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Efficacy of Crude Bark Extract of Acacia Polyacantha Against Leishmania Donovani in Mice

Christine Salbei[a]*, Moses Ngeiywa[b], Judith Makwali[c]

[a]*Kenya Medical Training College, 520, Eldoret 30100, Kenya*

[b]*University of Eldoret, 1125, Eldoret 30100, Kenya*

[c]*University of Eldoret 30100 Kenya*

[a]*Email: christinesalbei@gmail.com*

[b]*Email: mngeiywa@yahoo.com*

[c]*Email: makwali70@gmail.com*

Abstract

The template is used to format your paper and style the text. All margins, column widths, line spaces, and text fonts are prescribed; please do not alter them. Paper size is A4 (page layout→ size→A4). Use the normal margins (page layout→ margins→normal). Paragraph sittings (paragraph→line spacing 1.5, after and before the paragraph is automatic). Visceral leishmaniasis (Kala Azar) is the most severe form of leishmaniasis and is the second largest parasitic killer disease in the world after malaria.Conventional treatment of Kala Azar is with Pentavalent antimonials and Amphotericin B but these are toxic, expensive and the causative parasites are becoming resistant to the drugs. Affordable non- toxic alternative drugs are needed.This study investigated the in vitro and in vivo efficacy of methanolic and aqueous crude bark extracts of Acacia polyacantha against Leishmania donovani .SAB , BALB/c and Swiss Albino mice infected with Leishmania donovani were treated with the extracts and compared with conventional drugs and PBS.Splenomegaly and parasite loads in the spleens of the mice were determined and compared between the groups.Analysis of data was done using T test and ANOVA to determine any statistical significant differences in the tested parameters. P values of ≤ 0. 05 were considered to be significant.The study found that the crude bark extract of A .polyacantha is efficacious but less active against L .donovani with promastigote (MIC) of 1.47mg/ml compared to Pentostam with promastigote (MIC) of 0.03mg/ml and Amphotericin B with promastigote (MIC) 0.08mg/ml.

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* Corresponding author.

There were no significant differences in reduction of splenomegaly and spleen weights in L. donovani infected mice that were treated with the crude extracts compared to the controls.However, the study showed that there were significant differences in reduction of Leishmania donovani Units (LDUs) ,the spleen parasite loads reduced by 34% in SAB mice and by 63% in BALB/c mice when compared with PBS treated groups when using methanolic crude bark.There was also significant reduction of LDUs in SAB mice when treatment was given through the intra-peritoneal route (LDU mean=5.99) compared to the oral route(LDUmean=15.34) .In conclusion the crude bark extract of A. polyacantha is efficacious but less potent against L. donovani compared to both Penstotam and Amphotericin B. The study recommends that further studies should be done on root and leaf extracts of A. polyacantha to establish if they have better action against L. donovani.

Keywords: Efficacy; Leshmaniasis; Acacia Polycantha; Treatment; Splenomegaly; Antimonials.

1. Introduction

The name Kala-azar means “black fever” in Hindi because patients with the disease in India developed hyperpigmentation [1].Kala azar is also known as visceral leishmaniasis (VL) and is caused by *Leishmania donovani* which is the major risk factor for the disease [2]. Kala-azar was a lethal disease in colonial India until Charles Donovan of the Indian Medical Service (IMS) in Madras discovered the parasite independently in 1903 while William BoogLeishman did the same as he was carrying out his research in Great Britain [3]. Donovan's discovery ended the confusion prevalent over the anomalous and puzzling cases of malarial fevers in India and proved that all fevers were not related to malaria. This discovery added to the promotion of medical knowledge, initiated further research and created enthusiasm among medical scientists throughout the world. Donovan was the first person to see the Kala-azar parasite in the peripheral blood and thus provided a clue to the carriage and transmission of the Kala-azar parasite by the insect vector through peripheral blood [3]. The parasite was then named after the two discoverers, hence the name *Leishmania donovani*[1,3]. The leishmaniases occur in many forms. The visceral forms in the Old World are caused by *L. donovani* and*L. infantumchagasi* Nicolle. The cutaneous forms (CL) are caused by *L. tropica* Wright within Northern Africa, the Middle East and Asia, *L. major* Yarkimoff and Schokhor in Africa and in Asia and *L. aethiopic*a in Kenya and Ethiopia [4]. Infections in human beings are mainly dictated by the presence of susceptible animal reservoirs and vector phlebotomine sand flies (Diptera: Psychodiade) [5]. *Leishmania major* in Kenya is endemic in only one focus in Baringo County, Rift Valley, where it is transmitted by female sand fly *Phlebotomusduboscqi* [6]. This sand fly is a sylvatic species found mainly in animal burrows where it rests and feeds on rodents such as *Arvicanthisniloticus* Geoffrey, *Taterarobusta* Cretschmar, *Aethomyskaiseri* Noack, *Taterillusemini*Thomas and*Mastomysnatalensis* (Rodentia: Muridae) Smith which have been incriminated as *L. major* reservoir hosts [7,8]. Human CL caused by *L. tropica* is endemic in Laikipia and Nakuru Counties, Rift Valley, Kenya [9]. In these areas, mainly in caves and rock crevices the sand fly that has been incriminated as the vector is *Phlebotomusguggisbergi*[10]. No animal reservoir has been found suggesting that the disease could be anthroponotic[11].*Leishmania aethiopica* is also endemic in only one part of Kenya,Mt Elgon in Bungoma County, Western Kenya (12,13).In this focus, two sand fly species have been incriminated as vectors, *P. elgonensis* and *P. pedifer*[14,13]. The reservoir host for the parasite have been shown to be the rock hyrax,*Procaviajohnstoni*Thomas, the tree hyrax,*DendrohyraxarboreusA.Smith*(Hyracoiddea: Procaviidae), and the giant

