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Experimental therapeutic studies of *Solanum aculeastrum* Dunal. on *Leishmania major* infection in BALB/c mice

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ABSTRACT

Objective(s): *Solanum acueastrum* Dunal. has been shown to have some chemotherapeutic value. Leaf and berry water and methanol compounds of *S. acueastrum* were evaluated for possible antileishmanial activity *In vivo* on BALB/c mice and i*n vitro* against *Leishmania major* promastigotes, amastigotes and vero cells.

Materials and Methods: Dry *S. aculeastrum* berry and leaf material were extracted in methanol and water. *L. major* parasites were exposed to different concentrations of *S. aculeastrum* fruit and leaf compounds and the IC_{50} on the promastigotes, percentage of infection rate of macrophages by amastigotes and the toxicological effect on vero cells were determined. BALB/c mice were infected subcutaneously with 1×10^6 promastigotes and kept for four weeks to allow for disease establishment. Infected mice were treated with fruit and leaf methanolic and water compounds, amphotericin B (AmB), and sterile phosphate buffered saline (PBS).

Results: Fruit methanol compound was most effective in inhibiting the growth of promastigotes with IC_{50}78.62 µg/ml. Fruit water compound showed the best activity in inhibiting infection of macrophages by amastigotes. Fruit methanol compound was more toxic at Ld_{50}=8.06 mg/ml to vero cells than amphotericin B. Analysis of variance computation indicated statistically significant difference in lesion sizes between experimental and control mice groups (P=0.0001). Splenic impression smears ANOVA indicated a highly significant difference in parasitic numbers between the experimental and the control groups (P=0.0001).

Conclusion: The results demonstrate that compounds from *S. aculeastrum* have potential anti-leishmanial activities and the medicinal use of the plant poses considerable toxicity against dividing vero cells.

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Introduction

Leishmaniases consist of chronic human infections, developed by types of obligate parasites that are intracellular protozoan parasites of the genus *Leishmania* (1). Natural transmission of *Leishmania* parasite is carried out by sandflies of the genus *Phlebotomus* (Old world) or *Lutzomyia* (New World) (2, 3). Leishmaniasis is considered endemic in 16 developed countries, and 72 developing countries in 5 continents: Africa, Asia, Europe, South and Central America. A total of 350 million people are at risk of infection, while 14 million are directly infected by the disease (4, 5). Leishmania infection causes a group of diseases ranging from a self-healing cutaneous lesion to potentially fatal visceral form of disease, known as Kaka-azar (visceral leishmaniasis) (6). Cutaneous leishmaniasis (CL) is caused by infection with *Leishmania tropica* and *Leishmania braziliensis*. Cutaneous leishmaniasis caused by *L. tropica* is common along the shores of Mediterranean, throughout Middle East, Africa and parts of India (7). Cutaneous leishmaniasis caused by *L. braziliensis* is mainly confined to C. America and S. America. In Kenya, CL is endemic in Rift Valley, Eastern, Central and Western Provinces (8). Leishmaniases are considered neglected diseases, particularly in terms of new drug development pegged on its poor financial returns. Cutaneous leishmaniasis which forms the bulk of the infection, has a self-healing nature and as a result, people do not seek medical attention despite the associated chronic suffering (9). Leishmaniases control strategies are not always successful (6). The situation has been aggravated by the fact that pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), amphotericin B, pentamidine, miltefosine and paromomycin, the drugs of choice for the treatment of leishmaniases are expensive and need multiple injections, exhibit considerable toxicity, variable and limited efficacy and recently there is the emergence of antimony-resistant Leishmania strains in some endemic areas (10-13).

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In this regard, The Tropical Diseases Programmes of the WHO have considered the investigation of medicinal plants for the treatment of leishmaniases as essential and of high priority (14, 15). This is because herbal regimens are relatively cheap, less toxic and takes shorter time to develop and market as opposed to the conventional drugs that take over ten years to develop. In the family *Solanaceae,* a Bolivian plant species, *Saracha punctata* has been indicated to completely inhibit the growth of promastigote forms of *Leishmania braziliensis, Leishmania donovani* and *Leishmania amazonensis* (16). Different sub species of *S. aculeastrum* plants are found in many parts of Kenya including areas where *L. major* infection is common but there are no trial reports on the use of the plant as a medicinal herb against leishmaniasis despite having been used traditionally against inflammatory-related ailments and successfully validated against cervical and breast cancer in South African studies. It is essential that the new treatment options become truly accessible and available in endemic areas (17).

