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*Full Length Research Paper*

# Anti-inflammatory and analgesic activities of the aqueous leaf extract of *Agave sisalana* in rats

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***Agave sisalana*** **is gaining popularity in controlling gastro-intestinal parasites in chickens and treatment of local inflammatory conditions. However, little is known about its anti-inflammatory and analgesic properties. These properties were determined using the carrageenan, formaldehyde and histamine-induced oedema, as well as formalin, tail flick and acetic acid-induced pain tests.** ***A. sisalana*** **extract was administered** ***per os*** **at 50, 100, 200 and 400 mg/kg body weight of rats. Indomethacin was used as positive control. The 400 mg/kg dose caused 93.4% inflammation inhibition of the carrageenan-induced odema, while 100 mg/kg had 84.9% for the formaldehyde-induced paw oedema. Inflammation inhibition was low (8.1%) for histamine-induced oedema at 400 mg/kg. For tail flick test analgesic activity was low, 55.8 and 0% at 100 and 400 mg/kg, respectively. As for the acetic acid test, analgesic activity was high; 77.2, 81.0, 84.8 and 84.8% for 50, 100, 200 and 400 mg/kg doses, respectively, and for the formalin test it was 66.7 and 100% at 200 mg/kg in phases 1 and 2, respectively. Findings of this study demonstrated that** ***A. sisalana*** **possesses some anti-inflammatory and analgesic properties. Further investigation is necessary to determine the nutritional composition of** ***A. sisalana*** **that may augment its pharmacological properties.**

**Key words:** Medicinal plant, oedematous inhibition, pain relief.

## INTRODUCTION

*Agave sisalana* (Agavaceae) is a short-stemmed aloe-like plant exotic to South Africa (van Wyk and Gericke, 2003). It is mainly planted as a source of food, beverages (mescal and tequila liquors), ornamental succulents, fibre and medicine (Gentry, 2004; Iwu, 1993). The plant is now widely distributed in Southern Africa (van Wyk and Gericke, 2003). Leaves of *A. sisalana* are long and firm, making them the main source of strong fibre for strings, ropes and mats (van Wyk and Gericke, 2003). Currently, *A. sisalana* is gaining popularity as a medicinal pant. In East Africa, the sludge obtained from the sap is used to prepare a lotion that is used for the treatment of local inflammatory conditions (Iwu, 1993).

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Resource-limited farmers in the Eastern Cape Province of South Africa use *A. sisalana* leaves to control gastro-intestinal parasites in village chickens (Mwale and Masika, 2009). The fresh leaves of the plant are crushed and mixed with water which is given to chickens to drink until the signs and symptoms caused by gastro-intestinal parasites infection disappear (Mwale and Masika, 2009).

Gastro-intestinal (GI) parasites cause marked economic losses to village chicken production through loss of blood, reduced weight gains and causing gastric pains and inflammation (Phiri et al., 2007). Use of conventional drugs to control GI and their effects such as pain and inflammation has become expensive, especially for resource-limited farmers. The farmers thus resort to the use of medicinal plants such as *A. sisalana*, which are cost-effective (Khatun et al., 2011; Mwale and Masika, 2009). However, there is dearth of information on the anti-inflammatory and analgesic properties of the

plant. Therefore, the objective of the current study was to evaluate the anti-inflammatory and analgesic properties of *A. sisalana* using carrageenan, formaldehyde and histamine to induce rat paw oedema, and histamine, tail flick and acetic acid tests to induce pain.

This was tested through the hypothesis that *A. sisalana* does not possess anti-iflammatory and analgesic properties.

## MATERIALS AND METHODS

### Plant

Fresh leaves of *A. sisalana* were collected from Centane district (32°38'63''S and 28°24'36''E; elevation 50 m) in the Eastern Cape Province of South Africa in October 2007. The material was identified at Selmar Schonland Herbarium, at Rhodes University's Botany Department. A voucher specimen (MMAN 2007/02) was deposited in the Giffen Herbarium at the University of Fort Hare.

### Extract preparation

The extract was prepared based on the proportions and methods used by resource-limited farmers and herbalists. The spines around the leaves were removed using a knife after which the leaves were sliced. The sliced material, 200 g, was mixed with 100 ml of distilled water to obtain 200% (w/v) extract and milled in an electric blender for 5 min (Githiori et al., 2003). The milled 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. One percentage concentration was used to reconstitute the extract (re-suspended or re-dissolved in water to make stock solution; 50, 100, 200 and 400 mg/kg doses of *A. sisalana* aqueous extract).

