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ORIGINAL PAPER

Erythrina abyssinica prevents meningoencephalitis in chronic *Trypanosoma brucei brucei* mouse model

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Abstract Human African trypanosomiasis is prevalent in Sub-sahara African countries that lie between 14° North and 29° south of the equator. Sixty million people are at risk of infection. Trypanosoma brucei gambesience occurs in West and Central Africa while *Trypanosoma brucei rhodesience* occurs in East and Southern Africa. The neurological stage of the disease is characterized by neuroinflammation. About 10 % of patients treated with the recommended drug, melarsoprol develop post treatment reactive encephalopathy, which is fatal in 50 % of these patients, thus melarsoprol is fatal in 5 % of all treated patients. This study was aimed at establishing the potential activity of *Erythrina abyssinica* in reducing neuroinflammation following infection with Trypanosoma brucei brucei. Swiss white mice were divided into ten groups, two control groups and eight infected groups. Infected mice received either methanol or water extract of *Erythrina abyssinica* at 12.5, 25, 50 or 100 mg/kg body weight. Parasite counts were monitored in peripheral circulation from the third day post infection up to the end of the study. Brains were processed for histology, immunohistochemistry scanning and transmission electron microscopy. Following infection, trypanosomes were observed in circulation 3 days post-infection, with the parasitaemia occurring in waves. In the cerebrum, typical brain pathology of chronic trypanosomiasis was reproduced. This was exhibited as astrocytosis, perivascular cuffing and infiltration of inflammatory cells into the neuropil. However, mice treated with *Erythrina abyssinica* water extract exhibited significant reduction in perivascular cuffing, lymphocytic infiltration and astrocytosis in the cerebrum. The methanol extract did not have a significant difference compared to the non-treated group. This study provides evidence of anti-inflammatory properties of *Erythrina abyssinica* and may support its wide use as a medicinal plant by various communities in Kenya.

Keywords *Erythrina abyssinica* · Trypanosomiasis · Neuroinflammation · Neurodegeneration · Flavonoids · Mouse model

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Introduction

Trypanosomiasis is an important zoonotic disease commonly referred to as Nagana in domestic animals and sleeping sickness or Human African trypanosomiasis in humans (Steverding 2008). Trypanosomiasis is caused by protozoan parasites of the genus *Trypanosoma.* In animals, the culprit species are *Trypanosoma brucei brucei (TBB), Trypanosoma vivax (TV), Trypanosoma simiae (TS), Trypanosoma brucei rhodesiense (TBR)* and *Trypanosoma congolense (TC),* while sleeping sickness is caused by two sub-species of *Trypanosoma brucei* i.e. *Trypanosoma brucei gambiense (TBG)* and *Trypanosoma brucei rhodesiense (TBR)*. Trypanosomiasis is transmitted by infected tsetse fly (Glossina sp) (Steverding 2008). The rhodesian form of disease is lethal if left untreated (Stich et al. 2002). However, there are reports of TBG disease infection undergoing spontaneous cure without treatment (Jamonneau et al. 2012).

Human African trypanosomiasis has two stages, the early stage (hemolymphatic) and the late stage (meningoencephalitic).

After inoculation by tsetse flies, the parasites multiply at the site before moving to lymph nodes through the lymphatics, from where they move into the blood stream. In the late stage, parasites migrate into all organs including the brain (Enanga et al. 2002). In the brain, the parasites localize early during infection, to the choroid plexus and circumventricular organs, which lack a blood–brain barrier (Schultzberg et al. 1988), and later during infection, trypanosomes and white blood cells pass across the blood–brain barrier into the brain parenchyma (Grab and Kennedy 2008). The trypanosomes cross the blood–brain barrier near intercellular junctions, by an active process (Masocha et al. 2007). The pathogenesis is acute in *TBR* infections, taking only a few weeks, but chronic in *TBG* infection.

