classes were then statically compared within and between each experimental group by one-way ANOVA or Kruskal–Wallis one-way ANOVA—depending on whether

or not the necessary assumptions for the parametric tests were met. This procedure enabled assessment of whether the quantified tri-variant distribution was statically significant for each experimental group and whether or not there was a different in the size classes between the different experimental groups. Because the number of fibers ascribed to each of the constructed size classes differed, a random set of fibers was chosen for each size class of each group so that equal sample sizes could be compared. This enabled the assumption of equal variance to be ignored.

## Results

#### White blood cell counts

White blood cells were counted in blood smears to confirm the asthmatic status of the animals when compared with control animals, and to confirm the anti-asthmatic effects of the plant, the selenium, and the combination. Results from the blood smears showed that cell counts in control and hydrocortisone blood smears were decreased compared with that of the asthma group (Fig. 1). Also, results from the animals treated with *W. somnifera* and *W. somnifera* combined with selenium showed significantly reduced numbers of white blood cells, suggesting that *W. somnifera* indeed has an anti-inflammatory potential and it, in combination with an anti-oxidant, for example selenium, might successfully be used in the treatment of asthma.

#### SEM of platelets and fibrin networks

Fibrin networks are shown in Fig. 2a–f and platelets are shown in Fig. 3a–f. Transmission electron microscopy

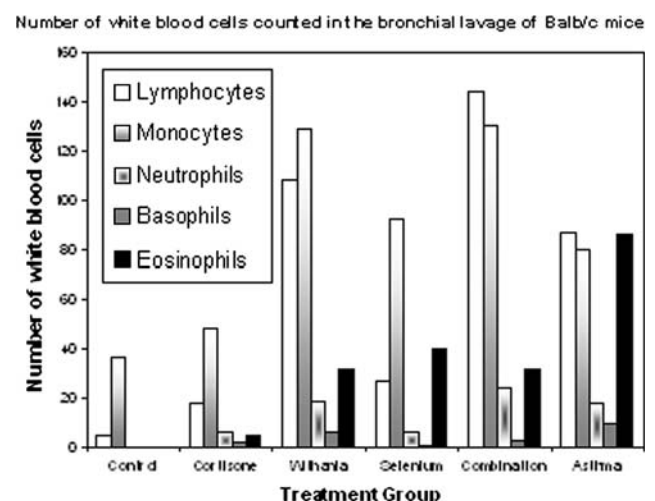
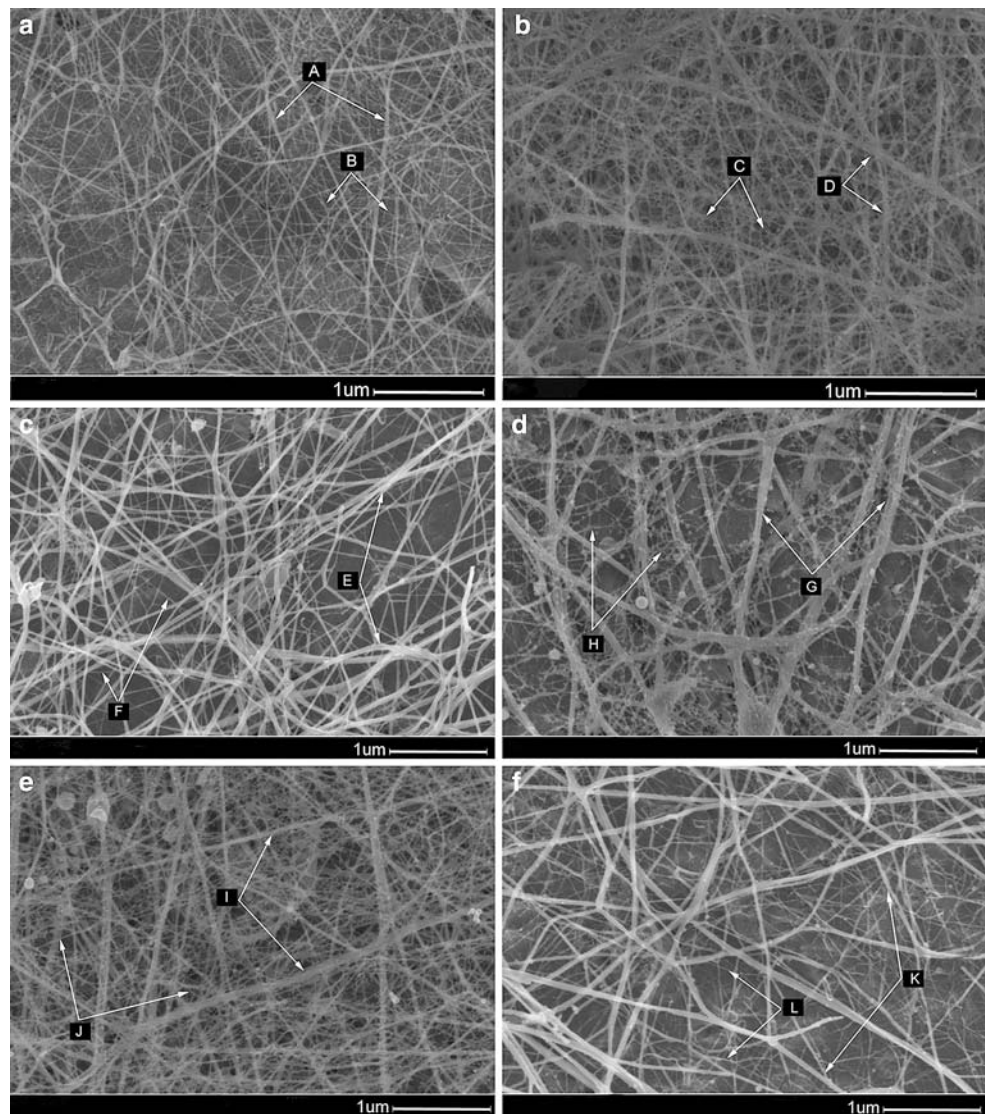


