Evaluation of Aster bakerianus Burtt Davy ex C.A. Sm. Crude Root Extract for Acute Antiinflammatory Activity in Rats

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Abstract: The crude extract of Aster bakerianus roots used for treatment of a variety of ailments in Lesotho was evaluated for anti-inflammatory activity and phytochemical content. Extract was first tested for toxicity at oral dosages of 0 (negative control), 1000, 2000, 3000 and 5000 mg/kg bw with five groups of female mice, each group having four mice. Negative control group received sterile distilled water (1.0 ml/kg bw). Mice were observed for general symptoms of toxicity for 24 hours and left for a further 14 days for any delayed toxicity. None of the extract doses induced toxicity. Anti-inflammatory activity of A. bakerianus was determined using the carrageenan-induced rat paw oedema assay. Five groups of six mice each were orally pre-treated as follows: Negative control group (group 1) received sterile distilled water (1.0 ml/kg bw). Positive control group (group 2) received indomethacin (10mg/kg bw). Three test groups (group 3, 4 and 5) received A. bakerianus extract, 100, 200 and 400 mg/kg bw respectively. After one hour of pre-treatment, all test groups and controls were injected with 0.1 ml carrageenan subcutaneously in the right hind paw and paw thicknesses recorded at the following time intervals: 0, 1, 2, 3, 4, 5, 6, and 24 hours. Statistically (p<0.05) there was no difference observed in the antiinflammatory activity profile of A. bakerianus and that of the drug indomethacin at the different time intervals of the study, implying same efficacy. The anti-inflammatory activity of extract was attributed to presence of terpenoids, saponins, sterols, simple phenols, coumarins, polyphenols, flavonoids, tannins, phlobatansins, antioxyacids, alkaloids, glycosides and amino acids. The results of this study justified the documented use of this plant by Basotho for treatment of inflammatory disorders.

Keywords: Eicosanoids, Cyclooxygenase, Phytochemicals, Indomethacin, Rat Paw Diameter

1. Introduction

Acute inflammation is a rapid, short-lived initial response of tissue to harmful stimuli (such as injury) initiated by cells constituting the relevant tissues (Ambriz-Pérez et al., 2016) and is characterised by accumulation of fluid, plasma proteins and the leukocytes which release a large number of soluble inflammatory mediators which are responsible for the initiation, progression, persistence, modulation (regulation) and eventual resolution of the acute state of inflammation (Oguntibeju, 2018; Nguyen et al., 2020). The classical signs of acute inflammation include oedema, erythema, pain, heat, and primarily loss of function of the affected part of the body (Nathan, 2002; Husein et al., 2012).

The acute inflammatory process involves a cascade of biochemical events comprising the local vascular system, the immune system and different cell types found in the injured tissue (Kulinsky, 2007). Numerous proinflammatory mediators are released during an inflammatory response, including the vasoactive amines; histamine, serotonin, bradykinin (Kulinsky, 2007; El-Shitany et al., 2014) and the cytokines, interleukin 1 β (IL-1 β), IL-6, IL-8, IL-12, tumour necrosis factor-α (TNF-α) and interferon-γ (INF-γ) as well as prostaglandins especially PGE2 through cyclooxygenase-2 (COX-2), leukotrienes through lipooxygenase (LOX), nitric oxide (NO) through the inducible nitric oxide synthase (iNOS) (Husein et al., 2012; Kulinsky, 2007; El-Shitany et al., 2014). Also released is the nuclear factor kappa B (NF-kB), a transcription factor that plays an important role in the transcription of genes, as it induces transcription of its target genes such as COX-2, iNOS, TNF-α, IL-1β, and IL-6, chemokines and adhesion molecules (Karim & Ben-Neriah, 2000) that cause acute inflammation (Nguyen et al., 2020). The cytokines play major roles in the initiation and amplification of inflammatory processes (Calixto et al., 2004). Nitric oxide (NO), a free radical generated by inducible nitric oxide synthase (iNOS), can act as a defence and regulatory molecule with homeostatic activities; however, it can also be detrimental when produced excessively (Xiong et al., 2000; Husein et al., 2012). Inflammatory reactions are supposed to
lead to either the resolution of tissue injury or complete eradication of pathogens in the body in case of infection (Husein et al., 2012). However, when the process of inflammation is not completely resolved, it can be detrimental to tissues and the body as a whole (Ben et al., 2016).

The different reactions in the inflammatory cascade are therapeutic targets, which anti-inflammatory agents including medicinal plants interfere with to suppress exacerbated inflammatory responses usually invoked in such disorders as injury, rheumatoid arthritis and infection (Iwueke et al., 2006). Any interruption of the inflammatory sequence of events results in the reduction of the liberation of the mediators causing the microcirculation to come back to normal hemodynamic state (Danya, 2017).

Conventionally, inhibitors of proinflammatory cytokines and cyclooxygenase (COX) enzymes such as the nonsteroidal antiinflammatory drug (NSAID) indomethacin, is currently the choice of anti-inflammatory agents (Lucas, 2016). The NSAIDs block prostaglandin and thromboxane formation by inhibiting cyclooxygenase activity (Danya 2017) and inhibit the NF-kB pathway and various inflammation-associated genes (Yamamoto & Gaynor (2001). However, these drugs, proven to be effective in many cases, can cause undesirable side effects to some degree (Lucas, 2016) and in some instances exhibit low potency (Husein et al., 2012; Talluri et al., 2016; Oguntibeju, 2018). The development of safer and efficacious alternative anti-inflammatory agents is therefore necessary (Ponmathi et al., 2017; Rajanandhini & Musthafa 2017; Sathiyabalan et al., 2018) hence the increase in studies on plant derived medicines and on creation of herbal formulations that could be used in the treatment of inflammatory diseases with less or no side effects (Talluri et al., 2016; Ponmathi et al., 2017; Rajanandhini & Musthafa 2017; Sathiyabalan et al., 2018; Oguntibeju, 2018). Also, traditional medicines derived from plant extracts are increasingly being used to treat a wide variety of diseases; many being prescribed broadly for the treatment of inflammatory conditions though relatively little knowledge about their mode of action is available (Amala Hazel et al., 2018; Oguntibeju, 2018). While plant derived anti-inflammatories are rarely as immediately effective as the steroidal antiinflammatory drugs and NSAIDs, they are very rarely as toxic nor potentially life threatening (Talluri et al., 2016; Oguntibeju, 2018).