Early stage sleeping sickness is treated with suramin for TBR and pentamidine isethionate for *TBG* (Bouteille and Buguet 2012). Treatment of meningoencephalitic stage of the disease is very limited and relatively few new formulations are currently available (Enanga et al. 2002). Melarsoprol is used in the late-stage of both forms of the disease, while eflornithine (Ishiyama et al. 2008) and nifurtimox (Priotto et al. 2009) are only used in the late-stage of *TBG* infections. The organo-arsenical drug, melarsoprol, the drug of choice for use in the second stage of infection is toxic, causes a severe post treatment reactive encephalopathy (PTRE) in up to 10 % of cases, with a fatality rate of about 50 % (Pepin and Milord 1994). Efforts have been put to develop new therapeutic agents for trypanosomiasis including those originating from medicinal plants (Adamu et al. 2009; Ngure et al. 2009; Asuzu and Chineme 1990; Oboh et al. 2013). One of the approaches to treatment is targeting immune reactions, which are thought to be the trigger for the encephalopathy (Hunter et al. 1992a), associated with over-expression of pro-inflammatory cytokines and their mRNAs (Quan et al. 1999; Masocha et al. 2006; Hunter et al. 1991). There has also been much interest in the development of new drugs capable of preventing neuroinflammatory mediated brain injury (Vauzour and Minihane 2012) as increased production of proinflammatory cytokines in the central nervous system (CNS) is a common occurrence in many neurodegenerative diseases (Griffin et al. 1998; Mogi et al. 1996; Maimone et al. 1997).

Erythrina abyssinica (EA) is a medicinal plant, traditionally used for ear, nose and throat infections (Njoroge and Bussman 2006), diarrhoea (Njoroge and Kibuga 2007), pneumonia, sexually transmitted diseases, and prostate cancer (Wagate et al. 2009). Plants in the genus erythrina produce several secondary metabolites, some of which are active against pathogenic fungi and bacteria (Kone et al. 2011). Several species of *Erythrina* have been shown to have anti-infammatory effects (Njamen et al. 2004). This study aimed at analyzing potential anti-inflammatory properties of *Erythrina abyssinica* extracts in a chronic trypanosomiasis mouse model.

Materials and methods

Collection and processing of plant material

Erythrina abyssinica root bark was collected in Machakos County, Kenya. The plant was identified by a botanist at the School of Biological Sciences herbarium, University of Nairobi. The root bark was washed to remove soil particles, before being chopped into small pieces, approximately 1 cm in length. The chopped pieces were air dried under a shade and ground into fine powder, which was stored in an airtight container, placed in a cool dry place for at least 1 week, before further processing.

Extraction and concentration of crude aqueous extracts

Methanol extraction was undertaken by soxhlet method as previously described (Ahmad et al. 2010). Briefly, 100 g of the powdered *EA* root bark was put in Soxhlet extraction chamber and extracted with methanol for 24 h. The extract was filtered with Whatman No. 1 filter paper and the filtrate dried by evaporation at 40 °C in a rotary evaporator at reduced pressure. Extracts were kept at 40 °C in an oven for 1 week to remove any methanol residues. The percentage sample yield was determined and stored in dark bottles at 4 °C to avoid biological degradation before use. Water extraction was undertaken by decoction method as previously described (Chebaibi and Filali 2013). Briefly, 600 g of the sample was soaked in 3 l of water and boiled for 2 h. After cooling, the sample was filtered and the filtrate frozen for freeze drying. Finally, the sample was weighed to determine the percentage yield then stored at 4 °C to avoid biological degradation.

Phytochemical screening

Qualitative phytochemical screening was done on *EA* extracts using standard tests to screen for the presence of alkaloids and flavonoids. Flavonoids were tested by two methods: Sodium hydroxide test (Mojab et al. 2003), where sodium hydroxide was added to a small amount of the extract. Formation of yellow orange color confirmed presence of flavonoids.

Ammonium test was performed as previously described (Saeed et al. 2012); briefly, 1 ml of the extract was added to 4 ml of ammonium solution and observed for formation of yellow color.