Solanum aculeastrum (Solanaceae) commonly known as *Omotobo* by the Abagusii community of Kenya is also known as soda apple or goat bitter apple or poison apple. The species name *aculeastrum* refers to the thorns that adorn most parts of the shrub. It is a shrub or small tree native to tropical Africa down to South Africa, in a wide range of soil, terrain and climatic conditions (18). *S. aculeastrum* occurs naturally in grassland, woodland and in forest margins, and also in disturbed places. It has been recorded from gentle to steep slopes and on all aspects, on various soil types e.g. sandy soils, reddish brown clay-loam and brown sandy loam (19, 20).

Among the Abagusii community of Nyamira County, the fruits and leaves of *S. aculeastrum* are used fresh, dried, boiled, or charred (ashed) for the treatment of jigger infestations and wounds (*Tungiasis*), swollen joints in fingers, gangrene, toothaches, gonorrhea, bronchitis, rheumatism and in ringworm in cattle, they are also used as eyewash. A decoction of the root bark is used in Kenya for sexually transmitted bacterial diseases including gonorrhea as well as acne (21). Ethnobotanical survey revealed that the berries are used in the treatment of breast cancer (22, 23). Methanol and aqueous extracts of the berries have been shown to have moderate antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa* and *Bacillus subtilis* bacteria (24- 27). The objective of the present study was to determine the effect of extracts of *S. aculeastrum In vitro* and *In vivo* in BALB/c mouse systems.

Materials and Methods

Leishmania parasites

Leishmania major parasites, strain IDUB/-KE/94=NLB-144 were used for this study. Parasites were prepared from a *Leishmania major* infected BALB/c mouse at Kenya Medical Research Institute (KEMRI) by cutting tissues from an infected footpad and culturing as previously described by Wabwoba *et al* (27). Briefly, parasites were cultured in RPMI 1640 (Sigma-Aldrich® chemical) and liquid phase Schneider's Drosophila medium (Sigma-Aldrich®/USA) supplemented with 25% foetal bovine serum (FBS) (Gibco Invitrogen Corporation, New York, USA), penicillin G (100 U/ml) (Gibco®), and streptomycin (100 µg/ml) (Gibco®) at 25˚C. The tubes were inspected frequently for any changes in cultivated parasites. Stationary phase promastigotes were harvested from 5 to 7 day old cultures and washed 3 times in phosphate buffered saline (PBS) by centrifugation at 2500 rpm for 15 min at 4˚C. The parasites were counted in hemocytometer and used for i*n vitro* and i*n vivo* experiments.

Plant material

Fresh ripe berries and leaves of *S. aculeastrum* Dunal. plant naturally occurring in the wild and from homestead hedges were collected from Nyakwerema village of Nyamira County, Kenya, during the months of June-July. The plant was positively identified at the Chiromo Campus herbarium, University of Nairobi. A voucher specimen LLT 2012/001 was prepared and submitted to the herbarium of the same university for preservation as reference material.

Preparation of plant material and extraction

Dried berry and leaf extracts were prepared according to the method described by Koduru *et al* (22). Briefly, 500 g of fresh ripe berries or leaves were chopped into small pieces. The chopped berries and leaves were air dried to constant weight under shade. The dried parts of the plant were ground using an electric blender and soaked in 1000 mg of fresh absolute methanol on a mechanical shaker with intermittent shaking for 48 hr. The solvent was filtered with a Buchner funnel (Global Scientific Supply) and Watman No 1 filter paper (Aldrich) and the filtrate concentrated to dryness under reduced pressure at 40˚C using a rotary evaporator to the required doses for further investigation. The final dry extract was stored at 4˚C in air tight bottles until used for bioassay investigations.

