International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 4 (5), pp. 001-007, May, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Analgesic and anti-inflammatory activities of *Aloe ferox Mill.* aqueous extract

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Accepted 16 January, 2015

Pharmacological activities of leaf gel and pulp of *Aloe ferox* have been extensively evaluated. However, there is scanty information on the pharmacological activities of the whole leaf of *A. ferox*. Carrageenan, histamine and formaldehyde- induced rat paw oedema were conducted to evaluate anti-inflammatory activity of *A. ferox* whole leaf aqueous extract. Tail flick, formalin and acetic acid tests were conducted to assess the analgesic activity of the plant. *A. ferox* exhibited highest anti-inflammatory and analgesic activities in the highest dose (400 mg/kg) tested. This dose level exerted highest anti-inflammatory activity; 78.2 and 89.3% for carrageenan and formaldehyde-induced rat paw oedema, respectively. The analgesic activity was 57.1 and 67.3% for the 400 mg/kg dose in phase 1 and 2, respectively of formalin test and 88.2% in acetic acid test. *A. ferox* reduced inflammation and relieved pain in rats at the highest dose level studied. This supports the extensive use of the plant as an antihelmintic reducing the inflammation and pain that might have been caused by gastro-intestinal parasite infections.

Key words: Leaf extract, pain reduction, oedema inhibition.

INTRODUCTION

Many plants have been part of the traditional healing practices among the indigenous people for centuries. One such plant is *Aloe ferox* Mill. or *Cape Aloe* found abundantly in the Southern, Western and Eastern Cape Provinces of South Africa (Van Wyk et al., 2002). The inhabitants of Southern Africa have known the healing and regenerative powers of *A. ferox* for centuries and the plant has maintained its popularity over the course of time. *A. ferox* has three usable parts: the green epidermis as fibre, the yellow, aloin rich bitter juice under the skin and the white inner flesh, rich in minerals, vitamins, amino acids, polysaccharides, enzymes and lipids (Viljoen, 2008). Today the plant has been accepted world wide for its superior antiseptic, cleansing, moisturizing and anti-inflammatory properties (Van Wyk et al., 2002).

One of the major uses of *A. ferox* is the control of gastro-intestinal parasites in village chickens (Mwale and Masika, 2008). These parasites cause marked economic losses; worms are particularly known to damage the intestinal lining leading to poor feed conversion efficiency and utilization (Mungube et al., 2008). This subsequently affects adversely the contribution of chickens to the livelihoods of the most vulnerable rural households in developing countries (FAO, 2003; Mack et al., 2005). To ameliorate the problem of gastrointestinal parasite infestation, resource-limited farmers have resorted to the extensive use of medicinal plants (Thamsborg et al., 1999; Siamba et al., 2007; Exner, 2008), *A. ferox* in particular. Medicinal plants are opted to commercial drugs due to the exorbitant cost of commercial drugs, residual effects, parasites resistance and inaccessibility to most farmers. The pharmacological activities of the plant have been evaluated on the leaf gel and the pulp (Vazquez et al., 1996; Van Wyk et al., 2002; Yagi et al., 2003; Langmead et al., 2004) . Yagi et al. (2002) worked on Aloesin derivatives that are reported to possess strong DPPH radical and superoxide anion scavenging

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activities. Yagi and Takeo (2003) and Speranza et al. (2005) reported on the anti-inflammatory compound aloeresin I, while Yates et al. (1992) worked on polysaccharides that are reported to act as immunostimulants. No work has been done on the whole leaf of *A. ferox* which the famers are using. It is, therefore, imperative to evaluate the pharmacological properties of the aqueous extract of the whole leaf of *A. ferox*'s to recommend its use in the control of gastrointestinal parasites in chickens for the subsequent improvement of rural livelihoods. In the current study, the analgesic activity of *A. ferox* using the formalin, tail flick and writhing test was investigated, together with the anti-inflammatory activities of the plant using the carrageenan, formaldehyde and histamine tests. These pharmacological experiments were conducted to determine if the plant has the capability of reducing pain and gastric oedema which might be caused by gastro-intestinal parasite infestation.