rat,*Cricetomysgambinus*Waterhouse (Rodentia: Nesomyidae) [12]. Female sand flies act as vectors of transmission. On rare occasions transmissions occurs in utero or at the time of birth [15], as a result of blood transfusion [16], by direct person-to-person contact [17],or as a consequence of laboratory accident [18].Kala-azar is endemic in west Bengal where it was first discovered, but it is seen in its most deadly form in North and East Africa. It can also be found throughout the Arab world,Sudan and Southern Europe [19]. But while the disease's geographical range is broad it is not continuous[20, 21]. The disease clusters around areas of drought, famine and high population density in Africa; this meant a knot of infection centers mostly in Sudan, Kenya and Somalia [22]. In Kenya Kala-azar is endemic in hot semi-arid lowlands of the Rift valley, Eastern and North Eastern regions of Kenya. In Kenya, although the disease burden is largely unknown it is estimated that every year about 4000 people are infected with the disease. The main areas affected are; West Pokot, Turkana, Samburu, Baringo,Isiolo and Wajir counties [7]. In India and in East Africa, visceral leishmaniasis is typically caused by *L. donovani*. It is responsible for sporadic cases of visceral leishmaniasis in Ethiopia, Somalia, Kenya and adjacent countries and it has caused major epidemics among refugees in the Sudan [23]. Rats, gerbils, ground squirrels and small carnivores are potential reservoirs [24]. The incubation period of kala-azar is typically weeks to several months, but it may be as short as ten days or as long as several years [25]. The onset of kala-azar is usually insidious but can be abrupt with high fever suggesting malaria or another acute infection [26]. The onset of kala-azar is a spectral of syndromes with majority of infections being inapparent, self-resolving and a subset smolder with mild symptoms [26]. Only a minority of victims progress to full-blown kala-azar which is characterized by fever, weight loss, hepatosplenomegaly, neutropenia and hypergammaglobinemia[27].The gold standard for diagnosis is visualization of the amastigotes in splenic aspirate or bone marrow aspirate [28]. This is, a technically challenging procedure that is frequently unavailable in areas of the world where visceral leishmaniasis is endemic [29]. Serological testing is frequently used in areas where leishmaniasis is endemic [29] but this is not very reliable. The convential treatment of kalaazar is with Pentavalent antimonials such as Sodium Stibogluconate and MeglumineAntimoniate[27]Leishmaniasis is considered as an emerging neglected, uncontrolled disease and is endemic in 98 countries. Annually, about 2 million cases of cutaneous and 500 000 cases of visceral-type leishmaniasis are recorded and 60 000 persons die from the disease [2]. *Acacia polyacantha* ,also known as white thorn is a flowering tree that can grow up to 25 metres tall [30]*A. Polyacantha* has the meaning "many thorns" in Latin [31]. The tree is native to Africa, India, the Indian Ocean and Asia but it has also been introduced to the Caribbean [2].*A polyacantha's* roots and its bark perhaps have medicinal uses. The root extract is useful for snakebites [32] and is applied to wash the skin of children who are agitated at night but there is no published use(s) of the bark extract