For water extraction, 500 mg of cold water were added to 100 g of ground berry powder and 900 mg cold water were added to 50 g of ground leaves. The two mixtures were filtered into conical flasks (Pyrex®) and placed on a water bath for one hr and 30 min. The mixture were coated in acetone and dry ice and fixed on freeze drier machine for 48 hr. The final dry extract was stored at 4˚C in air tight bottles until used for investigations (22).

Preparation of the test drugs

Preparation of drug extracts was done as described by Dorin *et al* (28). Briefly, stock solutions of

the fractions were made in culture media for antileishmanial assay and re-sterilized by passing through 0.22 µm micro-filters under sterile conditions in a laminar flow hood. If the extracts were insoluble in water or culture media, they were first dissolved in 1% dimethyl sulfoxide, DMSO (Polymer Trading USA LLC) to avoid solvent carry over. The final concentration did not exceed 1%. This is because DMSO is toxic and thus can affect cells. All the prepared drugs were stored at 4^0C and retrieved only during use.

In vitro antileishmanial activity of S. aculeastrum

In *in vitro* bioassays, *L. major* parasites were exposed to different concentrations of *S. aculeastrum* fruit and leaf compounds and the IC_{50} on the promastigotes, percentage infection rate of macrophages by amastigotes and the toxicological effect on vero cells determined.

Anti-promastigote assay

Anti-promastigote assay was carried out as described by Ngure *et al* (8). Briefly, *L. major* promastigotes were cultured in RPMI 1640 media overlaid with 2 mg of Schneider's *Drosophila* insect medium supplemented with 20% *et al* bovine serum, 100 mg/ml streptomycin and 100 U/ml penicillin-G, and 5-fluorocytosine arabinoside. Promastigotes were incubated in 24-well plates. After five days of cultivation, aliquots of parasites were transferred and seeded in 96-well (Dynatech Laboratories, 17 USA) micro-titer plate at a density of 1×10^6 parasites/ml. The parasites were incubated at 27˚C for 24 hr and 300 µl of the highest concentration (3000 µg/ml) of each of the test samples was added in the first well in duplicate and serial dilution carried out to 62.5 µg/ml. The experimental plates were incubated further at 27˚C for 72 hr. The controls used were promastigotes with no drugs and medium alone (no drugs/extract and no cells). A positive control with amphotericin B 20 mg/ ml was used in the same plate. Blank wells contained MTT and Schneider's Insect Medium (SIM). Ten microlitres of MTT reagent (final concentration of 0.5 mg/ml) was added into each well and the cells incubated for 2 - 4 hr (29). The medium together with MTT was aspirated off from the wells, 100 µl of DMSO (0.2%) added and the plates shaken for 5 min. Absorbance was measured for each well at 562 nm using a micro-titer plate reader. The IC_{50} values were computed by entering the optical density readings in a preset MS excel template.

Anti-amastigote assay

The anti-amastigote assay was carried out as described by Delorenzi *et al* (30). Briefly, the body surface of BALB/c mice was disinfected with 70% ethanol (Sagatal®), the skin torn dorsoventrally to expose the peritoneum and a sterile syringe used to inject 10 ml of phosphate buffered saline (PBS) into the peritoneum. After 24 hr, mouse peritoneal macrophages were harvested by withdrawing the fluid into sterile centrifuge tubes. The cell suspension was centrifuged at 2500 rpm for 10 min and the pellet re-suspended in 5 ml of complete RPMI 1640 medium. Macrophages were adsorbed in 24-well plates and allowed to adhere for 4 hr at 37˚C in 5% CO_2. Non-adherent cells were washed with phosphate buffered saline (PBS), and the macrophages incubated overnight in RPMI 1640 media. Adherent macrophages were incubated with *L. major* promastigotes at a parasite/macrophage ratio of 6:1 at 37^0C in 5% CO_2 for 4 hr and free promastigotes removed by extensive washing with PBS. The cultures were further incubated in RPMI 1640 medium for 72 hr. Treatment of the infected macrophages with drugs was done by serial dilution from 1000 µg/ml to 125 µg/ml. Amphtericin B standard drug, was used as positive control for parasite growth inhibition. After 5 days, the monolayers of cells were washed with PBS at 37˚C, fixed in methanol and stained with 10% Giemsa solution. The number of amastigotes was determined by counting a total of at least 100 infected macrophages in duplicate cultures, and results expressed as infection rate (IR) (31) (% IR = No. of infected macrophages per 100 macrophages).