**Fig. 1** Numbers of white blood cells present in the bronchial lavage of BALB/c mice in the different treatment groups

**Fig. 2** **a** Control fibrin network with thick major fibers (A) and thin minor fibers (B). **b** Fibrin network from asthmatic BALB/c mice showing thick major fibers (D) and thin minor fibers forming a dense network (C). **c** Fibrin network from hydrocortisone-treated asthmatic BALB/c mice, forming flimsy fibrin network (100 mg/kg); E, thick major fibers; F, matted, thin minor fibers. **d** Fibrin network from asthmatic BALB/c mice treated with *Withania somnifera* showing fibrin network with thick major fibers (G) and thin minor fibers (H). **e** Fibrin network from asthmatic BALB/c mice treated with selenium showing fibrin network with thick major fibers (I) and thin minor fibers (J). **f** Fibrin network from asthmatic BALB/c mice treated with *Withania somnifera* and selenium, showing fibrin network with thick major fibers (K) and thin minor fibers (L)



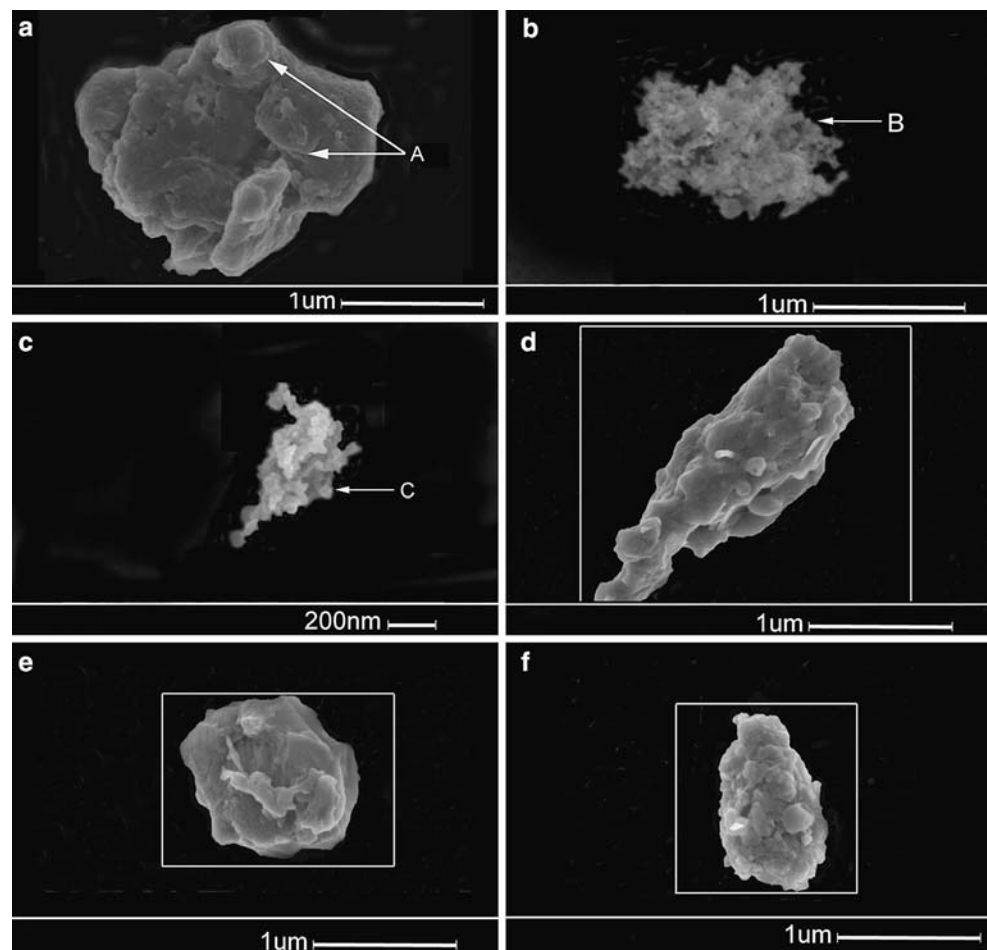
confirmed that the material studied was indeed platelets (results not shown). Control fibrin networks have major thick and minor thin fibers (Fig. 2a; labels A and B) whereas activated platelets form tight masses with pseudopodia protruding from the platelets (Fig. 3a; label A). Fibers of intermediate thickness are also present. Fibrin networks from asthmatic animals show thick major fibers (Fig. 2b; label D) and a thick, matted layer of thin minor fibers (Fig. 2b; label C). Platelets do not form a tight mass (Fig. 3b; label B).

Fibrin networks from hydrocortisone-treated animals showed a flimsy fiber network with both major and minor fibers present (Fig. 2c; labels E and F). Activated platelets also did not have a tight round appearance and were visible as granular, loosely arranged clumps and not attached closely to each other as seen in the controls (Fig. 3c; label C).