Research studies on medicinal plants used in traditional medicine represents a suitable approach for the development of new drugs (Ullah et al., 2014). Herbal medicine has been recognised by the World Health Organisation (WHO) as an important component of primary health care and as such efforts are being made to combine its therapeutic potential with that of orthodox medicine (Kaur and Jaggi, 2010). The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries (Ullah et al., 2014) and the carrageenan induced oedema model for acute peripheral inflammation is one of the in vivo models approved. Medicinal plants have therefore become the subject of intense pharmacological studies in the last few decades (Fernandes & Banu, 2012; Oguntibeju, 2018).

Many plant species have shown potential for anti-inflammatory activities (Oguntibeju, 2018) due to the presence of a wide variety of phytochemicals that can be a source of anti-inflammatories themselves and could also be used for the discovery of novel anti-inflammatory agents with fewer side effects (Talluri et al., 2016; Oguntibeju, 2018). The chemical compounds present in plant products are a part of the physiological functions of living organisms, and hence they are believed to have better compatibility with the human body (Prasad et al., 2012). The therapeutic potential of any herbal drug depends on its form; whether it is part of a plant, or isolated active constituents or crude extracts containing several constituents, which often work together synergistically (Bandaranayake, 2006).

In the Kingdom of Lesotho, two forms of health-care systems are used; namely the traditional system and the orthodox western system. Traditional medicine plays a vital role in the quest for well-being of the rural population in Lesotho, particularly where there is limited accessibility to clinics or health facilities (Seleteng-Kose et al., 2019; Shale et al., 1999).

*Aster bakerianus* (Asteraceae) is widely distributed in Southern Africa and is one of the important medicinal plants widely used by the Basotho in the Kingdom of Lesotho (Moteete & Seleteng-Kose, 2017; Oguntibeju, 2018) for the treatment of a variety of ailments. In the local dialect of Lesotho (Sesotho) *A. bakerianus* is called *phooa*, and its roots are used to treat a number of ailments (van Wyk et al., 1997; van Wyk et al., 1997). The denser cluster of roots, each of which is elongated and tapering on both ends (fusiform), is generally used in traditional medicine (van Wyk et al., 1997). As a traditional remedy for headache, the dried roots of *A. bakerianus* are powdered and used as snuff to induce sneezing (Hutchings and van Staden, 1994). The roots may also be pounded and mixed with water to clean the nostrils. *A. bakerianus* is also used locally for the...
treatment of snake-bites, asthma, venereal diseases, syphilis, urinary infections, anthrax, eye infections, stomach aches, colic, psychiatric disorders, short-sightedness and intestinal parasites (van Wyk et al., 1997). The liquid from crushed boiled roots is taken in doses of a teaspoonful once a day for chronic coughs or in larger doses as an emetic and have a purgative action (van Wyk et al., 1997).

According to Motetee and Seleleng-Kose (2017), *A. bakerianus* has not yet been evaluated for anti-inflammatory activity, and to date no other literature was found on anti-inflammatory activity of *A. bakerianus* hence this study.

The relationship between traditional use of a plant species and inflammatory processes has been studied using several species (Agnihotri et al., 2010; Ishola et al., 2014; Popoola et al., 2016). The carrageenan-induced rat paw oedema is used widely as a standard experimental model of aseptic acute inflammation (Di Rosa et al., 1971; Ponnathri et al., 2017; Danya 2017) used in the search for and evaluation of new orally active anti-inflammatory drugs or natural products (Amala Hazel et al., 2018; Panthong et al., 2007) which act through mediators of acute inflammation (Ben et al., 2016; Kumar and Jain, 2014), test new anti-inflammatory drugs for their potency, study the mechanisms involved in acute inflammation (Hassimotto et al., 2013; Khakimov et al., 2019) and evaluate NSAIDs and appears to have been the basis for the discovery of indomethacin (Falodun et al., 2006; Danya 2017). Moreover, this experimental model exhibits a high degree of reproducibility (Di Rosa & Sorretatino, 1968). In this method, reduction in paw oedema in this triphasic model is a good indicator of the protective effect of an anti-inflammatory substance (Rizvi et al., 2014; Rajanandhini & Mustafa, 2017). Carrageenan induced rat paw aseptic inflammation oedema leads to an increase in the production of NO, PGE2, TNF-α, and IL-6 in plasma, and also an increase in the expression of iNOS, COX-2, TNF-α, and IL-6 in paw tissue due to the late phase of acute peripheral inflammation due to activation of the nulear factor kappa B (Salvemini et al., 1996; Hussein et al., 2012; Talluri et al., 2016).

2. Materials and Methods

2.1 Chemicals

Indomethacin (Sigma-Aldrich, St Louis, MO, USA), carrageenan (Sigma, USA), Lambda-carrageenan Type IV (Sigma Chemical Co., St. Louis, USA), digital analytical balance, sodium chloride (Associated Chemical Enterprises (Pty) Ltd., Johannesburg, South Africa), methanol (Associated Chemical Enterprises (Pty) Ltd., Johannesburg, South Africa), micropipettes (Eppendorff), 1.0ml hypodermic syringes, sterile single use (Promex, Jhb, RSA), vernier calipers micrometer (0–150mm/0.02mm; Mitutoyo, Japan).