Safety evaluation assay

Vero cells were cultured and maintained in Minimum Essential Medium (MEM) (Sigma Aldrich®), supplemented with 10% FBS as described by Ngure *et al* (8). Briefly, vero cells were cultured at 37˚C in 5% CO_2, harvested by trypsinization, pooled in a 50 ml vial and 100 µl cell suspensions (1×10^5 cell/ml) put into duplicate wells of rows A-H in a 96-well micro-titer plate for one sample. The cells were incubated at 37˚C in 5% CO_2 for 24 hr to attach, the medium aspirated off and 150 µl of the highest concentration (20 mg/ml) of each of the test samples were put in wells and serially diluted to 0.625 mg/ml. The experimental plates were incubated further at 37˚C for 72 hr. A positive control with amphtericin B (20 mg/ml) was used in the same plate. The negative controls were cells with no drugs, and medium alone (no drugs and no cells), while blank wells contained MTT and Schneider's Insect Medium (SIM). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium Bromide (MTT) reagent (10 µg/ml) was added into each well and the cells were incubated for 2-4 hr until a purple precipitate was observed under a microscope. The medium together with MTT were aspirated off from the wells, after which 100 µl of DMSO was added and the plates shaken for 5 min. Absorbance was measured for each well at 562 nm using a micro-titer plate reader and LD_{50} values determined.

In vivo antileishmanial activity of S. aculeastrum

For *In vivo* bioassays, BALB/c mice were infected subcutaneously with 1×10^6 promastigotes and kept for four weeks to allow for disease establishment. Infected mice were treated with fruit and leaf methanolic and water compounds, AmB, and sterile phosphate buffered saline (PBS).

Experimental design

Sixty male BALB/c mice, 6-9 weeks of age and weighing 20-30 g were obtained from the animal house facility of KEMRI. The animals were housed in clean cages placed in a well-ventilated house for the whole period of the study. The mice were also allowed free access to food and water *ad libitum*. The study using mice was done following approval by the Ethical Committee on Animal Care and Use of KEMRI. The infection, lesion development monitoring and treatment of BALB/c mice procedure was carried out as described by Wabwoba *et al* (27). Briefly, the left hind footpads of BALB/c mice were inoculated with 1 x 10^6 stationary phase culture of *L. major* promastigotes in 40 µl phosphate buffered saline (PBS). Lesion development was monitored by measuring the thickness of the infected footpad weekly using a Vernier caliper. The mice were randomized into 6 cages comprising of 9, 9, 9, 9, 11 and 12 mice respectively. Group I was treated with fruit water extract, Group II was treated with leaf water extract, Group III was treated with fruit methanol extract, group IV was treated with methanol leaf extract, Group V was treated with AmB and Group VI was treated with PBS. Treatment with the extract, PBS and the standard drug started one month after infection, and continued for four weeks through intraperitoneal injections of 100 µg/ml. To compare the drug effects, the lesion size were measured and expressed as the difference in thickness between the infected and the uninfected contralateral footpad.

Quantification of L. major parasites in thespleen of BALB/c mice

At the end of the experiment (after 28 days) all mice were sacrificed by ether anaesthesia. At necropsy, the spleens of all the treated animals were weighed and impression smears made as described by Wabwoba *et al* (27). Briefly, the impression smears were fixed in methanol and stained with Giemsa. The slides were examined under a compound microscope in an oil immersion lens to enumerate the number of amastigotes per 1000 host nuclei. At least, 100 microscopic fields were examined before an imprint was reported negative. The total numbers of parasites in the spleen were expressed as splenic cell infection by amastigotes.

Statistical analysis

Data were analysed using GraphPad InStat software programme utilizing One Way Analysis of Variance (ANOVA) and Tukey-Kramer test statistic was used as *Post hoc* where applicable. Descriptive statistics were used where appropriate. A *P*-value of less or equal to 0.05 was considered significant. Data were organized into tables or bar graphs.