Fibrin networks from asthmatic mice treated with *W. somnifera* were similar in appearance to those from control mice, with major, thick fibers and few, thin minor fibers. Therefore, it seems as if *W. somnifera* stabilized the fibrin networks in the asthmatic mice (Fig. 2d; label G and H). The platelet shown in the block is similar in appearance to control platelets.

Platelet morphology of animals treated with selenium differed in ultrastructure from that of asthmatic animals (Fig. 2c, e). The platelets appeared similar to those of controls (Fig. 3a). Therefore, it is suggested that selenium promoted platelet stickiness, as opposed to the granular, loosely arranged platelet clumps as found in asthma. *W. somnifera*-treated platelets also showed this tight platelet arrangement. This plant is known for its anti-oxidant and anti-inflammatory potential. This therefore suggests that the anti-oxidant function might be the reason

**Fig. 3** **a** Control activated platelet; A, pseudopodia. **b** Activated platelet from asthmatic BALB/c mice; B, granular, loosely arranged platelet. **c** Activated platelet from hydrocortisone-treated asthmatic BALB/c mice (100 mg/kg); C, granular platelet. **d** Activated platelet from asthmatic BALB/c mice treated with *Withania somnifera*, forming platelet with pseudopodia; platelet in *block*. **e** Activated platelet from asthmatic BALB/c mice treated with selenium, forming tight platelet with pseudopodia; platelet in *block*. **f** Activated platelet from asthmatic BALB/c mice treated with *Withania somnifera* and selenium, forming platelet with pseudopodia, aggregate in *block*



why platelets of asthmatic mice treated with selenium and *W. somnifera* regain their tightly bound platelet structure.

Fibrin networks and platelets of asthmatic animals treated with *W. somnifera* in combination with selenium showed the same ultrastructure as those of the *W. somnifera*-treated group (Figs. 2f, 3f), where the thin fibers of the fibrin networks appeared the same as those of the controls (Figs. 2a, 3a).