2.2. Plant Material

The whole plant of *Aster bakerianus* was collected at Ha Nkhema village located north east of the National University of Lesotho Roma Campus with the following co-ordinates: 29° 26’ 56” S, 27° 43’ 18” E. Altitude: 1683 m above sea level. The roots were obtained by digging-out the whole plant after which the roots were separated and used in the study. The plant was authenticated by the herbarium curator (Mr. M. Polaki) of the Department of Biology, National University of Lesotho. A voucher specimen of the *A. bakerianus* plant was deposited in the herbarium in the Department of Biology.

2.2 Preparation of crude extract of *A. bakerianus* roots

The soil-free roots of *Aster bakerianus* were gently washed in distilled water and dried in an oven (Labcon) equipped with a fan at 35 °C for two days and then ground to fine powder with a pestle and mortar (Magama et al., 2017). The powdered material (40 g) was extracted with 400 ml methanol (95% v/v in distilled water) for 72 hours at room temperature on an orbital shaker at speed of 120 rotations per minute. The extract was then filtered under suction and the filtrate was concentrated to about a quarter of its original volume under vacuum in a Gallenkamp (Germany) rotary evaporator (Magama et al., 2017). The resultant crude extract was then dried in the oven (Labcon) with fanning at 35 °C until brittle which took 72 hours. The dried extract was stored at 4 °C until use.

2.3 Animals

Inbred 20 female nulliparous and non pregnant mice of NIH strain albino mice ranging from 8-12 weeks and weighing between 22-24g were used in the toxicity assay and 36 male Adult Wistar Albino rats between 8-12 weeks old and weighing between 150 and 200 g were used in the carrageenan-induced rat-paw oedema model for acute inflammation. The animals were bred and kept in the animal house of the Department of Biology and allowed free access to food (meadow sheep pellets) and water *ad-libitum*.

2.4 Toxicity assay

The method of the Organization for Economic Cooperation and Development-423 (OECD-423) guidelines (2002) dosing schedule was used to determine the acute oral toxicity of *A. bakerianus* root extract. Briefly, Twenty (20) healthy female
Evaluation of *Aster bakerianus* Burtt Davy ex C.A. Sm. Crude Root Extract for Acute Antiinflammatory Activity in Rats

Albino mice NIH strain ranging from 8-12 weeks old nulliparous and non pregnant, were obtained from the National University of Lesotho animal house, Department of Biology. The animals were then randomly selected and divided into four groups of four mice each (n=4). Prior to testing, the animals were fed classic horse feed (12% maintenance cubes) and had free access to drinking water but were starved for 12 hours before testing. After oral administration with different fixed single dosages of the *A. bakerianus* root extract; 1000, 2000, 3000 and 5000 mg/kg body weight and sterile distilled water in the control group using a bulb-ended steel needle, the animals were observed for any toxicological signs and symptoms, and mortality continuously for 1 hour and then hourly for 6 hours and finally after every 24 hours up to 14 days altogether for any delayed toxicity manifestations such as modifications of the skin, the hairs (piloerection), eyes, motor activity and behavior. Emphasis was on the observation of various manifestations of toxicity such as tremors, convulsions, salivation, diarrhoea, lethargy, piloerection, lacrimation, nasal secretion, cyanosis, sleep, coma and mortality (N’Goka et al., 2018; Zahra et al., 2020).

### 2.5 Acute inflammation test

For the determination of the anti-inflammatory activity of the crude extract of *A. bakerianus* roots, the following safe oral doses were used: 100, 200 and 400 mg/kg bw., which were 1/10 for the first two and 1/12.5 of the 4th (5000 mg/kg bw) doses used in the acute toxicity tests. In this study, the in vivo acute anti-inflammatory activity of *A. bakerianus* root extract was evaluated using the inhibition of carrageenan-induced rat hind paw oedema test used in many similar studies (Winter et al., 1962; Rodrigues et al., 2016; Sathiabalan et al., 2018). Five groups of six male albino Wistar rats per group (groups 1-5) were set-up in six metal cages and maintained at 26 ± 2°C with free access to food and water.

The animals were orally administered with *A. bakerianus* crude root extract (groups 3,4,5 at 100, 200 and 400 mg/kg bw respectively) and indomethacin 10mg/kg bw (group 2) (Rodrigues et al., 2016; Sathiabalan et al., 2018). The negative control group (group 1) was orally given 1.0 mL/kg bw H2O (Husein et al., 2012). After one hour of oral administration of the test samples (different doses of *A. bakerianus* root extract) and controls (positive control: indomethacin and negative control sterile distilled water); 0.1ml of 1% (w/v) carrageenan suspension in normal saline (0.9% w/v NaCl) solution was injected into the sub-plantaee tissue of the right hind paw of the animals in groups 1 to 5 for the induction of acute inflammation (Husein et al., 2012; Sathiabalan et al., 2018; Khakimov et al., 2019). The needle was inserted to a depth of approximately 1mm into the callus to deliver an accurate and uniform amount of carrageenan into the subplantar tissue (Ishola et al., 2014).