Results

Activity of S. aculeastrum extracts on L. major promastigotes

Following incubation of promastigotes with various drug compounds, drug concentration inhibiting parasite growth by 50% (IC_{50}) was established and results indicated fruit methanol compound with an IC_{50} of 78.62 µg/ml was more effective than other test compounds in inhibiting promastigotes growth. Fruit water, leaf methanol and leaf water were least effective in the control of promastigotes growth in an *in vitro* system indicating IC_{50} of 94.56 µg/ml, 450.98 µg/ml and 486.79 µg/ml respectively. However, the reference drug, AmB, remained more effective (IC_{50} = 4.38 µg/ml) than fruit methanol in inhibiting parasite growth as it showed 17 fold strength than the fruit methanol drug (Table 1).

Activity of S. aculeastrum extracts on L. major amastigotes

Drug activity which was recorded as percentage macrophage infection rate by *L. major* amastigotes cells following 72 hr incubation with the various drugs used at concentrations ranging from 1000 µg/ml to 125 µg/ml indicated that none of all the test compounds inhibited all amastigotes completely at any given concentration. The test drug concentrations demonstrated that infection of the macrophages with *L. major* amastigotes was dependent on the concentration of the drug treatments whereby decreased infectivity was observed with increased concentration as indicated by reduced mean amastigote percentages at 1000 µg/ml in comparison with 125 µg/ml in all treatments. Fruit water and fruit methanol demonstrated the best activity towards amastigotes with fruit water showing better efficacy. The leaf water and leaf methanol extracts treated macrophages were as twice infected by amastigotes as compared to their fruit compounds.

Table 1. The IC_{50} values of *Solanum aculeastrum* extracts and amphotericin B on *Leishmania major* promastigotes

Treatment	*Leishmania major* promastigotes
	IC_{50} (mean±SD µg/ml) No. of samples = 4
Fruit water	94.56±16.95
Leaf water	486.79±125.46
Fruit methanol	78.62±22.27
Leaf methanol	450.98±99.65
Amphotericin B	4.38±2.8

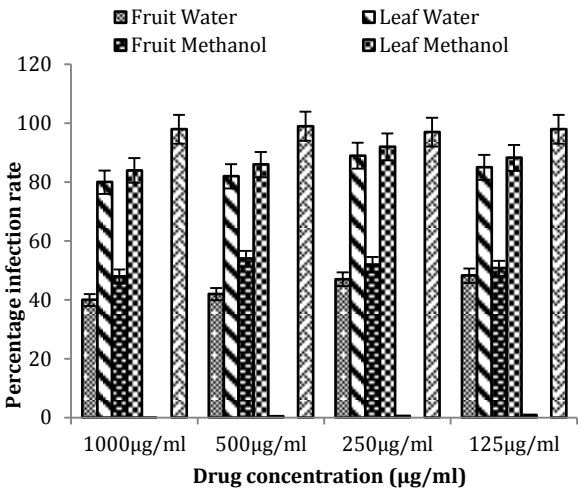

Figure 1. Activity of *Solanum aculeastrum* extracts and amphotericin B against *Leishmania major* amastigotes. Data represent percentage infection rates

Fruit water demonstrated the best results on the activity of amastigotes as indicated with an infection rate of 40% at the highest concentration of 1000 µg/ml, and an infection rate of 48.25% at the lowest concentration of 125 µg/ml, this was followed by fruit methanol which also demonstrated good results as indicated by infection rate of 48% at the highest concentration of 1000 µg/ml, and 50.75% at the lowest concentration of 125 µg/ml. Leaf water demonstrated infection rate of 80% at the highest concentration of 1000 µg/ml, and 85% at the lowest concentration of 125 µg/ml, while leaf methanol demonstrated least activity on amastigotes which was demonstrated by an infection rate of 84% at the highest concentration of 1000 µg/ml and 88.25% at the lowest concentration of 125 µg/ml. The control