Presence of alkaloids was tested as previously described (Mojab et al. 2003): Briefly, dissolved samples were placed in two test tubes, one ml of Hager's reagent (saturated aqueous solution of picric acid) and Wagner's reagents (1.27 g iodine and 2 g potassium iodide in 100 ml of distilled water) were each added into one of the test tubes. Formation of a yellow precipitate in any of the test tubes confirmed presence of alkaloids.

Experimental animals

A total of 58, swiss white male mice, weighing 23–30 g obtained from the Department of Medical Physiology Animal Facility, University of Nairobi, were used for the experiment. The animals were housed in polycarbonate cages and fed on mice pellets (Mice pellets®, Unga feeds Ltd, Kenya) and provided with water ad libitum. Animals were acclimatized to experimental conditions for 1 week before starting the experiment. Bedding (wood shavings) was changed daily. Animal care protocols and procedures used were according to the University of Nairobi, Faculty of Veterinary Medicine Animal Use and Care Committee.

Infection, parasitaemia and treatment

Cryopreserved *TBB* (GUTat 3.1) stabilate was obtained from International Livestock Research Institute trypanosome bank (ILRI, Kabete, Kenya). Two irradiated (600 rads) donor Swiss White mice were each inoculated intraperitoneally with 0.2 ml of the thawed *TBB* stabilates, diluted in phosphate saline glucose (PBS with 1 % glucose) (Caljon et al. 2006; Tyler et al. 2001). Parasitaemia in these mice was assessed as described by Herbert and Lumsden (1976) and when it reached antilog 7.8–8.1, they were bled from the heart and the blood was used to infect the experimental groups. Blood from donor mice was diluted in 1 ml of phosphate saline glucose to attain a concentration of 5×10^4 parasites/ml. Each experimental mouse was infected with 0.2 ml of the diluted blood i.e. 1×10^4 parasites. Infection was confirmed by examining a drop of blood obtained from tail snip, and parasitaemia estimated by the rapid matching method (Herbert and Lumsden 1976). Infected mice were randomly divided into ten groups (Table 1). Treatment with EA aqueous extract, methanol extract and placebo (distilled water) through intraperitoneal route was done every second day, beginning on day 12 post infection, up to the end of the study. The aqueous extract was reconstituted with distilled water, while the methanol extract was reconstituted in 5 % dimethyl sulfoxide. At 21 days post infection, infected mice were treated with suramin, at a dose of 20 mg/kg body weight. This was according to the established protocol of the model (Keita et al. 1997).

Table 1 Experimental groups showing those infected and the treatment given for each group

Group	Number of animals	Infection (1×10^4 parasites)	Treatment and dosage (mg/kg)	
1	7	Infected	12.5	Aqueous extract
2	5	" "	25	" "
3	5	" "	50	" "
4	5	" "	100	" "
5	7	" "	12.5	Methanol extract
6	7	" "	25	" "
7	5	" "	50	" "
8	6	" "	100	" "
9	5	" "	1 ml	Distilled water
10	6	Non-infected	1 ml	Distilled water

Table 2 Criteria for grading brain pathology in experimental mice

Grade	Characteristics
0	No pathological changes
1 (Mild)	Several mononuclear cells (2–3) in a few perivascular spaces.
2 (Moderate)	Up to 6 mononuclear cells in several perivascular spaces. Slight meningoenchephalitis
3 (Severe)	Heavy infiltration of mononuclear cells in perivascular spaces with complete occlusion. Mononuclear cell aggregates in parenchyma. Presence of meningoenchephalitis