The different groups were pre-treated orally as follows:

- **Group 1:** Distilled water, vehicle of drug (1 ml/kg bw) + carrageenan injection sc after 1 hour (negative control)
- **Group 2:** Indomethacin (10 mg/kg bw) + carrageenan injection sc after 1 hour (positive control)
- **Group 3:** *A. bakerianus* extract (100 mg/kg bw) + carrageenan injection sc after 1 hour
- **Group 4:** *A. bakerianus* extract (200 mg/kg bw) + carrageenan injection sc after 1 hour
- **Group 5:** *A. bakerianus* extract (400 mg/kg bw) + carrageenan injection sc after 1 hour

The linear paw thickness (dorso-ventral diameter) for each rat was measured before aseptic injection of carrageenan and after injection of carrageenan (Ponnmathi et al., 2017) using a vernier callipers at 0 hr, then at hourly intervals for six hours, at 1, 2, 3, 4, 5, 6 hours (Bamgbose & Noamesi, 1981) and finally at the 24th hour (Husein et al., 2012; Ganesh et al., 2013; Ishola et al., 2014; Khakimov et al., 2019) post sc injection. The increase in paw thickness was taken as an indicator of the degree of acute inflammation. The mean oedema value of each root extract treated group (group 3, 4 and 5) and the indomethacin treated group (group 2) was compared with that of the negative control group (group 1).

Anti-inflammatory activity was calculated as a percentage (%) inhibition of paw oedema when the drug indomethacin (or the root extract) was present, relative to the negative control (Group 1) as follows:

\[
\text{Percentage (\%)} \text{ inhibition of paw oedema} = \frac{(V_c-V_t)}{V_c} \times 100
\]

Where:

- \(V_c\) is the inflammatory increase in paw thickness in control group of animals (Group 1, given only the vehicle of both drug and extract).
- \(V_t\) is the inflammatory increase in paw thickness in drug (or extract) treated animals.

### 2.6 Qualitative phytochemical tests

The qualitative tests for relevant phytochemical classes in the crude methanolic extract of *A. bakerianus* roots were performed according to Thilagavathi et al., 2015; Gul et al., 2017; Bharathi & Udayakumar (2019). The following classes were tested for: Flavonoids (Thilagavathi et al., 2015; Gul et al., 2017), Terpenoids (Bharathi & Udayakumar, 2019), Phenolics (Bharathi & Udayakumar, 2019).
Evaluation of *Aster bakerianus* Burtt Davy ex C.A. Sm. Crude Root Extract for Acute Antiinflammatory Activity in Rats

Glycosides (Thilagavathi et al., 2015; Gul et al., 2017; Bharathi & Udayakumar, 2019), Tannins (Thilagavathi et al., 2015; Bharathi & Udayakumar, 2019), Coumarins (Bharathi & Udayakumar, 2019), Alkaloids (Thilagavathi et al., 2015), Reducing sugars (Thilagavathi et al., 2015). The results were noted as either negative (-) meaning not detected because either absent or below the detection limit) or positive (+) for the particular class of compounds. If positive, the colour intensity was classified as + for low intensity, ++for medium intensity, +++ for high intensity.

2.7 Data analysis

Results for evaluation of *A. bakerianus* root extract for anti-oedematogenic activity were expressed as the mean value ± standard deviation of the mean paw thickness. Treated groups were compared with the controls for statistically significant differences between the means of each group of 6 rats. (*p* < 0.05) using paired Student’s *t*-test and the Tukey multiple comparisons analysis of variance. Statistical differences with *P* < 0.05 were considered significant.

3. Results

The yield of the crude root extract from the dried powdered roots of *A. bakerianus* roots after extraction with 95% Methanol (v/v) in distilled water was 8.62%.

3.1 Toxicity test for *A. bakerianus* crude root extract

At the oral dosage range of *A. bakerianus* crude root extract (1000-5000 mg/kg bw) at which mice were challenged with the extract, no symptoms of toxicity in mice were observed at all dosages of the extract during the 14 days of monitoring, therefore the LD<sub>50</sub> was estimated to be higher than 5000 mg/kg bw.

| Parameters                  | Control group | *Aster bakerianus* root extract |
|-----------------------------|---------------|---------------------------------|
|                              | Sterile distilled water | Test group 1 | Test group 2 | Test group 3 | Test group 4 |
| Doses                        | 20ml/kg bw     | 1000 mg/kg bw                  | 2000 mg/kg bw | 3000 mg/kg bw | 5000 mg/kg bw |
| Behaviour                    | N              | N                               | N             | N             | N             |
| Mobility                     | N              | N                               | N             | N             | N             |
| Condition of stools          | C              | C                               | C             | C             | C             |
| Convulsion                   | A              | A                               | A             | A             | A             |
| Tremor                       | A              | A                               | A             | A             | A             |
| Sleep                        | A              | A                               | A             | A             | A             |
| Hair appearance              | N              | N                               | N             | N             | N             |
| Aspect of the skin           | N              | N                               | N             | N             | N             |
| Condition of eyes            | N              | N                               | N             | N             | N             |

N=Normal, A= Absent, C= Compact

3.2 Weight Progression

The mice of groups 1, 2, 3, and 4 were treated orally with doses of 1000, 2000, 3000 and 5000 mg / kg bw of methanolic root extract of *A. bakerianus* respectively. The mice of the control group were treated orally with sterile distilled water. The daily weighings of mice during the 14 days of testing for acute toxicity were used to monitor weight progression of the treated and control mice. Figure 1 shows the daily percentage (%) changes in weight for the 14 day study period for acute toxicity. The treated animals gained weight in a dose dependent manner though they were given the same feed. There was no significant (*p* <0.05) difference in increase in the weight of the mice within groups and between groups compared to the control during the 14 day period of the acute toxicity study in the ANOVA Tukey multiple comparisons.
3.3 Antiinflammatory activity of *A. bakerianus* crude root extract

In Table 1 is presented the results of comparison of mean rat paw thicknesses (mm±SD) of the indomethacin treated group (2) and each extract treated test groups (3-5) with the negative control (group 2) as well as between test groups themselves, at the seven different time intervals of the experiment and percent inhibition of inflammation in groups 2, 3, 4, 5 and 6 at the time intervals 0, 1, 2, 3, 4, 5, 6 and 24 hours.