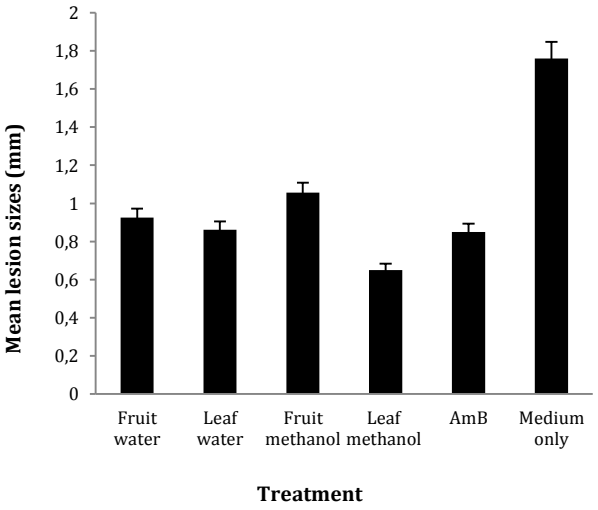

Figure 2. The effect of intraperitoneal treatment of *Leishmania major* infected mice with *Solanum aculeastrum* and amphotericin B on the progression of footpad lesions. Data represent mean lesion sizes ± S.D. (n = 5)

media demonstrated an average of 98% infection rate in all wells while AmB displayed the best activity against amastigotes with infection rates of 0.1% at the highest concentration of 1000 µg/ml and 0.9% at the lowest concentration of 125 µg/ml (Figure 1).

Safety of S. aculeastrum on vero cells

The lethal dose 50 (Ld_{50}) killing half the number of vero cells indicated that leaf water was the most safest drug requiring to be in a concentration of 13.89 mg/ml to kill vero cells from 1×10^5 to half the number and was followed by leaf methanol which required to be in a concentration of 11.11 mg/ml to reduce vero cells from 1×10^5 to half the number. Fruit water and fruit methanol were most toxic requiring being in 10.42 mg/ml and 8.06 mg/ml to kill vero cells from 1×10^5 to half the number respectively. However, AmB (Ld_{50} = 1.25 mg/ml) was 8 times and 6 times more toxic to health vero cells in an *in vitro* system than fruit water and fruit methanol respectively, which were the most toxic test drug compounds (Table 2).

Effects of intraperitoneal administration of S. aculeastrum lesion sizes (mm)

Intraperitoneal treatment with drug compounds which was used to evaluate drug efficacy *in vivo* by measuring lesion sizes (mm) indicated that leaf methanol, AmB, leaf water, fruit water recorded lesion sizes of 0.65±0.041 mm, 0.8500±0.1118 mm, 0.8620±0.101 mm, 0.9260±0.07987 mm, and 1.0560±0.22898 mm respectively, while the control group demonstrated a two fold increase in lesion sizes of 1.76±0.062 mm (Figure 2). Analysis of variance computation indicated statistically significant difference in lesion sizes between experimental and control (PBS) mice groups (F=11.118; *P*=0.0001). Tukey-Kramer multiple comparisons test indicated that lesion sizes were comparable (*P*>0.05) in all mice groups following treatment with test drug compounds. Results also indicated that there was no significant difference in lesion sizes in mice groups treated with any of the test drug as compared to treatment with AmB (*P*>0.05). However, treatment of mice with any of the drug compound was significantly protective against cutaneous disease as compared to placebo (PBS) (*P*>0.001).

Table 2. The Ld_{50} values of *Solanum aculeastrum* extracts and amphotericin B on vero cells

Treatment	Vero cells
	Ld_{50} (mean±SD mg/ml) No. of samples = 4
Fruit water	10.42±2.51
Leaf water	13.89±0.15
Fruit methanol	8.06±3.15
Leaf methanol	11.11±0.62
Amphotericin B	1.25±5.00

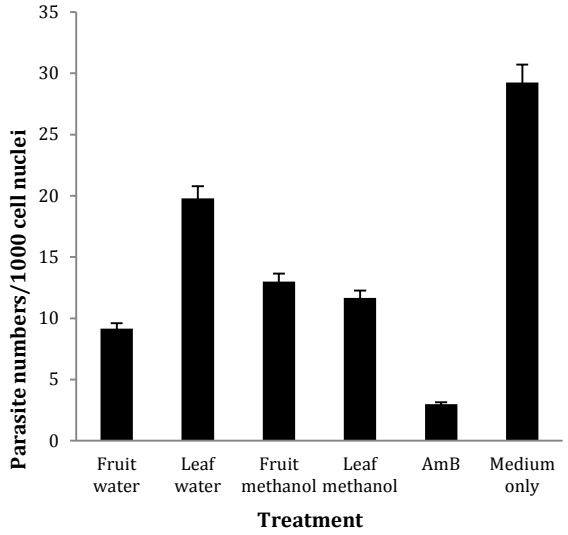

Figure 3. Parasite burden in spleens of *Leishmania major*-infected BALB/c 7 days after the end of treatment. Data presented as mean ± SD