### Animals and experimental design

Thirty Wistar young rats of either sex, weighing 145 ± 20 g that were 6-8 weeks of age were used. The rats were bred in the Animal House at the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare under controlled environmental conditions (ambient room temperature 25 ± 2°C, and stand light from 0600 to 1800 h that is, 12 h light-dark cycle). For each one of the experiments conducted and explained below, except for the acute toxicity experiment, a completely randomised design was used in which the rats were randomly grouped into six categories of five rats each. Treatments were assigned to rats in groups 1 to 6 as explained below.

The rats were allowed free access to standard commercial rat pellets (EPOL Feeds Ltd, South Africa), except for the acute toxicity group where they were fasted for 16 h prior to the administration of the test aqueous extract. Clean water was provided *ad libitum* throughout the experimental period. Ethical procedures for using Wistar 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.

### Acute toxicity

Five groups of five rats each were used. The test was conducted according to the method of Sawadogo et al. (2006), where rats received a single dose of the graded doses of the test extract. The initial body weights of the rats were recorded. The control (group 1) received distilled water (5 ml/kg body weight), and groups 2 to 5 received aqueous extract of *A. sisalana* at doses 50, 100, 200 and 400 mg/kg body weight of the rat *per os* by means of a bulbed steel needle, 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.

### 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 *A. sisalana* extract (50, 100, 200 and 400 mg/kg body weight i.p.) was administered to rats in groups 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 Adedapo et al. (2008) formula:

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

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 to 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 sub-cutaneous (s.c.)]. After one hour, on the first and the third day of the experimental period, rats were injected with 0.1 ml of 2% formaldehyde [Solution of Paraformaldehyde (Paraformaldehyde is the powder/solid form of formaldehyde while formaldehyde is the gaseous form)] 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 days 2 to 7, paw oedema was measured daily an hour after the treatment with the test extracts, using a micrometer screw gauge (Sterling Manufacturing Co., SMC 20326, India). 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. Paw volume was recorded using a micrometer screw gauge (Sterling Manufacturing Co., SMC 20326, India) before 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 to 6 (50, 100 200 and 400 mg/kg body weight), respectively, an hour prior to treatment with histamine. The percentage inhibition of inflammation was calculated using the formula described above for the carrageenan-induced rat paw oedema experiment.

**The analgesic activities**

***The formalin test***

Pain was induced by injecting 0.05 ml of 2.5% formalin [a liquid form of formaldehyde (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 to 5 min (phase 1-neurogenic) and from 20 to 25 min (phase 2-inflammatory) (Lima et al., 2006) after intraplantar injection of formalin. Distilled water [5 ml/kg body weight intraperitoneally (i.p.)] and indomethacin (10 mg/kg body weight i.p.) were administered to the rats in groups 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 to 6, respectively. Percentage analgesic activity was calculated using the following formula (Asongalem et al., 2004):

$$\text{Percentage analgesic activity} = \frac{N\text{-}N^1 \times 100}{N}$$

Where: N is the average number of stretching of control per group and $N^1$ is the average number of stretching 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 to 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 min for 3 h. A cut off time of 25 seconds was used to avoid tissue damage (Dias et al., 2007; Lisoba et al., 2006).

Percentage analgesic activity was calculated as per the formula shown below (Langmead et al., 2004):

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

***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 treatments: 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 to 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-D*/C x 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).

**Statistical analyses**

Data obtained for anti-inflammatory and analgesic activities were analysed for the effect of *A. sisalana* on induced rat-paw oedema and pain, respectively using PROC GLM (General Linear Model) procedures of the Statistical Analyses System (SAS, 2004). Thereafter, Dunnett's t-test was computed for the comparison of treatment means against the control means.

## RESULTS

### Anti-inflammatory activities

***Carrageenan-induced rat paw oedema***

There was a significant difference in inflammation reduction from 2 to 4 h time intervals after inflammation was induced using carrageenan, between the extract (200 and 400 mg/kg doses) and the negative control ($P<0.05$; Figure 1).

The doses 200 and 400 mg/kg inhibited inflammation better than indomethacin at 2 to 4 h interval ($P<0.05$). The 50 and 100 mg/kg doses significantly exhibited anti-inflammatory properties at 3 and 4 h. Indomethacin was different from the negative control ($P<0.05$) at 4-h time interval in inhibiting inflammation. *A. sisalana* leaf extract showed some remarkable inflammation inhibition percentages of 53.6, 64.7, 85.4 and 93.4% for the 100, 50, 200 and 400 mg/kg body weight dose, respectively. Indomethacin showed an inflammation inhibition percentage of 31.2%.