#### Measurement of fibrin network thickness

For each of the experimental groups, the recorded fibrin diameters were assessed in their distribution with the utilization of a Shapiro–Wilk *W* test for normality. In all cases, as expressed in Table 1, the data were not normally distributed. The histograms revealed a distinct tri-variant count frequency distribution in the measured fibrin diameters, as seen in Fig. 4 (presenting control fibrin diameters, with each size class of this tri-variant distribution pattern being encompassed by a black line). Table 2 details the diameter ranges associated with each group's fibrin size classes. Three classes (size ranges) were visually

distinguishable (determined by the statistical method) within each of the analyzed groups. Table 3 shows, for each experimental group, the three fibrin size classes derived from the constructed histograms. For both the control and the *W. somnifera* and selenium combination group, the data failed to meet the assumption of normality, resulting in the non-parametric test—Kruskal–Wallis one-way ANOVA—being utilized. In all other cases, the necessary assumptions were met for utilization of parametric one-way ANOVA. It could thus be concluded that the fibrin fiber network conformed to a statically significant tri-variant size class distribution, because the fibrin fibers did not assume a normal distribution, with the three visually discernable size classes being significantly distinct from one another.

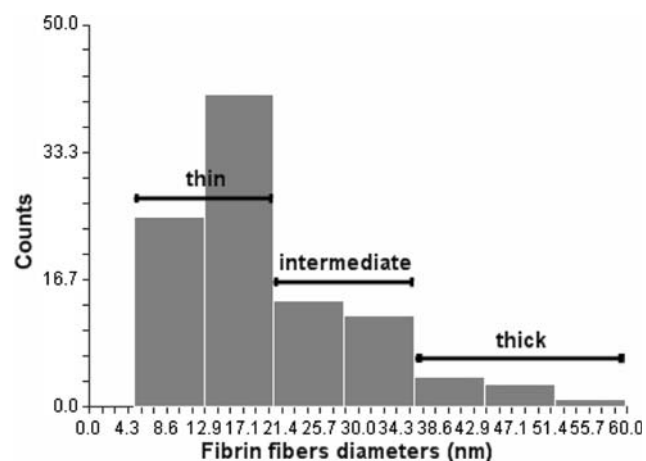
The corresponding fibrin size classes amongst some experimental groups seemed to vary from each other in terms of their size ranges. Table 4 expresses these differences quantitatively. Comparison of all six experimental groups' thin and thick fibrin fibers was performed with the aid of the non-parametric Kruskal–Wallis one-way ANOVA test, as the assumption of normality was not met

**Table 1** Analysis of the fibrin fiber diameter distributions for each experimental group, with the aid of a Shapiro–Wilk *W* test for normality

| Group       | <i>P</i> value* | Implication                       |
|-------------|-----------------|-----------------------------------|
| Control     | 1.01E–06        | Data assume a normal distribution |
| Asthma      | 3.93E–07        |                                   |
| Cortisone   | 1.20E–06        |                                   |
| WS          | 1.39E–06        |                                   |
| Sel         | 6.52E–08        |                                   |
| Combination | 9.52E–05        |                                   |

Sel, selenium group; WS, *Withania somnifera* group; Combination, selenium and *Withania somnifera* combination group

\*Significance was set at a level of 0.05

**Fig. 4** Histogram depicting the control group's fibrin diameters. The black lines indicate the three distinguishable fibrin fiber size classes present within the analyzed fibrin network

by the data in these two situations. The intermediate fibers were assessed between the six groups with the aid of a parametric one-way ANOVA, as the necessary assumptions for its utilization were met.

## Discussion

Oberholzer et al. (2009) have shown that bronchial lavage from asthmatic animals contains significantly more eosinophils. Also, bronchial lavage smears of the asthmatic group showed numerous platelet clumps that were distributed between white blood cells. Platelets were also found in the other treatment groups but are not as prevalent as in the asthmatic group. Results from the blood smears showed the same trends; cell counts in control and hydrocortisone blood smears were decreased compared with that of the asthma group (Fig. 1). *Withania somnifera* and *W. somnifera* combined with selenium significantly reduced the number of white blood cells in both bronchial