MATERIALS AND METHODS

Plant material collection

Fresh leaves of *A. ferox* were collected from Centane district (32°38'63'' S altitude and 28°24'36'' E latitude with the elevation of 50 m) in the Eastern Cape Province of South Africa in October 2007. The material was identified by Tony Dold of Selmar Schonland Herbarium at Botany Department, Rhodes University. A voucher specimen (MMAN 2007/01) was deposited in the Giffen Herbarium at the University of Fort Hare.

Aqueous extraction

The collected leaves were washed in cold water; spines around the leaves were removed using a knife after which the leaves were sliced. Two hundred grams of the sliced material were mixed with 100 ml of distilled water (as per the farmers or herbalists' procedure) and blended in an electric blender for 3 min (Githiori et al., 2003) to obtain 200% (w/v) extract. The blended material was squeezed through a muslin cloth. The filtrate was freeze-dried at -50°C under vacuum using a lyophiliser (Savant Refrigerated Vapor Trap, RVT 4104, USA) and kept in a freezer at −20°C until use. Various dose levels of the *A. ferox* were made by reconstituting the extract at a concentration of 1% (w/v).

Animals and experimental design

Thirty Wistar rats, of either sex, weighing 115 ± 35 g were used. The rats were bred in the Animal House at Agricultural and Rural Development Research Institute (ARDRI) Department, University of Fort Hare under standardised environmental conditions (ambient room temperature 25 ± 2°C and 12 h light-dark cycle). For each of the experiments conducted and explained below, a completely randomised design was used in which the rats were randomly grouped into six groups of five rats each. The rats were allowed free access to standard commercial rat pellets (EPOL Feeds Ltd, South Africa). Clean water was provided *ad libitum* throughout the experimental periods. Ethical procedures for using rats were according to the University of Fort Hare ethics committee's and international standards (Austin et al., 2004; Marie, 2006).

Chemicals and drugs

The chemicals used; carrageenan, formaldehyde, histamine, acetic acid, indomethacin and Tween 80 were all of analytical grade and from Sigma-Aldrich Chemie Gmbh, Steinheim, Denmark.

The anti-inflammatory activities

Carrageenan-induced rat paw oedema

Distilled water and indomethacin were administered intraperitoneally (i.p.) to rats in group 1 (negative control; 5 ml/kg body weight) and group 2 (positive control; 10 mg/kg body weight) respectively. The extract (50, 100, 200 and 400 mg/kg body weight i.p. (intraperitoneally)) was administered to rats in group 3 - 6 respectively. An hour later, the rats were injected with 0.05 ml of 1% carrageenan suspension into the foot pads of the left hind paws (Asongalem et al., 2004). Linear diameters of the injected paws were measured using a micrometer screw gauge (Sterling Manufacturing Co., SMC 20326, India) for four hours at one hour intervals. Increases in the paw diameter were taken as an indication of paw oedema. The percentage inhibition of inflammation was computed using the formula by Adedapo et al. (2008):

$$\% \text{ inhibition} = \left[\frac{D_0 - D_t}{D_0} \times 100\% \right]$$

Where: D_0 = the average inflammation (hind paw oedema) of the negative control group at a given time period; D_t = the average inflammation (hind paw oedema) of the treated group at a given time period.

Formaldehyde-induced paw oedema

The experimental rats in group 1 and groups 3 - 6 orally received 5 ml/kg body weight of distilled water and graded levels of the extract (50, 100, 200 and 400 mg/kg body weight) respectively, for 7 consecutive days. Rats in group 2 were administered with indomethacin (10 mg/kg body weight s.c. (sub-cutaneous)). After one hour, on the first and the third day of the experimental period, the rats were injected with 0.1 ml of 2% formaldehyde into the foot pad of the left hind paw according to Dharmasiri et al. (2003). On the first day, paw oedema was measured using a micrometer screw gauge (Sterling Manufacturing Co., SMC 20326, India) an hour before and 4 h after formaldehyde injection. On day 2 - 7 paw oedema was measured daily an hour after the treatment with the test extracts. The percentage inhibition of inflammation was calculated as in the Carrageenan-induced rat paw oedema.

Histamine-induced rat paw oedema

Using the method of Perianayagam et al. (2006), paw oedema was induced by the sub-plantar administration of 0.1 ml of a 0.1% freshly prepared solution of histamine into the right hind paw of rats.