***Formaldehyde-induced paw oedema***

As indicated in Table 1, paw oedema for rats on 100, 200

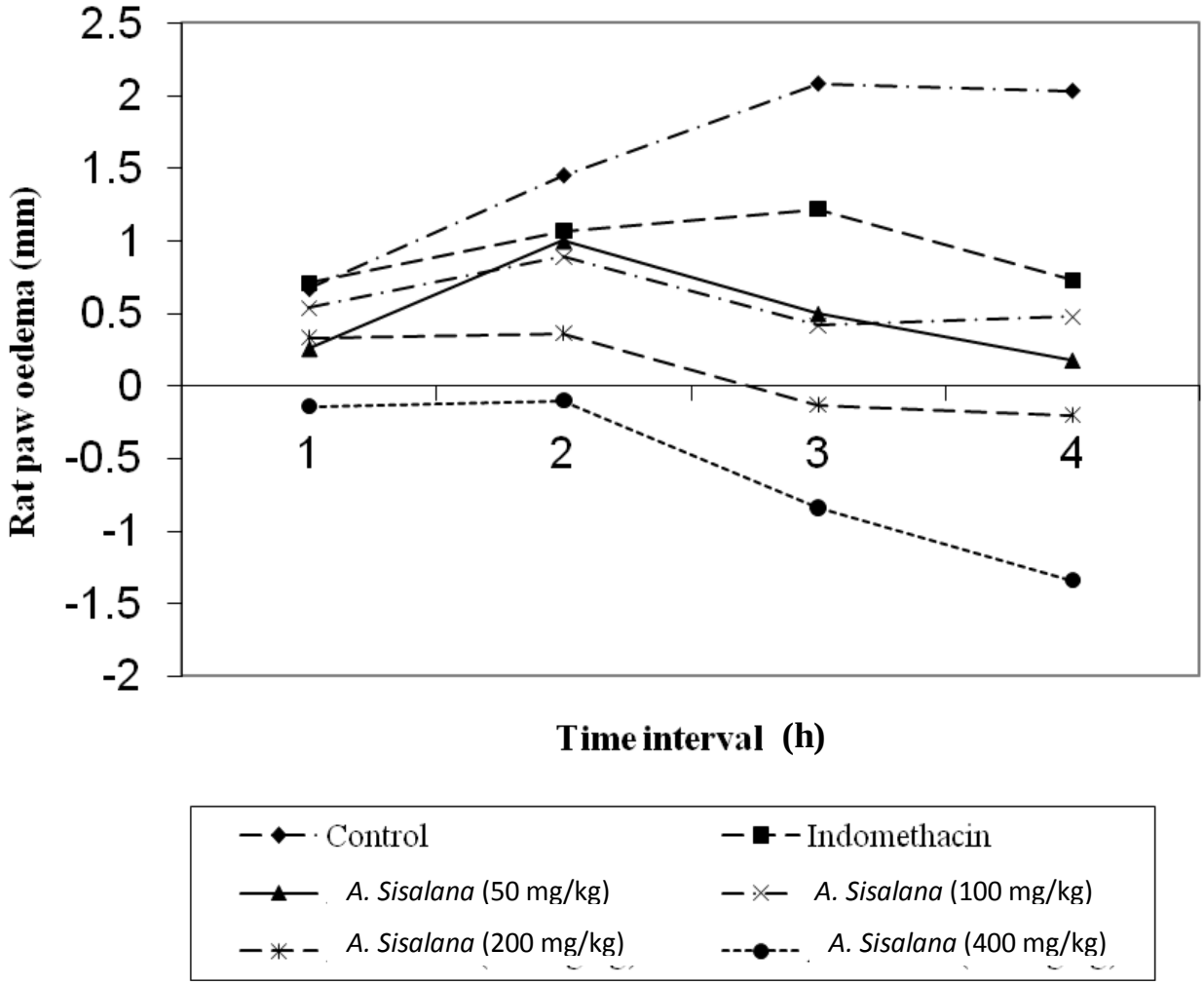


**Figure 1.** Effects of *Agave sisalana* aqueous extract and indomethacin on carrageenan-induced rat paw oedema

and 400 mg/kg body weight doses of *A. sisalana* aqueous leaf extract was different from the control ($P<0.05$) on day one. On day 2 to 7 there was no difference in the oedema of rat paws under the leaf extract and those under the control ($P>0.05$). The 100 mg/kg body weight dose level had the highest inhibition percentage (84.9%), whereas the 50 mg/kg body weight had the lowest (33.4%) compared to that of indomethacin (25.8%).