Tissue processing for histology and neuroinflammation scoring

Cerebral tissues from experimental mice were harvested on day 28 post infection and fixed in 10 % formalin by total immersion, for 2 weeks. Mid cerebral tissue blocks (through the hippocampus) measuring 1 cm^3 were washed under running water for 24 h, dehydrated in increasing concentrations of ethanol, cleared in two changes of methyl benzoate and embedded in paraffin wax. Sections were cut at 5 μm using a Reichert Jung microtome, and subsequently mounted on albumin coated glass slides. The sections were deparaffinized in xylene and rehydrated first in a series of decreasing concentrations of ethanol (100–50 %), then in distilled water. Staining was done using hematoxylin followed by eosin. Leica DM500 microscope was used to view stained slides. The tissues were analyzed semi quantitatively, in a blinded fashion, by modifying the technique used by Kennedy et al. (1997). The scores were 0, 1, 2 or 3, based on the degree of perivascular cuffing, presence or absence of meningoencephalitis and the extent of leucocytic infiltration in the brain parenchyma (Table 2). Neurodegeneration was analysed qualitatively.

Tissue processing for transmission electron microscopy

Tissues were processed as described by Makanya et al. (2011). Cerebral tissue blocks measuring 1×1 mm were fixed in 2.5 % glutaraldehyde in distilled water, washed in three changes of 0.1 M PBS and post-fixed in 1 % osmium tetroxide for 1 h and rinsed in two changes of 0.1 M PBS. Tissues were dehydrated in increasing concentration of ethanol, followed by clearing in

propylene oxide. The tissues were embedded in araldite resin and polymerized at 60 °C overnight. Semithin sections were made by cutting in a microtome, stained with 1 % toluidine blue, and viewed under a LEICA DM 500 light microscope, to identify areas of interest for ultrathin sectioning. Ultrathin sections of the areas of interest were cut using a microtome, placed on copper grids, stained with uranyl acetate and lead citrate, before viewing in Phillips 301 transmission electron microscope.

Tissue processing for scanning electron microscopy

This was undertaken as detailed in Kiama et al. (1994). Tissue blocks were preserved in 2.5 % glutaraldehyde in water, by total immersion. The tissues were washed in Millonig's phosphate buffer for 15 min while shaking. The tissues were stained with osmium tetroxide for 1 h then washed in Millonig's phosphate buffer for 20 min. This was followed by dehydrating in a series of increasing concentration of ethanol, followed by critical point drying. Tissues were mounted on aluminium stabs with silver conductive points and then sputter-coated with gold palladium complex and viewed in a JEOL-6010LA Scanning electron microscope.

Immunohistochemistry

Formalin fixed paraffin-embedded (FFPE) cerebral tissues were processed as described elsewhere (Jiao et al. 1999). Sections measuring 4 μm in thickness were mounted on gelatin coated slides and subjected to immunohistochemistry for glial fibrillary acidic protein (GFAP), a cytoplasmic intermediate filament specific for astrocytes (Balasingam et al. 1994). Three pairs of serial sections per animal were processed. Tissue sections were dewaxed in xylene. Antigen retrieval was performed for 20 min in citrate buffer heated at 95 ° C. After washing with phosphate-buffered saline (PBS), each section was treated for 10 min with blocking buffer (Envision™ Flex, Dako) to inhibit endogenous peroxidase prior to incubation with a rabbit anti-GFAP polyclonal antibody (1:300, Dako) for 30 min at room temperature. Following a brief rinse with PBS, a goat anti-rabbit immunoglobulin conjugated to horseradish peroxidase (Envision™ Flex/HRP, Dako) was introduced for 20 min. Diaminobenzidine (Dako), a HRP substrate was introduced for 5 min followed by thoroughly washing in PBS. Finally, tissues were dehydrated in 100 % ethanol, cleared in xylene and cover-slipped.

Stereological counts of astrocytes

The numerical density of astrocytes was determined using the physical disector method (Sterio 1984; Gundersen 1986). Two consecutive serial sections, 3 μm apart were used, one being the look up section and the other the reference section. Several fields of view were selected by systematic random method to count up to 20 astrocytes, the corresponding field in the reference section was selected and the image also taken.