1.1 Statement of the problem

Human leishmaniasis is an infectious disease caused by 20 different *Leishmania* species reported in 98 countries and territories spread across four continents (Africa, Americas, Asia, and Europe). Leishmaniasis is considered a major public health issue as it currently affects 12 million people. [33]. Leishmaniasis is a growing health problem in many parts of the world, with about 350 million people living in areas of disease endemicity and about 2 million new cases occurring each year [34]. However, only 600,000 cases are reported annually [33]. Socioeconomic conditions such as poverty and malnutrition, environmental changes such as atmospheric temperature and humidity, ecological conditions affecting the vector, parasite, and its reservoir, and population

movements caused by migration and tourism are all risk factors that directly interfere with the world's distribution of leishmaniasis [21]. As the leishmaniases continue to be one of the major tropical diseases affecting people in the Sub-saharan Africa, Middle East and South America, the drugs being used in the treatment are very expensive, toxic and require special administration in patients [33]. Also drug resistance is becoming a major concern [34]. There is therefore need to identify alternative less expensive non-toxic treatment option which can be prepared locally for the treatment of leishmaniasis

1.2 General Objective

To investigate the potency of crude bark extracts of *Acacia polyacantha* in treatment of SAB, BALB/c and Swiss Albino mice infected with *Leishmania donovani*

2. Materials and method

2.1 Study Site

The study was carried out in the Kenya Medical Research Institute (KEMRI), Centre for Biotechnology Research and Development (CBRD) in leishmaniasis laboratory .The facilities necessary for this study were availed in this laboratory.The plant material was collected from Baringo County and transported to KEMRI where it was dried under shaded conditions before grinding of the bark for extraction.

2.2 Experimental Mice

The mice were obtained and maintained at KEMRI animal house where controlled breeding was done through cross breeding between BALB/c and Swiss albino mice to obtain hybrids. The cross breeds were named SAB mice by Dr. Christopher Anjili. The F_6 generation was used in this study.

2.3 Cultivation of Leishmania parasites

Metacyclic promastigote of *L. donovani* strain NLB 144 were used. Parasites were maintained as described by Titus and his colleagues 1994. *Leishmania donovani* parasites were cultured in Schneider's insect medium supplemented with 20% Fetal bovine serum, glutamine (2µM), penicillin G (1000/ml) and streptomycin (100ug/ml).Stationary phase amastigotes were obtained from day five to seven old cultures. Metacyclics were isolated from stationary phase cultures by negative selection using peanut agglutination [35].

2.4 Extraction of the crude extract from the bark of Acacia Polyacantha

The ground material (100 g) was soaked in absolute methanol for 24 h for the methanolic extraction.The extract was filtered, dried with Na_2SO_4 and the solvent removed under vacuum in a rotary evaporator at 30-35^{0}C. For aqueous extraction, 100 g of ground material in 600 ml of water was placed in a water bath and maintained at 60^{0}C for 2 h. The filtrate was freeze-dried, weighed and stored at -20^{0}C until required for use. Reconstitution was done using methanol and water respectively according to the method of Kigondu and his colleagues 2009

[35].

3. Results

3.1 Promastigote MIC

The *in vitro* Minimum Inhibitory Concentration (MIC) of Pentostam ,Amphotericin B, *A . polyacantha* aqueous and methanolic extracts against *Leishmania donovani* promastigotes are as shown in figure 3.1. In general Pentostam was the most active against *L.donovani* MIC (3 µg /ml) followed by amphotericin B MIC (8 µg /ml) .Water extract of *A. polyacantha* was the least active with MIC (2.35 mg /ml) while the methanolic extract had MIC (1.47 mg/ml*). A. polyacantha* is far much less active against leishmania *donovani* than both pentostam and amphotericin B. Furthermore the methanolic extract is more active against *Leishmania donovani* than the water extract. These are depicted in figure 3.1.