### *Histamine-induced rat paw oedema*

There was no difference in rat paw oedema between the groups under indomethacin and *A. sisalana* leaf extract, and the control group ($P>0.05$; Table 2) throughout the experimental period.

The inflammation inhibition percentage due to the leaf extract was generally low 8.1, 3.9, 2.2 and 1.4% for doses 400, 100, 50 and 200 mg/kg body weight, respectively.

## Analgesic activities

### *Formalin test*

As indicated in Table 3, there was no difference in the reduction of pain of rat paws between the aqueous leaf extract of *A. sisalana* and the negative control ($P>0.05$). However, the analgesic activity was 66.7 and 100% for the 200 mg/kg dose level in phase 1 and phase 2, respectively. The 50 mg/kg body weight dose exhibited the lowest analgesic activity in both phase 1 (13.3%) and phase 2 (16.7%).

### *Tail-flick test*

An hour after test drug administration, the reaction time of rats was significantly different among the leaf extracts

**Table 1.** The anti-inflammatory effects of aqueous leaf extract of *Agave sisalana* and indomethacin on formaldehyde-induced rat paw oedema

| Treatment | Dose (mg/kg) | Paw diameter (mm) in days interval | | | | | | | Oedema Inhibition (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Control | 0 | 1.8±0.30[a] | 0.8±0.29 | 2.1±0.34 | 0.9±0.32[a] | 0.4±0.22 | 0.1±0.23 | -0.3±0.26 | - |
| Indomethacin | 10 | 1.9±0.48 | 0.5±0.45 | 0.8±0.54 | -0.8±0.51[b] | 0.3±0.34 | 0.2±0.37 | -0.4±0.42 | 25.8 |
| Extract | 50 | 1.8±0.30 | 0.5±0.29 | 1.4±0.34 | 0.8±0.32 | 0.1±0.22 | -0.2±0.23 | -0.1±0.26 | 33.4 |
| Extract | 100 | 0.5±0.30[b] | 0.3±0.29 | 1.7±0.34 | -0.1±0.32 | -0.4±0.22 | -0.7±0.23 | -0.4±0.26 | 84.9 |
| Extract | 200 | 0.6±0.30[b] | 0.4±0.29 | 2.0±0.34 | 0.6±0.32 | -0.1±0.22 | -0.3±0.23 | -0.1±0.26 | 58.3 |
| Extract | 400 | 0.4±0.30[b] | 0.2±0.29 | 1.5±0.34 | 0.3±0.32 | 0.2±0.22 | -0.4±0.23 | -0.7±0.26 | 68.3 |

[b]Values in a column with a superscript are 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.

**Table 2.** Anti-inflammatory effects of aqueous leaf extract of *Agave sisalana* and indomethacin on histamine-induced rat paw oedema

| Treatment | Dose (mg/kg) | Paw diameter (mm) at time T (h) | | | Oedema Inhibition (%) |
|---|---|---|---|---|---|
| | | 1st hour | 2nd hour | 3rd hour | |
| Control | - | 0.3 | 0.8 | 0.1 | - |
| Indomethacin | 10 | 0.8 | 1.1 | 0.2 | 87.8 |
| Extract | 50 | 0.3 | 1.0 | 0.8 | 2.2 |
| Extract | 100 | 0.4 | 0.7 | 0.1 | 3.9 |
| Extract | 200 | 0.8 | 1.1 | 0.6 | 1.4 |
| Extract | 400 | 0.7 | 0.5 | -0.1 | 8.1 |
| Standard error | | 0.24 | 0.30 | 0.22 | |

Negative values indicate that the absolute change in pain for the control was less than that of the treated groups.
T1: Time at the 1st hour; T2: Time at the 2nd hour; T3: Time at the 3rd hour.