A counting frame (0.198×0.278 mm) was drawn on images with the aid of Leica application suite program (LAS EZ version 1.8.0). The "forbidden line" rule was applied (Gundersen 1977), where cells touching the forbidden lines were omitted from the count (Fig. 5). Astrocytes appearing only on the look up section were counted and the numerical density obtained using the formula

$$\mathbf{Nv} = \sum \mathbf{Q} \Big/ \mathbf{h}.\sum \mathbf{a}$$

Where **Nv** is numerical density, **Q** is number of cells counted, **a** the area of counting frame=0.055 mm^2 (0.198× 0.278), **h** is the distance between tissue section pairs = Section thickness (because they were consecutive sections)= 0.003 mm

NB. Therefore in a single frame, cells were counted within a volume of 0.000165 mm^3

Data analysis

For both parasitaemia and neuroinflammatory scores, data were entered in excel data sheets (MS Excel, Microsoft USA), and line graphs plotted for parasitaemia. The data were then analyzed by comparing the means between the different groups using a one way ANOVA and a post hoc analysis using the Tukey method. This analysis was done with the aid of SPSS® computer program.

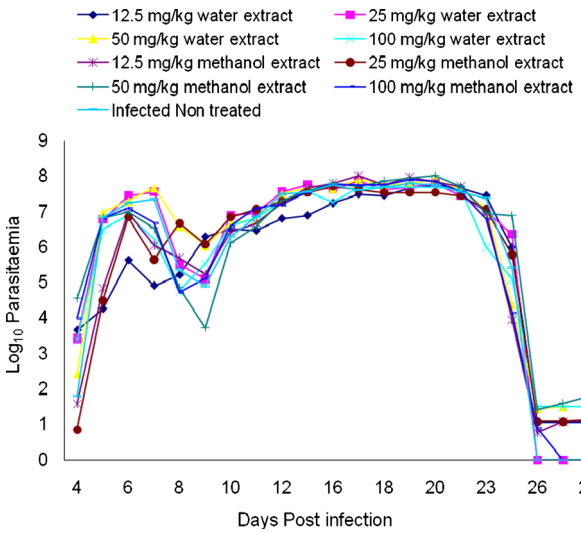

Fig. 1 Graph showing the parasitaemia pattern of *Trypanosoma brucei brucei* in mice treated with *Erythrina abyssinica* extracts. Mice were infected intraperitonealy with 1×10^4 blood stream forms of *Trypanosoma brucei brucei* (GUTat 3.1). A plot of parasitaemia against days postinfection shows fluctuation in parasite counts over the 28 days of the experiment in all experimental groups