The paw volume was recorded before the histamine injection (time 0) and 1, 2 and 3 h after the injection. Distilled water, indomethacin and the extract were intraperitoneally administered to the rats in group 1 (5 ml/kg body weight), group 2 (10 mg/kg body weight) and groups 3 - 6 (50, 100 200 and 400 mg/kg body weight) respectively, an hour prior to treatment with histamine. The percentage inhibition of the inflammation was calculated using the formula given above.

The analgesic activities

The formalin test

Pain was induced by injecting 0.05 ml of 2.5% formalin (20 µl 1%), through the subplantar route of the rats' left hind paws. Paired rats were placed in transparent Plexiglass cages (25 × 15 × 15 cm) observation chambers. According to Asongalem et al. (2004) the number of lickings were noted from 0 - 5 min (phase 1-neurogenic) and from 20 - 25 min (phase 2-inflammatory) (Lima et al., 2006) after intraplantar injection of formalin. Distilled water (5 ml/kg body weight i.p. (intraperitoneally)) and indomethacin (10 mg/kg body weight i.p.) were administered to the rats in group 1 and 2, respectively, 30 min before the formalin injection and the extract (50, 100, 200 and 400 mg/kg body weight i.p.) to groups 3 - 6, respectively. Percentage analgesic activity was calculated using the following formula (Asongalem et al., 2004):

$$\text{Percentage analgesic activity} = \frac{N - N1}{N} \times 100$$

Where: N is the average number of stretching of control per group and N^1 is the average number of stretchings of test per group.

The Tail-flick test

Distilled water (5 ml/kg body weight), indomethacin (10 mg/kg body weight) and graded dosage levels (50, 100, 200 and 400 mg/kg body weight i.p.) of extracts were administered to rats in group 1, group 2 and groups 3 - 6, respectively. The rats were held in position in a suitable restrainer with the tail extending out (Dharmasiri et al., 2003). The tail of the rat 4 - 5 cm from its tip was dipped into a water bath maintained at 55 ± 0.5°C. Each rat acted as its own control. Prior to the immersion of tails into the water bath, the reaction time was conducted at zero and 10 min interval. The average of the two was obtained as the initial reaction time (T_b). The reaction time (T_a) following the administration of the test drugs was measured. The time in seconds taken to flick or withdraw the tail out of the water was recorded as the reaction time due to the analgesia in the extracts and commercial drug. This was recorded after every 30 minutes for 3 hours. A cut off time of 25 seconds was used to avoid tissue damage (Lisoba et al., 2006; Dias et al., 2007). Percentage analgesic activity was calculated as per the formula shown below (Langmead et al., 2004):

$$\text{Percentage analgesic activity} = \frac{(T_a - T_b)}{T_b} \times 100\%$$

Acetic acid-induced writhing response in rats. To evaluate the analgesic effects of the plant extract, the method in earlier studies (Asongalem et al., 2004) was followed. Rats received the following treatment: distilled water at 5 ml/kg body weight (group 1), indomethacin at 10 mg/kg body weight (group 2) and plant extract at 50, 100, 200 and 400 mg/kg body weight (groups 3 - 6) intraperitoneally. Thirty minutes later, 0.6% acetic acid solution was administered intraperitoneally to all the experimental rats. The number of writhes occurring was counted for 30 min after a latency period of 5 min. A significant reduction of writhes in tested animals compared to those in the control group was considered as an anti-nociceptic response (reducing sensitivity to painful stimuli) and was calculated using the formula:

$$C\text{-}D/C \times 100$$

where C is the average number of writhings for the control group of rats and *D* is the average writhings of the extract treated rats (Asongalem et al., 2004; Gupta et al., 2005).

Acute toxicity

Five groups of five rats each were used. The test was conducted according to the method of Sawadogo et al. (2005), where rats received a single dose of the graded dose levels of the test extract. The control (group 1) received distilled water and group 2 - 5 received aqueous extract of *A. ferox* at doses 50, 100, 200 and 400 mg/kg body weight, respectively. Observations were made for any physiological and behavioural changes that included feeding behaviour, increased or decreased activity due to drug reaction, stress and rat mortality. The rats were observed continuously for 3 h soon after administering the extract, then hourly for 72 h.