Subcutaneous injection of the rat paw with carrageenan without treatment (group 1) as shown in Table 2 induced a time-dependent progressive inflammatory oedema which was recorded as mean paw thickness with a peak during the period from 4 till 6 hours as follows: at 1 hour: 6.31±0.84 mm, at 2 hours: 6.87±0.64 mm, at 3 hours: 6.78±0.67 mm, at 4 hours: 7.50±1.13 mm, at 5 hours: 7.47±1.57 mm, and at 6 hours: 7.59±1.15 mm post carrageenan injection. After the 6th hour, a very slight resolution of the inflammation continued till the end of the experiment at 24 hours (7.24±0.67 mm). The increases in paw thickness were less pronounced in the indomethacin and extract treated groups (groups 2-5) than in the negative control (vehicle) treated group (group 1). With reference to the negative control group, it was observed that from the time of subcutaneous injection of the rat paw with carrageenan, up to 2 hours post injection, there was an increase in paw thickness which plateaued slightly between 2 and 3 hours then began to increase again up till 6 hours and remaining high despite an insignificant decrease observed at 24 hours (Table 2 and Figure 2). This pattern of the graph was depressed in the treated groups (groups 2-5).

The results (Table 2) showed that all three test extract doses (100, 200 and 400 mg/kg bw) of *A. bakerianus* root extract protected the rats from carrageenan induced inflammation and the three test extract doses showed a moderate anti-inflammatory activity in a time and dose dependent manner during the period of study. All three doses of the extract and indomethacin induced a significant (p<0.05) time- and dose-dependent inhibitory effect against carrageenan-induced acute peripheral inflammation in the rat paw when compared with the water treated (vehicle) negative control group (group 1) at 1, 2, 3, 4, 6 and 24 hours except at the 5 hour time interval. The inhibitory effects were statistically the same for both *A. bakerianus* root and the drug indomethacin treated groups at each of the time intervals and highest during the period of 4-6 hours post carrageenan injection. Reduction of paw thickness or oedema (as an indication of reduction in acute inflammation) by the different doses of the crude extract of *A. bakerianus* roots (100, 200, and 400 mg/kg bw) and the drug indomethacin (10 mg/kg bw) was observed to be statistically similar (p>0.05) in the Tukey’s multiple comparisons analysis using ANOVA at all doses and time intervals as shown in Table 2.
Evaluation of *Aster bakerianus* Burtt Davy ex C.A. Sm. Crude Root Extract for Acute Antinflammatory Activity in Rats

was therefore no statistically significant difference in anti-inflammatory activity between indomethacin and the *A. bakerianus* extract at all time intervals and doses of the extract used in this study.

In Figure 1 is presented the curves of the changes in paw thickness with time for the three concentrations of *A. bakerianus* root extract (groups 3-5) and the reference drug indomethacin (group 2). As shown on the graphs, treatment with extracts and indomethacin gave more depressed curves than the negative control group 1 treated with the vehicle, distilled water.

Table 2: Group (n = 6) mean of rat paw thickness (mm) ± SD after oral administration with different dosages of *A. bakerianus* extract (100, 200, 400 mg/kg bw), indomethacin (10 mg/kg bw) and distilled water (1 ml/kg bw), followed one hour later by subcutaneous injection with 0.1 ml carrageenan solution in saline.

| Time (Hrs) | Distilled water (1ml/kg bw) | Indomethacin (10mg/kg bw) | Aster bakerianus root extract (mg/kg bw) | Time Interval F Values |
|------------|----------------------------|--------------------------|------------------------------------------|-----------------------|
| 0          | 3.15±0.19a 0.00§ (0.00)    | 5.33±0.28b 0.00§ (-68.92)*| 4.90±0.61b 0.00§ (-55.39)*               | 5.12±0.39b 0.00§ (-62.26)* 40.455¥ |
| 1          | 6.31±0.84 b 50.00§ (0.00)  | 6.09±0.32 b 12.58§ (3.38) | 6.38±0.57 b 25.49§ (-4.28)               | 6.27±0.38 b 14.29§ (0.55) 5.80±0.54 ab 11.83§ (7.98) 7.153¥ |
| 2          | 6.87±0.64 b 54.08 § (0.00) | 6.38±0.42 ab 16.51§ (7.08)*| 6.6±0.78b 26.46§ (2.96)                 | 6.74±0.24b 20.24§ (1.85) 6.08±0.60abc 15.80§ (11.50)* 4.689¥ |
| 3          | 6.78±0.67b 53.49§ (0.00)   | 6.56±0.53 ab 18.84§ (3.20) | 6.6±0.60 b 26.39§ (1.82 )               | 6.74±0.52 ab 20.24§ (0.59) 6.22±0.71ab 17.74§ (8.26) 2.941¥ |
| 4          | 7.50±1.13b 57.94§ (0.00)   | 6.8±0.43 ab 22.39§ (8.45)  | 6.74±0.57 ab 27.30§ (10.09)              | 6.78±0.562ab 20.66§ (9.61) 6.62±0.51 ab 22.71§ (11.69)* 3.641¥ |
| 5          | 7.47±1.57 bcd 57.77§ (0.00) | 6.49±0.58 acd 17.97§ (13.04) | 6.6±0.70 acd 26.72§ (10.45 )          | 6.6±0.45bcd 18.89§ (11.23) 6.2±0.100 acd 17.43§ (17.01) 2.315£ |
| 6          | 7.59±1.15b 58.44§ (0.00)   | 6.4±0.66ab 19.78§ (12.48)* | 6.9±0.77ab 28.99§ (9.05)               | 6.9±0.53 ab 22.04§ (9.10) 6.64±0.73 ab 22.98§ (12.43) 3.447¥ |
| 24         | 7.24±0.67 b 56.45§ (0.00)  | 6.73±0.88 b 20.54§ (7.00)* | 7.0±0.69 b 30.92§ (2.03)               | 6.9±0.48 b 23.07§ (3.48) 6.7±0.4 b 24.40§ (6.52) 7.116¥ |