**Table 2** The fibrin fiber diameter size ranges associated with each experimental group's three fibrin size classes

| Group       | Thin fiber diameter range (nm) | Intermediate fiber diameter range (nm) | Thick fiber diameter range (nm) |
|-------------|--------------------------------|----------------------------------------|---------------------------------|
| Control     | 4–21                           | 22–37                                  | 38–60                           |
| Asthma      | 7–21                           | 22–67                                  | 68–112                          |
| Cortisone   | 10–35                          | 36–73                                  | 74–99                           |
| WS          | 6–25                           | 26–80                                  | 81–136                          |
| Sel         | 4–35                           | 36–63                                  | 64–107                          |
| Combination | 4–26                           | 27–47                                  | 48–80                           |

Sel, selenium group; WS, *Withania somnifera* group; Combination, selenium and *Withania somnifera* combination group

lavage and blood smears, suggesting that *W. somnifera* indeed has an anti-inflammatory potential and it, in combination with an anti-oxidant, for example selenium, might successfully be used in the treatment of asthma.

Previous research by Pretorius et al. (2007) showed control mice possess major thick fibers, minor thin fibers, and tight round activated platelets with typical pseudopodia formation. Minor fibers of asthmatic mice have a netlike appearance covering the major fibers, while the platelets seem to form loosely connected, granular aggregates. Hydrocortisone, however, made the fibrin more fragile and platelet morphology changed from a tight platelet to a more granular platelet, not closely fused to each other. From previous research it was concluded that hydrocortisone affects the fragility of the fibrin (fibers disintegrated easily while viewing with SEM) and prevents the minor fibers from forming the dense netlike layer over the major fibers, as is seen in untreated asthmatic mice (Pretorius et al. 2007).

The question asked, however, is whether fiber thickness can be assessed statistically, and whether there are differences between controls, asthmatic, and treatment groups. The literature is very vague about classification of fiber thickness. Shah et al. (1982), suggest that the diameters of the fibrin fibers in a clot are not uniform, and that morphometric analysis of transmission electron micrographs shows a bimodal distribution. The authors investigated the effect of polydispersity of fiber diameter on mass-length ratio calculated from the turbidity and permeability of a clot, with the aid of a two-network model. They suggested the words major and minor network. According to the authors, the fibers in the major network are many times thicker than those in the minor network.

Statistical analysis of control fiber thickness in the current study, however, revealed a distinct tri-variant count frequency distribution in the measured fibrin diameters, as seen in Fig. 4 (which depicts control fibrin diameters with each size class of this tri-variant distribution pattern being encompassed by a black line). Therefore it seems as if there

**Table 3** The within-group statistical analysis of the three constructed fibrin size classes, performed in order to determine if they were statistically distinct from each other

| ANOVA                                                                                                                           |             |                              |           |                                                                                                         |
|---------------------------------------------------------------------------------------------------------------------------------|-------------|------------------------------|-----------|---------------------------------------------------------------------------------------------------------|
|                                                                                                                                 | Asthma      | One-way ANOVA                | 5.20E−05  | Thick fibrin fibers significantly larger in diameter than both the intermediate and thin fibrin fibers. |
|                                                                                                                                 | Cortisone   | One-way ANOVA                | 1.00E−06  |                                                                                                         |
| Sel, selenium group; WS, <i>Withania somnifera</i> group; Combination, selenium and <i>Withania somnifera</i> combination group | WS          | One-way ANOVA                | <1.00E−06 | Intermediate fibrin fibers significantly larger in diameter than the thin fibrin fibers                 |
|                                                                                                                                 | Sel         | One-way ANOVA                | 2.00E−06  |                                                                                                         |
|                                                                                                                                 | Combination | Kruskal–Wallis one-way ANOVA | 1.00E−06  |                                                                                                         |
| * Significance was set at a level of 0.05                                                                                       |             |                              |           |                                                                                                         |

\* Significance was set at a level of 0.05

**Table 4** The between-group statistical analysis of the three constructed fibrin size classes, performed in order to determine if they were statistically distinct from each other

| Fibrin fiber size class | Test utilized                | P value*  | Difference between compared groups determined, if applicable, with the aid of the Tukey–Kramer multiple-comparison test                                                                                                                                                                                                                                                                          |
|-------------------------|------------------------------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Thick                   | Kruskal–Wallis one-way ANOVA | 0.0112    | The control group was associated with thick fibers with significantly smaller diameters in comparison with the asthma, cortisone, Sel and WS groups. The combination group's thick fibers were significantly smaller than those of the WS group                                                                                                                                                  |
| Intermediate            | One-way ANOVA                | <1.00E–06 | The control group was associated with intermediate fibers with significantly smaller diameters in comparison with the asthma, Sel and WS groups. The combination group was not significantly different in its associated intermediate fiber thickness in comparison with the control and asthma groups but was significantly smaller than comparable fibers of the Sel, WS, and cortisone groups |
| Thin                    | One-way ANOVA                | <1.00E–06 | The control group was associated with thin fibers with significantly smaller diameters in comparison with the Sel, WS, and cortisone groups. The cortisone group's thin fibers were significantly thicker in comparison with the control, asthma, Sel, WS, and combination groups                                                                                                                |