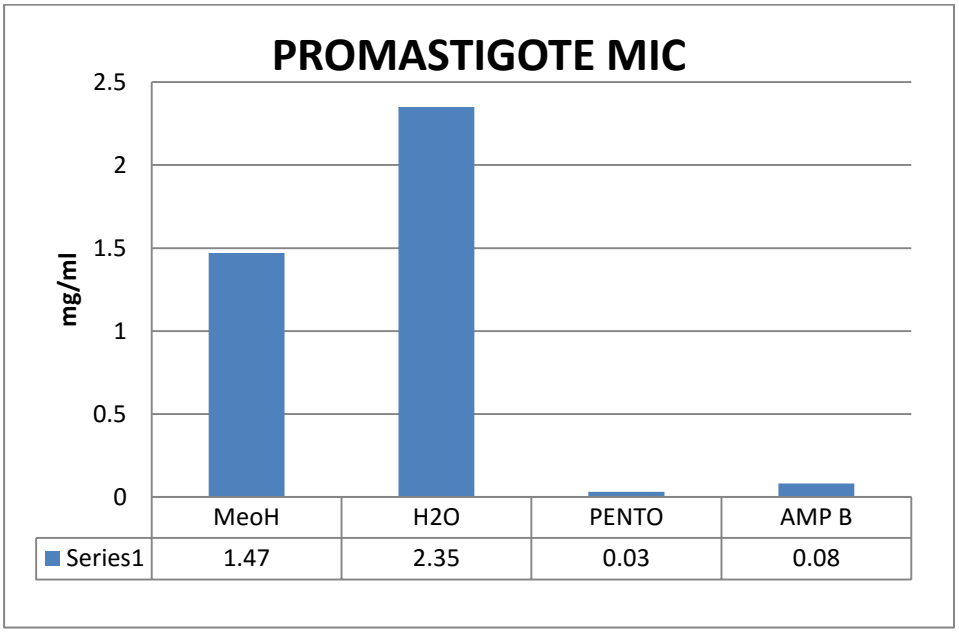

Figure 3.1: Promastigote MIC

Key: MeoH- Methanolic*A.polyacantha* bark extract

H2O-Aqueous *A.polyacantha* bark extract

PENTO-Pentostam

AMP B-Amphotericin B

3.2 Inhibition of amastigotes

The multiplication indices of the methanolic and aqueous bark extracts of *A.polyacantha* with conventional

drugs and negative control are as shown in table 3.1 and figure 3.2.In general the crude bark extracts of *A.polyacantha* inhibited *L.donovani* amastigotes but not as effectively as the conventional drugs,the highest concentration of methanolic crude bark extract of *A.polyacantha* (500µg/ml) had multiplication index of 13.9 almost similar to that of lowest concentration of Amp B (25µg/ml) with multiplication index of 13.8.

Table 3.1: Multiplication index of amastigotes in macrophage cells

DRUGS/EXTRACTS	CONCENTRATION	MULTIPLICATION INDEX
MeoH	500 µg/ml	**13.9**
	250 µg/ml	**31.5**
	125 µg/ml	**42.5**
H2O	500 µg/ml	**73.9**
	250 µg/ml	**80.8**
	125µg/ml	**86.3**
PENTOSTAM	100 µg/ml	**2.7**
	50 µg/ml	**7.1**
	25 µg/ml	**12.3**
AMP B	100 µg/ml	**10.3**
	50 µg/ml	**12.7**
	25 µg/ml l	**13.8**
RPMI		**100**