§  = Percent (%) changes in paw thickness;  
( ) = Numbers in parenthesis are percent (%) inhibition of oedema;  
*  = Percent (%) inhibition of oedema was significantly different (p< 0.05 in the t-test) compared to the negative control (saline);  
¥  = There is a significant difference in the Mean inhibition values of oedema at the given time interval of the different treatments (F5,29 = 2.54, P<0.05)  
£  = There is no difference in the Mean inhibition values of oedema at the given time interval of the different treatments (F5,29 = 2.54, P>0.05)  
a,b,c,d = Group Means of inhibition of oedema with the same letter at any given time interval do not differ statistically (p<0.05) in the Tukey multiple comparisons analysis.
3.3 Qualitative phytochemical tests

The phytochemical classes identified in the root extract are presented in Table 3. The relevant phytochemical constituents detected qualitatively in the crude root extract of *A. bakerianus* were mostly phenolics; both simple phenols (+++) and polyphenols (+++) including the flavonoids (+++), tannins (+++), phlobatannins (+++), anthocyanins (+++), betacyanins, coumarins (+++). Also present in high intensities were the terpenoids (+++), saponins (+++), phytoesters (+++), amino acids (+++), reducing sugars (+++). In lower intensities were the glycosides(++) and traces (+) of alkaloids.

### Table 3: Qualitative phytochemical profile of methanolic extract of *A. bakerianus* root extract

| Phytochemical class | Test                        | Positive (+)/Negative (-) |
|---------------------|-----------------------------|----------------------------|
| Alkaloids           | Wagner                      | +                          |
| Flavonoids          | Shinoda                     | +++                        |
| Saponins            | Foam/froth test             | +++                        |
| Tannins             | Ferric chloride/Braymer’s test | +++                      |
| Terpenoids          | Saikowski                   | +++                        |
| Simple phenols      | Ferric chloride             | +++                        |
| Polyphenols         | Ferric chloride             | +++                        |
| Sterols             | Saikowski/Chloroform & conc. H2SO4 | +++                      |

Glycosides | Fehling’s | ++
Quinones   | HCl       | -
Phlobatannins | HCl       | +++
Coumarins  | NaOH+ Chloroform | +++
Anthocyanins | NaOH      | +++
Betacyanins | NaOH      | +++
Reducing sugars | Benedict’s | +++
Amino acids | Ninhydrin | +++
Proteins   | Biuret    | -

Key: (-) = Negative; (+) = Positive; + = low colour intensity, ++ = medium colour intensity, +++ = high colour intensity

4. Discussion

In this study, the crude methanolic extract of *A. bakerianus* roots used in traditional medicine in the Kingdom of Lesotho for the treatment of various ailments was evaluated for acute toxicity and acute anti-inflammatory activity on the basis of inhibition of carrageenan-induced rat hind paw oedema. The presence of oedema is one of the major signs of inflammation and paw oedema volume has been increasingly used to test new anti-inflammatory drugs (Kumar & Jain, 2014).

In the acute toxicity study, six groups of five mice each were orally administered with sterile distilled water, indomethacin and the following four doses of the crude methanolic extract of *A. bakerianus* roots.
Ishola et al., 2014; Talluri et al. which are influenced by own in Table 2, intermediate phase (2 hours after injection of carrageenan). Talluri et al., 2016; Popoolaa et al., 2016; Goka et al., 2018 reported that in the methanolic extract, the extract was rich in amino acids and phytosterols (Table 3). It has been proven that phytosterols increase body mass and amino acids are required for growth too (Nsonde Ntandou et al., 2015). This effect could therefore be explained by the presence of the phytosterols in the methanolic extracts (N’goka et al., 2018). The oral doses of A. bakerianus root extract used in the anti-inflammatory tests were chosen as 400 mg/kg bw, 200 mg/kg bw and 100 mg/kg bw, these being much lower than the LD50.

The oral doses of A. bakerianus root extract administered in the anti-inflammatory tests were chosen as 400 mg/kg bw, 200 mg/kg bw and 100 mg/kg bw, these being much lower than the LD50.

As shown in Table 2 and in Figure 2, group 1 (negative control) rats, it was observed that from the time of sc injection with carrageenan, up to 2 hours post injection, there was an increase in paw thickness which slightly plateaued between 2 and 3 hours. Then, from the 3rd hour, it began to increase again remaining high till 24 hours. The peak period of carrageenan-induced inflammation was from 4 hours. The changes (increases) in paw thickness in group 1 rats (the negative control group), at the different time intervals as shown in Table 2 were as follows: at 1 hour, 50%; at 2 hours, 54.08%; at 3 hours, 53.46%; at 4 hours, 57.94%; at 5 hours, 57.77%; at 6 hours, 58.44% and at 24 hours to 56.45%. The period of maximum paw oedema was from 4 to 24 hours in the untreated group 1. A depressed, similar pattern was observed in the treated groups (groups 2-5). Based on such observations the initiation and progression of oedema after subcutaneous injection of carrageenan into the rat paw has been described as being triphasic and acute (Ishola et al., 2014; Talluri et al 2016; Kumar & Jain, 2014; Birhane et al., 2014; Popoolaa et al., 2016). The phases of inflammatory oedema were demarcated as follows: early phase (0-2 hours) (Talluri et al., 2016), intermediate phase (2-3 hours) Talluri et al., 2016), late phase (3-24 hours) (Talluri et al., 2016; Ishola et al., 2014). The early phase is mainly mediated by histamine and serotonin starts immediately after injection of carrageenan while the intermediate phase provides continuity between the early phase and the late phase and is mediated by kinins, mainly bradykinin (Ishola et al., 2014; Popoolaa et al., 2016). The late phase is associated with release of prostaglandins, leukotrienes and NO with increased expression of COX-2 and iNOS which are influenced by the nuclear factor kappa B (NF-kB) (Di Rosa et al., 1971; Sidhapuriwala et al., 2007) and also the release of protease, lysosomes, and migration of leukocytes into the inflamed site occurs during the late phase of oedema (Talluri et al., 2016). It has been reported that the suppression of carrageenan induced hind paw oedema after the fourth hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory drugs, both steroidal and non steroidal such as indomethacin (Panthong et al., 2007; Kumar & Jain, 2014). In our study, this suppression of carrageenan-induced oedema by A. bakerianus roots extract and indomethacin was maximal during the period from 4 hours to 6 hours post carrageenan injection corresponding to the late phase of inflammation.