Effects of S. aculeastrum extracts on parasite numbers

Splenic impression smears showed amastigotes both within infected cells and outside of cells following bursting of some infected macrophages and release of the intracellular amastigotes outside cells. Amastigotes in splenic tissues from treated mice indicated parasitic burden of 9.15±2.634 amastigote per 1000 splenic macrophages in the fruit water treated groups and 19.8±3.718 amastigotes per 1000 cell nuclei in the leaf water treated mice group while a parasitic burden of 3±0.909, 11.675±2.211, 13±3.916 amastigotes/1000 splenic cell nuclei was observed in the mice groups treated with AmB, leaf methanol and fruit methanol respectively. The mean parasite number in the control (PBS) mice group was 29.25±2.2172 per 1000 splenic cell nuiclei (Figure 3). Analysis with ANOVA indicated a highly significant difference in parasitic numbers between the experimental and the control groups (F=42.793; P=0.0001). Although treatment of mice with fruit water indicated significantly more protective capacity as compared to treatment with leaf water (P<0.001), mice group treated with fruit water showed comparable parasitic numbers as compared to mice treated with either leaf methanol or fruit methanol (P>0.05). Parasite numbers were also comparable in mice groups treated with leaf methanol or fruit methanol (P>0.05). However, treatment of mice with fruit methanol was also significantly more protective than treatment with leaf water (P<0.05). In addition, mice treated with leaf methanol were significantly more protected against cutaneous leishmaniasis than mice treated with leaf water compound (P<0.01). Remarkably, treatment of mice with fruit water showed comparable disease parasite outcome as compared to treatment with the positive control drug, AmB (P>0.05). However, treatment of mice with AmB was significantly more protective against disease than treatment with leaf methanol, leaf water, or fruit methanol (P<0.001). Treatment with any of the drug compound recorded significant protection level when compared with the untreated control mice group (P<0.01).

Discussion

The emergence of antimonial-resistant Leishmania strains is on the rise and natural products like *S. aculeastrum* extracts and other plant products that have been tested and found to possess antileishmanial activities may provide alternative treatment against leishmaniasis. In the family *Solanaceae,* a Bolivian plant species *S. punctata* has been indicated to completely inhibit the growth of promastigote forms of *L. braziliensis, L. donovani* and *L. amazonensis* (16). In the present study, these previous findings are extended to determine the safety and efficacy of *S. aculeastrum* water and methanolic compounds in *in vitro* and *in vivo* test systems against *L. major* parasites.

The lowest IC_{50} associated with fruit methanol compound is an indication of its effectiveness against *L. major* promastigotes in comparison to fruit water, leaf water and leaf methanol compounds. The fruit methanol compound was one and half times and at least six times more effective than fruit water and other test compounds in killing parasites, proves to be a potentially efficacious drug compound against leishmaniasis. The efficacy demonstrated by fruit methanol in this assay compares well with similar studies where *S. aculeastrum* fruit methanol compounds demonstrated the highest antiproliferative activity against cancer cells (32).

The phenomenon of substantial amount of polar constituents present in the *S. aculeastrum* plant could explain the higher anti-leishmanial activity of fruit water and fruit methanol compounds in comparison to other test compounds. The *S. aculeastrum* fruits, both mature and immature, contain steroidal alkaloids which have been the target of pharmacological investigations because of their structural similarities with anabolic steroids, steroidal hormones and corticosteroids (33). In the present study it was interesting to associate fruit water with the highest leishmaniacidal effect on mastigotes than all other test compounds. The fact that the fruit water compound was one times and at least two times more effective than fruit methanol and other test compounds in reducing macrophage infection by amastigotes proves to be a potentially efficacious drug compound against leishmaniasis.