**Table 3.** The effect of aqueous leaf extract of *Agave sisalana* and indomethacin on formalin-induced pain

| Treatment | Dose (mg/kg) | Number of licks (min) | | | |
|---|---|---|---|---|---|
| | | Phase 1 (0-5 min) | | Phase 2 (15-30 min) | |
| | | Number of lickings | % inhibition of pain | Number of lickings | % Inhibition of pain |
| Control | - | 1.2 | - | 8.4 | - |
| Indomethacin | 10 | 4.0 | 33.3 | 12.8 | 52.4 |
| Extract | 50 | 2.8 | 13.3 | 7.0 | 16.7 |
| Extract | 100 | 1.6 | 23.3 | 13.4 | 59.5 |
| Extract | 200 | 0.4 | 66.7 | 0.0 | 100.0 |
| Extract | 400 | 0.8 | 33.3 | 1.0 | 88.1 |
| Standard error | | 1.12 | | 4.05 | |

(50, 100 and 200 mg/kg body weight) and the control. The extract exhibited analgesic activity for the 100 mg/kg body weight at 3 h after test drug administration (P<0.05).

As shown in Table 4, the percentage analgesic activity of the extract was highest for the 100 mg/kg body weight dose level (55.9%) and lowest for the 400 g/kg body weight dose level (0%) after a 3 h interval.

### *Acetic acid-induced writhing response in rats*

There was a difference in the writhing response in rats of the test extract and indomethacin against the control (P<0.05; Table 5). Analgesic activity was high for the entire dose levels and was comparable to that of indomethacin.

**Table 4.** Percentage analgesic activity of aqueous leaf extract of *Agave sisalana* and indomethacin on pain using the tail flick test

| Treatment | Dose (mg/kg) | Analgesic activity percentage at 30 min interval | | | | | |
|---|---|---|---|---|---|---|---|
| | | 30 | 60 | 90 | 120 | 150 | 180 |
| Control | - | 16.5 | -35.0 | -26.1 | -28.9 | 1.4 | -20.9 |
| Indomethacin | 10 | 14.1 | -12.6 | -1.9 | -3.0 | 13.7 | 3.9 |
| Extract | 50 | -16.9 | -32.5 | -31.7 | -13.7 | -12.9 | -23.9 |
| Extract | 100 | -18.6 | -5.0 | -2.7 | 0.4 | -25.0 | 55.9 |
| Extract | 200 | -40.3 | -13.6 | -31.0 | -22.2 | -36.9 | -34.7 |
| Extract | 400 | 9.3 | 18.3 | 19.6 | 3.2 | 32.0 | -0.0 |

Negative values indicate that the absolute change in pain for the control was less than that of the treated groups.

**Table 5.** The effect of aqueous leaf extract of *Agave sisalana* and indomethacin on writhings induced by acetic acid

| Treatment | Dose (mg/kg) | Number of writhings within 30 mins | Inhibition (%) |
|---|---|---|---|
| Control | - | 15.8[a] | - |
| Indomethacin | 10 | 4.4[b] | 72.2 |
| Extract | 50 | 3.6[b] | 77.2 |
| Extract | 100 | 3.0[b] | 81.0 |
| Extract | 200 | 2.4[b] | 84.8 |
| Extract | 400 | 2.4[b] | 84.8 |
| Standard error | | 1.59 | |

[b]Values in a column with a superscript are different from the control (P<0.05).

## DISCUSSION

The aqueous leaf extract of *A. sisalana* showed some inflammatory inhibition properties when inflammation was induced by carrageenan (acute inflammation). Carrageean-induced paw oedema in rats is believed to be biphasic. The first phase is attributed to the release of histamine or serotonin and the second phase is caused by the release of bradykinins (vasoactive substances), protease, prostaglandins and lysosomes (Akindele and Adeyemi, 2007; Maleki et al., 2008; Saha et al., 2007).

Therefore, it can be inferred that the inhibitory effect of the aqueous leaf extract of *A. sisalana* was due to the inhibition of the enzyme cyclooxygenase that leads to the inhibition of prostaglandin synthesis, and also through blocking the release of the vasoactive substances.

The finding that the extract reduced inflammation beyond the normal paw size of the rats could be attributed to the adverse effects of non-steroidal drugs. When non-steroidal anti-inflammatory drugs are either taken in excess, for a long time period or when two or more non-steroidal anti-inflammatory drugs are taken at the same time they cause some side effects (Argoff, 2007). The extract concentration might have been too high for the rats, thereby causing neurogenic atrophy that occurs when there is injury to a nerve. This type of muscle atrophy is the most severe and tends to occur suddenly (Campellone, 2007). The actual causes of reduction in inflammation beyond the normal paw size warrant investigations.