The efficacy of A. bakerianus root extract and that of indomethacin were statistically (p<0.05) in this study. The trend observed in our study was in agreement with observations by other authors using the carrageenan test for acute inflammation. Sarada et al., 2012 studied the leaf and bark of extract of Naringi crenulata at oral doses of 250 and 500 mg/kg bw and reported that the pattern of anti-inflammatory activity and percent inhibition in paw volume induced by these extracts was similar to that of indomethacin (10mg/kg bw p.o.) which, to these authors suggested that, the activity of the leaf and bark extracts of Naringi crenulata used in the study could be mediated by COX-1 and COX-2 inhibition since indomethacin is a COX-1 and COX-2 inhibitor. In another study the oral doses 150 mg/kg and 300 mg/kg of ethanol extracts of Barleria courtillica prepared from stem, root and leaf produced significant inhibition of carrageenan induced paw oedema at 3rd hour comparable to that brought about by the reference drug indomethacin (10 mg/kg bw) (Ponnmathi et al., 2017). Rodrigues et al., 2016 observed that indomethacin was effective at reducing oedema at all time intervals of a 5 hour study with maximum activity at 5 hours in the same way as oral doses of Ocimum basilicum oil (100 and 50 mg/kg bw p). In a similar study, Ishola et al., 2014 observed that oral administration of methanol root extracts of
Evaluation of *Aster bakerianus* Butt. Davy ex C.A. Sm. Crude Root Extract for Acute Antiinflammatory Activity in Rats

Alafia barteri, Combretum mucronatum, and Capparis thonningii (100–200mg/kgbw) produced dose-related and time dependent significant suppression of carrageenan-induced inflammation in the middle and late phases when compared with the vehicle-treated negative control group with peak inhibition from the 3rd to the 24th hour at 200mg/kg p.o. treatment. Other reported studies of depression of carrageenan-induced rat paw acute inflammation include studies with methanolic extracts of *C. rottleri* root, leaf and stem at 125 mg/kg, 250 mg/kg and 500mg/kg bw by Talluri et al., 2016. These doses significantly inhibited the maximal paw edema response during the 6 hours study of the carrageenan-induced rat paw acute inflammation together with the standard inflammatory drug, indomethacin the authors were still to isolate the active compounds. Popoola et al., 2016 reported that the ethanol extracts of *Garcinia kola* stem bark, *Uvaria chamae* roots and *Olx subscorpioidea* roots produced significant time and dose dependent reduction in carrageenan-induced inflammation during the 6 hour study. For *G. kola* maximum inhibition of 73% was observed at 200 mg/kg, for *U. chamae* 86.7% produced by 100 mg/kg and for *O. subscorpioidea* at 92.31% produced by 400 mg/kg bw 6 hours post carrageenan injection.