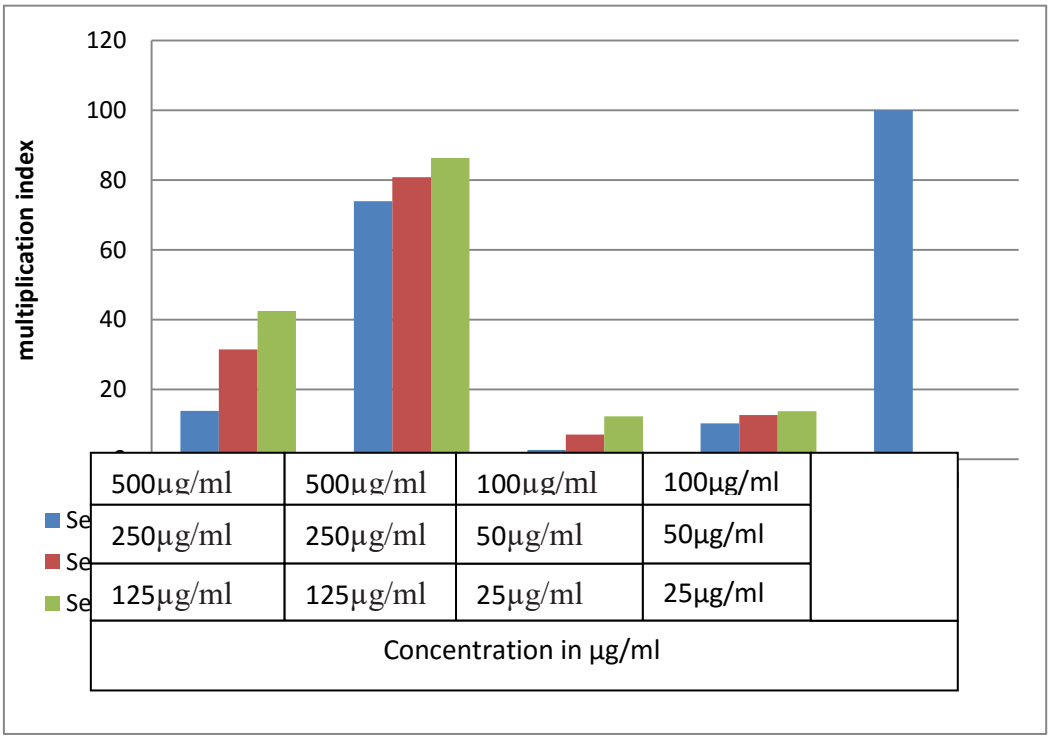

Figure 3.2: Multiplication index of amastigotes in macrophages at different dose concentrations of the drugs/crude bark extracts

The methanolic crude bark extract of *A. polyacantha* inhibited amastigote growth better than the aqueous with multiplication index of 13.9 at concentration of 500 µg/ml compared to multiplication index of 73.9 at same concentration when using aqueous extract at same concentration.

3.3 In vitro cytotoxicity studies

The *in vitro* cytotoxicity effects of varios concentrations of methanolic and aqueous crude bark extracts of *A.polyacantha* was carried out for vero cells.Several different concentrations of the extracts were used (0.01 µg/ml, 0.02 µg/ml, 0.06 µg/ml, 0.19 µg/ml, 0.56 µg/ml, 1.67 µg/ml and 5 µg/ml).The cytotoxicity potentials of various concentrations of the methanolic and acqueous bark extract of *A.polyacantha* with IC50 values are displayed in table 3.2 and figure 3.3.The IC50 values observed for vero cells against methanolic and acqueous crude bark extracts of *A. polyacantha* were 1.090 µg/ml and 1.127 µg/ml respectively.Results showed that the cytotoxicity rate was increased when the concentrations of crude bark extracts of *A.polyacantha* were increased .Maximum inhibition of growth occurred at concentrations of 0.06 µg/ml for both methanolic and aqueous extracts of *A.polyacantha*.

Table 3.2: Cytotoxicity effects of various concentrations of methanolic and aqueous crude bark extracts of A.polyacantha.

Extracts	Conc in µg/ml	0.01µg /ml	0.02µg /ml	0.06µg/ml	0.19µg/ml	0.56µg/ml	1.67µg /ml	5 µg/ml	IC50
Methanolic	Growth inhibition	0.09	1.02	1.26	1.15	0.74	0.35	0.84	1.090
aqueous		0.89	1.06	1.22	1.19	0.71	0.39	0.78	1.127

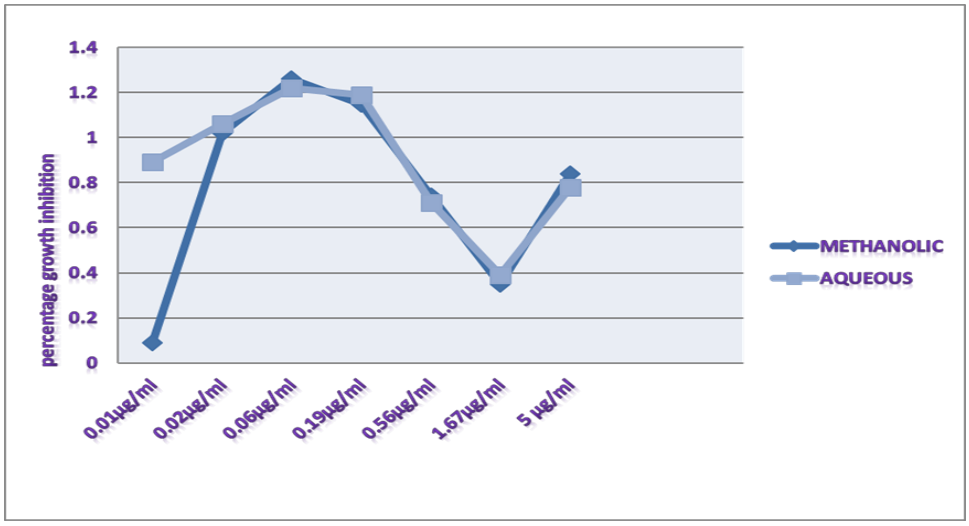

Figure 3.3: Percentage of cell growth inhibition of various concentrations of methanolic and aqueous *A.polyacantha* crude bark extracts against vero cells.