Statistical analyses

The obtained data was analysed using the GLM (General Linear Model) procedures of the statistical analyses system (SAS, 2004) followed by the Dunnett's t-test for the comparison of treatment means against the control mean.

RESULTS

Acute toxicity test

The aqueous extract of fresh leaves of *A. ferox* did not cause mortality of rats during the 72 h ($P < 0.05$) period. The tested dose level did not ($P > 0.05$) affect the behavioural nor physiological changes of the experimental rats.

The anti-inflammatory activities

Carrageenan-induced rat paw oedema

There was a difference in the inhibition of inflammation caused by carrageenan on paws of rats ($P < 0.05$; Figure 1) for all the tested time levels. The 400 mg/kg body weight *A. ferox* dose level had the highest ($P < 0.05$) percentage inhibition of 78.2% followed closely by the 100 mg/kg body weight with 72.1%. The percentage inhibition of inflammation (59.9%) exerted by the

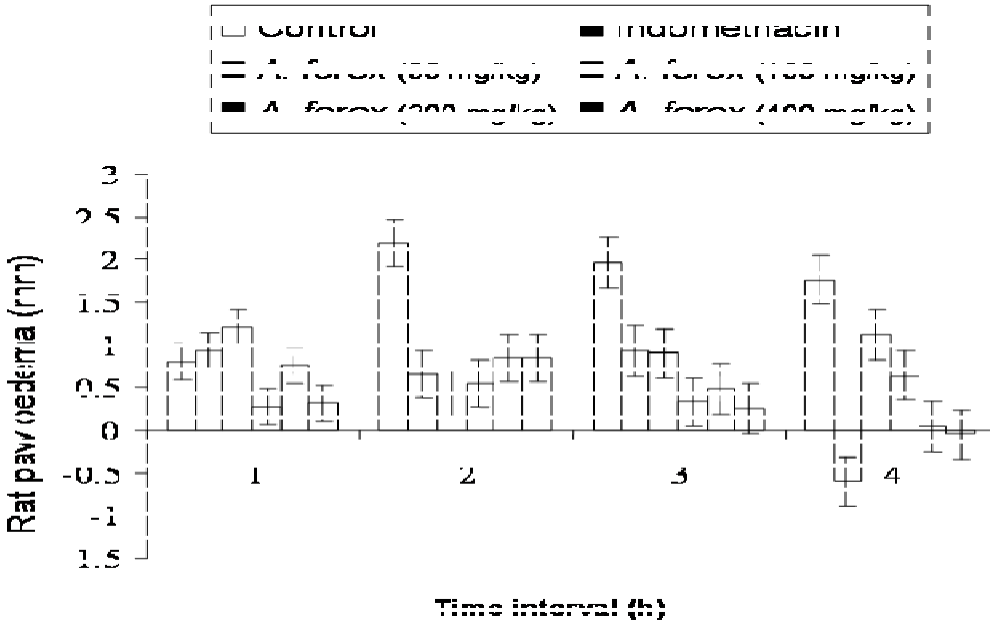

Figure 1. Effect of *A. ferox* aqueous extract and indomethacin on carrageenan-induced rat paw oedema (Error bar represents standard error of mean for a given dose at any given time interval).

200 mg/kg dose level was comparable to the commercial drug; indomethacin (60.3%) (Figure 1). The 50 mg/kg dose had the least inhibition percentage (30.6%).

Formaldehyde-induced paw oedema

The *A. ferox* aqueous extract significantly reduced inflammation of rat paws, in the 4^{th} and 7^{th} observation interval (Table 1). The 400 mg/kg body weight dose level had the highest ($P < 0.05$) inhibition percentage (89.3%).

Histamine-induced rat paw oedema

There was no significant difference in the paw oedema between the treated groups and the control group. The inflammation percentage inhibition was lowest; (5%) for the 400 mg/kg dose and highest (19.4%) for the 50 mg/kg dose rate. Generally inflammation increased from the first hour to the second hour interval and then it decreased at the third hour interval.

The analgesic activities

The formalin test

The aqueous extract of *A. ferox* did not ($P > 0.05$) reduce or eliminate pain of the rat paws that had formalin-induced pain. However, the analgesic activity was 57.1 and 67.3% for the 400 mg/kg dose in phase 1 and 2, respectively.