\* Significance was set at a level of 0.05

is a natural division of fiber thickness which allows the fibers to be divided into thin (minor) fibers, intermediate fibers and thick (major) fibers. The black lines seen in Fig. 4, enclosed the fibrin diameter ranges that could be ascribed to each fibrin size class. Table 2 shows the fibrin size classes and diameter ranges associated with each of the treatment groups and the asthmatic group whereas Table 3 shows the analysis of the three fiber size classes within each experimental group. The three fiber size classes derived from the constructed histograms are significantly different from one another—the diameters of the thick fibers being significantly larger than those of the intermediate fibers, which in turn are significantly larger than those of the thin fibers. For both the control and combination groups the data failed to meet the assumption of normality,

resulting in the non-parametric test—Kruskal–Wallis one-way ANOVA—being utilized. In all other cases, the necessary assumptions were met for utilization of parametric one-way ANOVA.

Table 4 shows results from between-group statistical analysis of the three constructed fibrin size classes, performed to determine if they were statistically distinct from each other. The diameters of the thick fibers of the control group were of significantly smaller than those of the asthma, hydrocortisone, selenium, and *Withania* groups. The thick fibers of the *Withania* and selenium combination group were significantly thinner than those of the *Withania* group. The diameters of the intermediate fibers of the control group were significantly smaller than those of the asthma, selenium, and *Withania* groups. The thickness of

intermediate fibers of the *Withania* and selenium combination group was not significantly different from those of the control and asthma groups but was significantly less than those of the selenium, *Withania*, and hydrocortisone groups. The control group was associated with thin fibers with significantly smaller diameters than those of the selenium *Withania* and hydrocortisone groups. The thin fibers of the hydrocortisone group were significantly thicker than those of the control group and all the other groups. It is therefore suggested that asthma causes changes in fiber thickness and it seems as if the combination *Withania* and selenium treatment has the best potential to stabilize these fibers.

Although selenium seems to play an important role in stabilizing asthma and it is known that asthma patients have decreased selenium levels, it is known that taking selenium supplementation on its own will not reduce the symptoms of asthma (Kadrobova et al. 1996; Jahnova et al. 2002; Allam and Lucane 2004). Results from the current study showed that the fibrin networks appeared similar to that of asthmatic mice. Therefore, selenium did not stabilize or protect the fibrin network in asthma. However, it seems as if selenium stabilized platelet structure. This might be because of its anti-oxidant potential.

*Withania somnifera* is both an anti-oxidant and anti-inflammatory product (Mishra et al. 2000). Fibrin fiber morphology and platelet morphology were both affected in animals treated with the plant. Hydrocortisone is a potent anti-inflammatory product but does not have anti-oxidant potential. It is therefore suggested that anti-inflammatory products affects fiber morphology whereas antioxidants promote platelet stickiness and stability in asthma.

From previous bronchial lavage and blood cell counts (Oberholzer et al. 2009) and results shown in Fig. 1 it was seen that *W. somnifera* on its own and in combination with selenium does have anti-inflammatory potential. The current research shows that the plant extract, alone and in combination with selenium, does has a stabilizing effect on fibrin networks and platelet structure. Selenium alone does not affect fibrin networks but activated platelets seemed to be stabilized. It is therefore concluded that the anti-inflammatory potential of *W. somnifera* and its possible use for treatment of asthma, should be investigated further. Furthermore, it is suggested that the effect of *W. somnifera* on the fibrin networks is because of its anti-inflammatory potential, and the stabilizing effect on the platelets is because of its anti-oxidant potential. It is known that selenium is a scavenger of free radicals, and previous research has shown that asthmatic patients have reduced selenium levels. From the current research we conclude that selenium has a stabilizing effect of the platelets but not

on fibrin networks. Its specific role in the physiology of asthma, however, should be investigated further.

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