The safety evaluation assay which demonstrated that fruit compounds were more toxic than leaf compounds towards health vero cells compares well with prior studies which showed that solasodine and tomatidine, compounds found in *S. aculeastrum* fruit

possess significant cytotoxic activity against various cancer cell lines (34). The effect of *S. aculeastrum* extracts on health cells in an *in vitro* test system implies that in addition to efficacy, safety considerations should govern possible therapeutic uses of these plant extracts. However, from the results it is clear that *S. aculeastrum* compounds were much safer than AmB and therefore can be used as alternative clinical drug for leishmaniasis. A key question for the possible use of the *S. aculeastrum* extracts evaluated in this study against *L. major* treatment should take into consideration the ratio of the effective therapeutic to toxic dose. Although compounds found in *S. aculeastrum* are potent inhibitors of vero cells, it is still imperative to ascertain that any potent inhibitor of *L. major* cells must possess low IC_{50} values when tested against the parasitic actively dividing cells and a high LD_{50} values against confluent cells (cells in a cell culture dish or a flask).

The leaf methanol contribution in significantly reducing disease progression in the BALB/c mice in comparison to other compounds with one fold strength in comparison to the reference drug, AmB by reduced mean lesion sizes is a probable indication that the therapeutic effect of *S. aculeastrum* leaf can also be considered when targeting cutaneous leishmaniasis. These results are in agreement with an earlier study which has demonstrated that besides steroidal alkaloids, *S. aculeastrum* leaves also possess alkanes, aldehydes, aromatic hydrocarbons, phthalic acid, methyl salicylate and terpenoids (18, 25, 27), which could be contributing to the medicinal property of this part of the plant.

The significant protection against progression of cutaneous leishmaniasis in mice treated with fruit water as opposed to the other drug compounds was an indication of the advantages of the fruit water compound against the disease. The fruit water compound demonstrated a one time and two times more efficacy than leaf methanol, fruit methanol and leaf water test compounds in reducing parasite burdens which is an indication of a potentially efficacious drug compound against leishmaniasis. This observation may be partly due to different modes of action of fruit water drugs which may effectively reduce parasite resistance. The observation of the fruit water compound being effective in reducing parasite burden in BALB/c mice is consistent with the fruit water activity on amastigotes with general agreement that fruit water compounds were still most effective against amastigotes. Furthermore, in an earlier study, it has already been indicated that fruit water compound showed higher anticancer property than fruit methanol compound (35).

A recent study on the antiproliferative activity of the aqueous, methanol and acetone extracts of the leaves and berries of *S. aculeastrum* against HeLa, MCF-7 and HT-29 human cancer cell lines has shown that the extracts of the leaves possess no anticancer property while the methanol and aqueous extracts of the berries possess the highest antiproliferative activity (22). The effect of combined compounds of solasodine and tomatidine, steroidal alkaloids isolated from the berries of *S. aculeastrum* has also indicated pronounced activity against human cancer cells rather than the individual compounds (32). It therefore follows that a combination of *S. aculeastrum* leaves and fruits may be very effective for treatment of cutaneous leishmaniasis depending on the formulation used if the more effective compound can be used at a higher concentration than the less effective drug to formulate a combination with a desired efficacy.

Conclusion

Infections caused by the genus parasites of Leishmania are major worldwide problems with high endemicity in developing countries. Treatment of leishmaniases suffers from problems of misdiagnosis and the situation has been aggravated by the fact that the drugs of choice for leishmaniases are expensive, exhibit considerable toxicity, require prolonged parenteral administration, show variable efficacy and recently, there is the emergence of antimony-resistant Leishmania strains. With no real promising prospects of an effective vaccine against leishmaniasis in the near future and the fact that these antimony drugs have been in use for over 50 years despite the underlying problems, scientists have underscored an urgent need to investigate medicinal plants for the treatment of leishmaniases as essential and of high priority. This is because herbal regimens are relatively cheap, less toxic and takes shorter time to develop and market as opposed to the conventional drugs that take over ten years to develop. This was the basis of this study with *S. aculeastrum* Dunal. plant which has proved that it possesses some pharmacological properties which can be exploited for development of a probable treatment for cutaneous leishmaniasis.

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