The Tail- flick test *A. ferox* did not ($P > 0.05$) show differences from the control in the reduction of reaction time of rat tails immersed in water at 55 ± 0.5°C. As indicated in Table 2; the dose 400 mg/kg body weight had a better analgesic percentage (56.1%) only at latency period 1.5 (one and half hours after treatment with the test drug).

Acetic acid-induced writhing response in rats

There was no difference in the writhing (stretching) response in rats of the test drug against the control ($P > 0.05$). However, the analgesic activity was high (88.2%) in the dose 400 mg/kg body weight; comparable to the commercial drug (indomethacin) which was 91.2% (Table 3).

DISCUSSION

The inhibition of inflammation in carrageenan-induced paw oedema of rats by the *A. ferox* aqueous extracts is concurrent with observations by Steenkamp and Stewart (2007). The anti-inflammatory activity of *A. ferox* is attributed to its gel containing three malic acid acylated carbohydrates: Veracylglucan A, B and C that demonstrate anti-inflammatory effects (Steenkamp and Stewart, 2007). In addition, the plant also contains the

Table 1. Inhibition of Inflammation (%) by the aqueous extract of *A. ferox* and indomethacin on formaldehyde-induced rat paw oedema.

Treatment	Dose (mg/kg)	Interval for paw oedema measurement (days)							Oedema Inhibition (%)
		1	2	3	4	5	6	7	
Control	-	0.64	1.06	1.82	-1.04	-0.77	-0.58	-0.43*	-
Indomethacin	10	0.64	0.54	1.58	-0.15*	-0.73	-0.30	-0.05	41.6
Extract	50	1.09	0.48	1.23	-0.39	-1.18	-0.76	-0.87	-15.2
Extract	100	0.77	0.47	1.46	0.76*	-0.77	0.30	-0.43	54.5
Extract	200	0.69	0.73	1.96	1.31*	0.09	0.46	0.60	11.5
Extract	400	1.15	0.52	1.53	0.53*	-0.29	0.31	0.73*	89.3
Standard error		0.298	0.314	0.280	0.202	0.231	0.396	0.280	-

*Values in a column with an asterisk are significantly different from the control ($P < 0.05$)
Negative values indicate that the absolute oedema paw volume change for the control was less than that of the treated groups.

anti-inflammatory compound aloeresin I (Speranza et al., 2005). Furthermore, *Aloe* is reported to contain the enzymes carboxypeptidase and bradykinase that tend to reduce inflammation and swelling (Duke, 1997). Inhibition of inflammation was observed also in the formaldehyde-induced rat paw oedema and in the histamine- induced oedema particularly for the highest dose level studied (400 mg/kg body weight); further supporting the presence of anti-inflammatory substances in the plant.

Since the anti-inflammatory activity of *A. vera* is attributed to the inhibition of arachidonic acid pathway through cyclo-oxygenase (Vazquez et al., 1996); the same can be accredited to *A. ferox*, since both plants are in the same family and of similar chemical constituencies (Viljoen, 2008). Also, the aqueous extract of *aloe* gel is reported to have inhibited the production of prostaglandins E2 from arachidonic acid *in vitro* (Vazquez et al., 1996). Prostaglandins tend to stimulate nerves that signal pain to the brain and are involved in the swelling of the blood vessels at the injured site, opening space in the capillary walls for the white blood cells to enter (Vazquez et al., 1996). Therefore, reduction in the level of prostaglandins results in reduction of paw oedema.

However, the low anti-inflammatory activity in both the histamine-induced rat paw oedema and the early phases of the formaldehyde-induced oedema could be attributed to the fact that the extract does not posses antihistamine properties and could not initiate leukocyte adhesion, respectively (Adedapo et al., 2008). The same authors sated that histamine is an important inflammation mediator, potent vasodilator substance and increases vascular permeability. Therefore, *A. ferox* aqueous leaf extract failed to inhibit the synthesis, release or action of inflammatory mediators such as histamine. However, the fact that at least some anti-inflammatory activities were noted indicates that apart from the use of anti-inflammatory compounds, the plant extract could have enhanced the immunity of the rats due to the immunomodulatory properties of *Aloes (*Zhang et al., 2006).