Splenomegaly mean indices in SAB,Swiss Albino and BALB/c mice infected with *L.donovani* are as shown in Table 3.3

Table 3.3: Splenomegaly mean indices in SAB ,Swiss Albino and BALB/c mice infected with *Leishmania dnovani* and treated with crude extract from the bark of wild *A. Polyacantha* and controls**.**

		Splenomegaly		
Route	**SAB mice**	**Swiss Albino**	**BALB/c**	
	Extract/controls	**Mean±SE**	**Mean±SE**	**Mean±SE**
Oral	Aqueous	0.0054±0.001b	0.0027±0.000a	0.0062±0.001c
	Methanol	0.0056±0.001b	0.0035±0.001a	0.0064±0.000c
	PBS	0.0055±0.000b	0.0042±0.000ab	0.0066±0.002c
IP	Aqueous	0.0058±0.000b	0.0054±0.000c	0.0052±0.001ab
	Methanolic	0.0052±0.000b	0.0053±0.001c	0.0048±0.000a
	PBS	0.0057±0.002b	0.0055±0.002c	0.0055±0.000b
	PENTO	0.0046±0.001b	0.0026±0.000a	0.0048±0.001a
	AMPHO	0.0053±0.000b	0.0054±0.001c	0.0048±0.001a

n general splenomegaly development was reduced while treating with Pentostam in all three strains of mice SAB (splenomegaly mean=0046) ,Swiss Albino (splenomegaly mean=.0026) and BALB/c (mean=.0048).There was no significant difference in reduction of splenomegaly development in SAB ,Swiss Albino and BALB/C mice on treating with crude extracts from the bark of wild *A polyacantha*. The crude extract from *A polyacantha* is not more potent against development of splenomegaly in SAB ,Swiss Albino and BALB /c mice compared to the conventional drugs but better than PBS

Spleen weight mean indices of SAB,Swiss Albino and BALB/c mice infected with *L.donovani* that were treated with crude extracts from the bark of wild *A.polyacantha* and controls are as shown in Table 3.4

Table 3.4: Spleen weight mean indices of SAB, Swiss Albino and BALB/c mice infected with *L donovani* and treated with crude bark extracts of *A. polyacantha* and controls.

Route		SAB mice	Swiss Albino	BALB/c
	Extract/control	**Mean±SE ()**	**Mean±SE()**	**Mean±SE()**
Oral	Aqueous	0.14±0.035a	0.07±0.000a	0.16±0.007ab
	Methanolic	0.15±0.029a	0.09±0.028a	0.19±0.014b
	PBS	0.15±0.006a	0.11±0.000a	0.15±0.000ab
IP	Aqueous	0.16±0.021a	0.15±0.001b	0.15±0.001ab
	Methanolic	0.16±0.007a	0.15±0.001b	0.16±0.000ab
	PBS	0.16±0.014a	0.15±0.002b	0.16±0.001ab
	PENTO	0.12±0.035a	0.07±0.000a	0.14±0.000a
	AMPHO	0.14±0.027a	0.14±0.001b	0.15±0.001ab

In general spleen weight mean indices of Swiss Albino mice were lower compared to those of SAB and BALB/c mice**.** Swiss Albino mice are resistant to infection with *leishmania donovani*. Spleen weight mean indices of SAB (spleen weight mean=0.12), Swiss Albino (spleen weight mean=0.07) and BALB/c (mean=0.14) mice treated with positive control (Pentostam) were the lowest. There is no significant difference of spleen weights in SAB; Swiss Albino and BALB/c mice treated with crude extract from the bark of wild *A. polyacantha* .Crude extracts from the bark of wild *A. Polyacantha* is not potent in reducing spleen weights of SAB, Swiss Albino and BALB/c mice infected with *L. donovani*.