In Table 3 is presented the results of the qualitative phytochemical analysis of *A. bakerianus* root extract. The following relevant classes of phytochemicals were detected in the root extract: alkaloids, glycosides, reducing sugars, terpenoids, saponins, phytosterols, simple phenols, polyphenols, flavonoids, tannins, phlobatannins, anthocyanins, betacyanins, coumarins and amino acids. All these compounds have been reported to exhibit antiinflammatory activity (Kosala et al., 2018; Yoon & Baek, 2005; Popoola et al., 2016; Agnihotri et al., 2010; Rodrigues et al., 2016; Owolabi et al., 2018; Aquila et al., 2009; Suyyah et al., 2004; Street et al., 2013; Bribi et al., 2015; Zhang et al., 2018; Su et al., 2019; Garcia et al., 1999). These compounds have also been found in roots of other Asteraceae species (Ugwoke et al., 2017; Senguttuvan et al., 2014; Cheng & Shao, 1993; Kwon et al., 2003; Wang et al., 2003; Liu et al., 2007; Su et al., 2019). Most of the phytochemicals identified in the *A. bakeriatus* root extract were polyphenolic in nature and as phenolics, they are known antioxidants (Zelice da Cruz de Moraes et al., 2020; Senguttuvan et al., 2014). Antioxidants are known to exhibit anti-inflammatoriy activity (Yoon and Baek, 2005; Wu et al., 2006; Zelice da Cruz de Moraes et al., 2020; Barragán-Zarate et al., 2020). Antioxidant phytochemicals in plant extracts are reported to reduce oxidative stress resulting from neutrophil activity during inflammation by possibly increasing the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione (GSH) and by reducing ROS generation by neutrophils (Akamatsu et al., 1991; Barragán-Zarate et al., 2020). Alkaloids are said to reduce the intensity of oedema caused by carrageenan by inhibiting vascular permeability induced by histamine (Perez, 2001; Owolabi et al 2018). Some alkaloids may also prevent inflammation through blocking the metabolic pathway of arachidonic acid (Barik et al., 1992; Ullah et al., 2014) and also through inhibition of production of nitric oxide, IL-6 and through down-regulating messenger ribonucleic acid expression of pro-inflammatory key players such as IL-6, IL-1β, inducible nitric oxide synthase, TNF-α, and cyclooxygenase-2 (Bribi et al., 2015). Compounds with aromatic rings and alcohol groups as seen in phenols and polyphenols (such as coumarins, flavonoids, tannins, phlobatannins, anthocyanins, betacyanins) have been shown to modulate inflammation at different levels by decreasing the production of reactive nitrogen and oxygen species (Kim et al., 2006; Arulselvan et al., 2016; Hassimotto et al., 2013), limiting the activity of NF-κB, iNOS and COX-2, limiting mRNA expression levels of nitric oxidesynthase (iNOS) and cyclooxygenase-2 (COX-2) in cells (Khan et al., 2015; Ahad et al., 2014; Rizvi et al., 2014), suppressing inflammatory chemokines and cytokines such as interleukin -6 (IL-6), TNF-α, IL-1 β synthesis, as well as controlling pathways for NF-κB signaling (Mansouri et al., 2015; Arulselvan et al., 2016; Santangelo et al., 2007; González et al., 2011; Ghorbanzadeh et al., 2015; Ben Saad et al., 2017; Rizvi et al., 2014). Phenolic glycosides and kaempferol glycosides are reported to inhibit nitric oxide (NO) production and nitrite production which is the stable end product of NO and TNF-α (Agnihotri et al., 2010; Liu et al., 2016; Hien et al., 2018). Terpenoids and saponins have been reported to exhibit antiinflammatory effects through nuclear factor kappa B (NFkB)-inhibiting mechanisms and inhibition of production of pro-inflammatory cytokines and iNOS production (de las Heras & Hortelano 2009; Kim et al., 2009; Owolabi et al., 2018). Saponins consist of triterpenoids or steroid aglycons and oligosaccharide substituents (Kosala et al., 2018). Triterpenoids may perform their anti-inflammatory action by reducing the number of cells that expresses inducible nitric acid synthase (iNOS) (Lucetti et al., 2010; Owolabi et al., 2018) or by inhibiting the production of nitric oxide (NO) through decreasing iNOS expression (Schmide et al., 2009; Owolabi et al 2018). Terpenoids in general have a high tendency to inhibit inflammatory processes by

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Inhibiting the production of pro-inflammatory cytokines and iNOS production (Kim et al., 2009; Owolabi et al., 2018). Phytotherapies are known to reduce inflammation through the inhibition of Phospholipase A2, which hydrolyses arachidonic acid from membrane phospholipids. This prevents the formation of prostaglandins and leukotrienes via the cyclooxygenase and lipooxygenase pathways respectively, and this could result in reduction of inflammation (Yuan et al., 2019). Phytotherapies, just like polyphenols, have also been reported to possess anti-inflammatory activity by decreasing various mediators of inflammation such as prostaglandins, NO, cytokines TNF-α IL-6, and IL-1 (Rizvi et al., 2014).

The observed anti-inflammatory activity of the *A. bakerianus* root extract in this study could therefore be ascribed to terpenoids, alkaloids, simple phenols including coumarins and polyphenols, including flavonoids and tannins as well as glycosides among other bioactive compounds acting either as distinct entities or a combination of these phytochemicals acting synergistically (Barragán-Zarate et al., 2020; Popoola et al., 2016). Kosala et al., 2018 attributed the antiinflammatory activity of the methanolic extract of *Coptosapelta flavescens* roots that they observed to the presence of polyphenols, terpenoids, steroids, anthraquiones and saponins. In a study of the anti-inflammatory activity of ethanolic extracts roots of *Cichorium intybus* (chicory roots) by Rizvi et al., 2014, the observed anti-inflammatory activity was attributed to the presence of polyphenols, flavonoids, sterols, glycosides, tannins, and terpenoids reported in chicory roots (Street et al., 2013).

The findings of this study on *A. bakerianus* extract suggest that the bioactive phytochemical constituents in the crude extract of *A. bakerianus* roots suppressed all the phases of acute inflammation, with the inhibition being more effective in the late phase, probably by interfering with the release and/or activity of the chemical mediators, such as histamine, serotonin, bradykinin, prostaglandins. Similar to the mechanisms of action of other NSAIDs, both the therapeutic and adverse event profiles of indomethacin are caused by decreased production of prostaglandins (Lucas 2016). For this purpose, ethnobotanical studies represent an increasingly attractive approach for applying indigenous knowledge of plant use to modern societies, with the final aim of developing new remedies.

5. Conclusion
The anti-inflammatory activity of *A. bakerianus* root extract in this study was statistically similar in efficacy to that of the reference drug indomethacin, a COX-1 and COX-2 inhibitor. All doses of *A. bakerianus* root extract used in this study statistically had the same efficacy as the reference drug indomethacin at all the time intervals of the study. Administration of *A. bakerianus* root extract inhibited the carrageenan-induced inflammatory oedema starting from the first hour after aseptic subcutaneous injection of carrageenan into the rat hind paw and during all phases of inflammation. The inhibition of inflammation was attributed to the presence of various phytochemical classes in the extract, most of which are known to exhibit antioxidant activity. These detected phytochemicals might have reduced the plasma concentrations of pro-inflammatory mediators of the early, intermediate and late phase mediators as well as oxidant species in all phases. The present study confirms the efficacy of the root extract of *A. bakerianus* as an efficient acute phase anti-inflammatory medication, traditionally used by Basotho in the treatment of various types of inflammation.

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Conflict of interests
There is no conflict of interests among the authors.

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Volume 9 – September 2020 (09)
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http://www.ijSciences.com Volume 9 – September 2020 (09)
Evaluation of Aster bakerianus Butt dy ex C.A. Sm. Crude Root Extract for Acute Antiinflammatory Activity in Rats

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