Previously it was reported that reduction in inflammation is also enhanced through the stimulation of the immune system to release white blood cells (Steenkamp and Stewart, 2007). It, therefore, could be that the immune cells were initiated already such that a sub-class of cytokines called *leukotrienes* (or *interleukins*) which ensures that the immune response is checked before it destroys outlying healthy cells and tissue called off the inflammatory response. In addition, it was reported that polysaccharides in *A. vera* act as immunostimulants, enhancing the release of cytokines, which in turn stimulate an increase in the replication of the fibroblast that are partially responsible for reduction in inflammation (Yates et al., 1992).

Although there was no difference in the elimination of pain of the test extract and the control under the formalin, tail flick and acetic acid test, the highest dose of the *A. ferox* aqueous extract showed some remarkable and commendable analgesic activities. This proved that at the highest dose level tested, the plant could be safely said to possess analgesic properties. The analgesic activity of the plant is attributed to the presence of the enzymes carboxypeptidases and bradykinase that tend to relieve pain (Duke, 1997). The plant is known to contain some alkaloids and steroidal substances responsible for the release of pain. In addition, the presence of two dihydrocoumarins with immunomodulatory and antioxidative properties has been reported in earlier studies (Zhang et al., 2006). These tend to assist in the reduction of pain through the stimulation of the immune system and the reduction of prostaglandins that are responsible for the pain. Thus, combination of the fairly non-toxic nature of the plant and the identified pharmacological properties is crucial for the plant as an antihelmintic (Magwa et al., 2006; Steenkamp and Stewart, 2007). The plant ultimately helps stop the bleeding as the plant cause increase in blood platelets level, damage and leakage of the intestinal wall of parasite infested chickens (Mwale and Masika, 2008), thereby taking the stress off the

Table 2. The effect of aqueous extract of *Aloe ferox* and indomethacin on pain using tail flick test.

Treatment	Dose (mg/kg)	Latency period (h)						
		0	0.5	1	1.5	2	2.5	3.0
Control	-	5.60	4.40 (-17.3)	4.40 (-19.5)	5.20 (-10.8)	5.20 (-11.5)	4.40 (-24.1)	4.00 (-29.5)
Indomethacin	10	7.10	6.60 (-12.2)	6.60 (-10.6)	7.80 (22.2)	7.00 (-0.6)	7.40 (12.8)	7.20 (3.3)
Extract	50	5.60	2.80 (-50.8)	4.00 (-28.8)	4.60 (-15.9)	4.80 (-14.7)	2.80 (-50.1)	3.40 (-39.7)
Extract	100	4.50	2.40 (-47.9)	4.20 (-2.1)	5.20 (16.5)	5.00 (14.1)	4.00 (-11.5)	4.00 (-10.9)
Extract	200	3.60	2.00 (-39.6)	3.00(33.3)	3.40 (-2.3)	4.20 (17.9)	4.00 (12.9)	4.20 (20.7)
Extract	400	3.60	2.80 (-23.2)	4.60 (33.7)	5.40 (56.1)	4.20 (18.9)	5.00 (42.1)	4.60 (29.2)
Standard error		1.375	1.424	1.439	1.378	1.458	1.400	1.420

Values in parenthesis represent inhibition of inflammation percentage.

Table 3. The effect of aqueous extract of *Aloe ferox* and indomethacin on writhing induced by acetic acid**.**

Treatment	Dose (mg/kg)	Number of writhings within 30 min	Inhibition (%)
Control	0	6.80	0
Indomethacin	10	0.60	91.2
Extract	50	2.80	58.9
Extract	100	4.00	41.2
Extract	200	5.60	17.7
Extract	400	0.80	88.2
Standard error		2.166	

immune system (Steenkamp and Stewart, 2007).

Conclusion

The *A. ferox* plant possesses some anti-inflammatory and analgesic activities. It effectively reduced inflammation and moderately relieved pain in rats at the highest dose level studied. Studies are underway to evaluate the nutritional composition of this plant that might synergistically assist in the relief of pain and inflammation in the gastro-intestinal tract due to the effect of gastro-intestinal parasite infestation.

ACKNOWLEDGEMENTS

The authors are indebted to the National Research Foundation of South Africa which funded the project. The Department of Livestock and Pasture Science, Microbiology and Biochemistry, Botany and the Agricultural and Rural Development Research Institute (ARDRI) staff and students for the invaluable technical support.

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