The LDUs of SAB,Swiss Albino and BALB/c mice infected with *L.donovani* and treated with crude extracts from the bark of *A.polyacantha* and controls are as shown in Table 3.5

Table 3.5: LDUs of SAB, Swiss Albino and BALB/c mice infected with L. *donovani* and treated with crude bark extracts of *A .polyacantha* and controls

Route		**SAB mice**	**Swiss Albino**	**BALB/c**
	Extract/control	**Mean± SE(10^6)**	**Mean± SE(10^6)**	**Mean± SE(10^6)**
Oral	Aqueous	13.66±7.063d	5.838±0.772c	15.899±8.817c
	Methanolic	12.54±6.595d	4.842±3.482c	17.362±0.342c
	PBS	21.536±2.896e	9.922±3.311c	16.651±1.762c
IP	Aqueous	6.590±2.567c	9.271±2.423c	19.138±0.138c
	Methanolic	7.725±1.566c	7.527±3.456c	10.561±1.231c
	PBS	11.711±1.670d	9.264±2.329c	28.167±7.429d
	PENTO	3.281±1.980b	0.042±0.001a	6.021±1.372b
	AMPHO	0.641±0.281a	0.252±0.003b	0.331±0.002a

In general Swiss Albino mice recorded lower LDUs compared to SAB and BAlB/c mice. BALB/c mice recorded higher LDUs compared to other experimental mouse models, the highest being (LDU= 28.187) after treatment with negative control using PBS . BALB/c mice are more susceptible to infection with *L. donovani* compared to the other mouse models used in this study. Amphotericin B was the most active against *L .donovani* with (LDU = 0.641, 95% protection) in SAB mice while in BALB/c mice (LDU = 0.331, 99% protection).Treatment with Pentostam reduced spleen parasite load markedly (LDU=3.281,76% protection) in SAB mice while in BALB/c mice (LDU= 6.021,79% protection).There was significant difference in reduction of spleen parasite load ($p < 0.05$) while treating with methanolic bark extract of *A.polyacantha* although it was less potent compared to conventional treatment with SAB mice (LDU=7,725,34% protection) and BALB/c mice (LDU=11.711 ,63% protection).

Spleen weights, LDUs and development of splenomegaly of SAB, swiss Albino and BALB /c mice infected with L*donovani* and treated with crude extracts from the bark of wild *A. polyacantha* using oral and intra-peritoneal routes are as shown in Table 6.6

Table 3.6: Effect of route of administration (oral or peritoneal) of crude bark extracts of *A. polyacantha* on spleen weight ,LDU and development of splenomegaly in SAB ,Swiss Albino and BALB/c mice infected with *L donovani*

Mice strain	Route of administration	Spleen weight	Splenomegaly	LDU(10^6)
SAB mice	Oral	0.14±0.024b	0.0055±0.001b	15.34±6.52c
	IP	0.14±0.027b	0.0045±0.002b	5.99±2.76a
Swiss Albino	Oral	0.09±0.022b	0.0033±0.001a	6.26.±2.76a
	IP	0.13±0.034b	0.0048±0.001b	5.27±2.73a
BALB/c	Oral	0.17±0.019b	0.0061±0.001b	16.63±4.47c
	IP	0.15±0.011b	0.0050±0.001b	13.89±4.62b

In general there were no significant differences ($p>0.05$) in spleen weight and in development of splenomegaly in SAB, Swiss Albino and BALB/c mice infected with *L donovani* whether the crude extracts from *A. polyacantha* were administered orally or intra- peritoneally. However there was a significant difference ($p<0.05$) in LDU mean when intra- peritoneal route was used to administer the bark extracts of *A. polyacantha* in SAB mice with(LDU = 5.99) compared to (LDU=15.34) when using oral route.There were no significant differences in LDU mean in Swiss Albino or BALB/c mice whether treatment was administered by oral or intra- peritoneal route .The most effective route to administer crude extract from the bark of wild *Apolyacantha* is the intra -peritoneal route.

4. Conclusions

A.Polyacantha is efficacious but less active against *L.donovani* compared to the conventional treatment using Pentostam and Amphotericin B. The methanolic extract of *A.Polyacantha* is more active against *L.donovani* compared to the aqueous extract. Crude extract from *Acacia polyacantha* is not potent in reducing spleen weights and splenomegaly of SAB, Swiss Albino and BALB/c mice infected with *leishmania donovani*.The most effective route to administer the crude extract is the intra-peritoneal route.

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