Formaldehyde-induced oedema has also been shown to be biphasic; originating mainly from a neurogenic inflammation (up to 24 h post injection) followed by participation of kinins and leukocytes with their pro-inflammatory factors that include prostaglandins (Popov et al., 2005). These authors reported that leukocyte adhesion represents one of the first steps in the inflammation response initiation and is essential for the accumulation of active immune cells at sites of inflammation. Accordingly, the extract showed the capability of enhancing leukocyte adhesion mechanism leading to the reduction of inflammation in the paw of the rat. However, the extract exerted effect more of the non-steroidal anti-inflammatory substances (acute inflammation) than for the steroidal drugs (chronic inflammation) since the inflammation inhibition was observed in day one of seven for the formaldehyde induced oedema.

The inflammation inhibition percentage was low for the histamine induced-paw oedema. Nonetheless, the anti-inflammatory findings of the current study indicate that the aqueous leaf extract of *A. sisalana* possesses some inflammatory inhibition properties. Iwu (1993) supported this, reporting that *A. sisalana* sap was used to make

lotion for the treatment of local inflammatory conditions. The plant is said to be comprised of diosgenin and steroidal saponins such as sisalagenin, tigogenin and neotigogenin that are responsible for reducing inflammation, wounds and stomach ailments (FAO, 2009; Iwu, 1993). The chemical constituents of *A. sisalana* that reduce inflammation and the extent to which they reduce inflammation is not known. Therefore, it is imperative to conduct research in order to elucidate this information.

The observation that the reaction time of rats under the tail flick test was slightly increased and there was no trend in the tolerance of thermal effect by the rats might indicate that the reaction time of the rats and pain reduction effect of the plant did not coincide. The low activity of the extract could be attributed to the fact that it was not yet absorbed into the system to reveal its analgesic properties. However, the intramuscular and intraperitoneal route of administration is considered for immediate analgesic effects (Gupta et al., 1998) compared to oral, rectal or topical administration methods. Nevertheless, aqueous extracts are absorbed faster than other drug preparations such as ethanol extracts, depending on route of administration. The analgesic activity of the plant could be confounded with haemolytic and toxic substances that the plant potentially has (FAO, 2009). Nonetheless, the findings show that the plant extracts increased the stress tolerance capacity indicating that the plant possesses moderate analgesic effects (Ghule et al., 2007).

*Agave. sisalana* significantly reduced the number of writhes caused by acetic acid injection, an indication of anti-nociceptive activity. Acetic acid causes acute inflammatory reaction related to the increase in the levels of prostaglandins E2 and Fα in the peritoneal fluid (Ghule et al., 2007; Perianayagam et al., 2004). The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. Consequently, the test is useful for evaluating mild analgesic non-steroidal anti-inflammatory substances (Perianayagam et al., 2004; Saha et al., 2007). It is, therefore, postulated that extract response to acetic acid effect involved local peritoneal cells and is mediated by the prostaglandin pathways, and simulation of the action of salicylates that particularly are effective in relieving pain associated with inflammation.

The extract inhibited both phases of the formalin-induced pain with a more potent effect on the second phase than the first phase. The formalin-induced pain test is useful for evaluating the mechanism of pain and analgesia. Formalin induces persistent pain leading to two distinct phases, namely the neurogenic and then inflammatory phase that is accompanied by the release of inflammatory mediators (Ghule et al., 2007). Drugs which act mainly centrally such as narcotic analgesics inhibit both phases of pain. Peripherally acting drugs, for example acetylsalicylic acid or indomethacin, only inhibit the late phase of pain. In the current study, *A. sisalana* aqueous leaf extract performed similarly to centrally acting drugs.

Gastro-intestinal parasites cause pathological damage to chickens through marked thickening and inflammation of intestinal walls, or nodulation in the cecal walls. In addition, the parasites cause ulceration, maceration or mild lesions of the proventriculus, anemia and hemorrhagic intestines. Sloughing off of intestinal cells occur at the parasitic loci (Kahn, 2008). These infection effects of GI parasites subsequently elicit inflammation and pain in the gastro-intestinal walls of chickens, which could be ameliorated by the *A. sisalana* plant.

## Conclusion

The aqueous leaf extract of *A. sisalana* showed some anti-inflammatory and analgesic properties. The 100 or 200 mg/kg body weight doses reduced pain and inhibited inflammation remarkably. Thus, it is advisable for farmers to use the plant at 100 or 200 mg/kg body weight doses in order to reduce pain and inhibit inflammation. Doses above 200 mg/kg are detrimental to animals and should not be used. There is need for further studies that determine the nutritive value of *A. sisalana* that may aid the pharmacological properties of the plant in controlling gastro-intestinal parasites in village chickens.

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