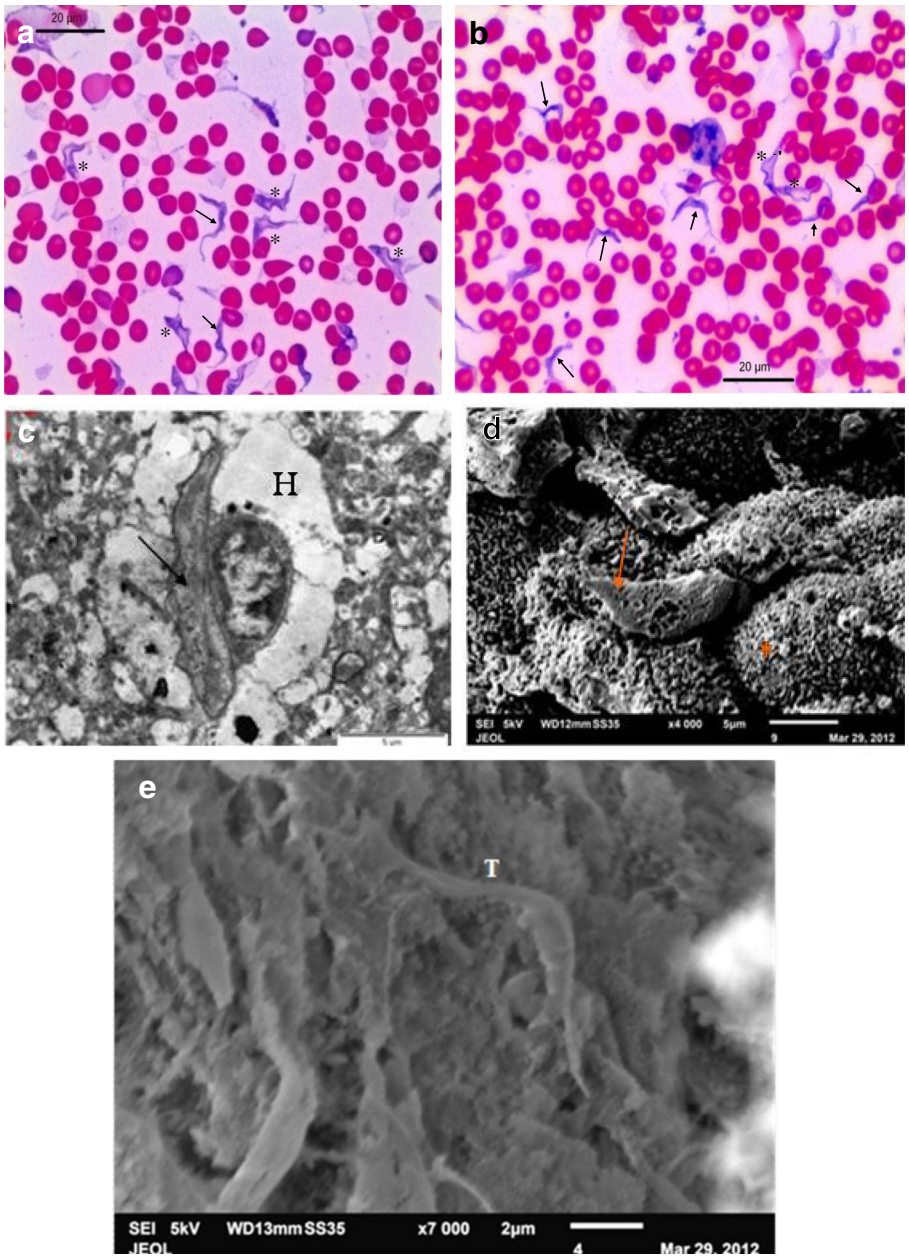

Fig. 2 Micrographs of cerebrum and blood smears from mice infected with *Trypanosoma brucei brucei* (GUTat 3.1). Note the two forms of parasites, blunt (*asterix*) and sharp forms (*arrow*). The blunt forms were higher in number during the peak of parasitaemia wave (**a**), while the slender form abundant in early phase (**b**). Trypanosomes (*arrow* and T) were seen within cerebral parenchyma (**c**), surface of choroid plexus (**d**), and within third ventricle (**e**). Note the presence of halo (H) around the parasite in cerebral parenchyma

Results

Yields from extraction

The water extract had a yield of 7.66 %, while methanol extraction had 6.13 % yields.

Phytochemical screening

Qualitative phytochemical tests revealed presence of alkaloids and flavonoids in both water and methanol extracts of *Erythrina abyssinica*. In Hager's test, addition of saturated aqueous solution of picric acid to the extract sample formed a strong yellow precipitate confirming presence of alkaloids; Wagners test produced a reddish brown precipitate. Sodium hydroxide or ammonia solution was added to the extracts and a yellow color was formed in both tests confirming the presence of flavonoids.

Parasitaemia

Parasites were detected in peripheral circulation on the fourth day post-infection. The mean prepatent period was 4 days post infection. Parasitaemia course during the study was similar in all the groups (Fig. 1), appearing in waves peaking on days 6 and from day 12 to 23 post-infection. From 22 days post infection, there was a sharp decline in parasitaemia, this was expected, because at 21 days post infection, the animals were injected with an anti-trypanosome drug (suramin). Two forms

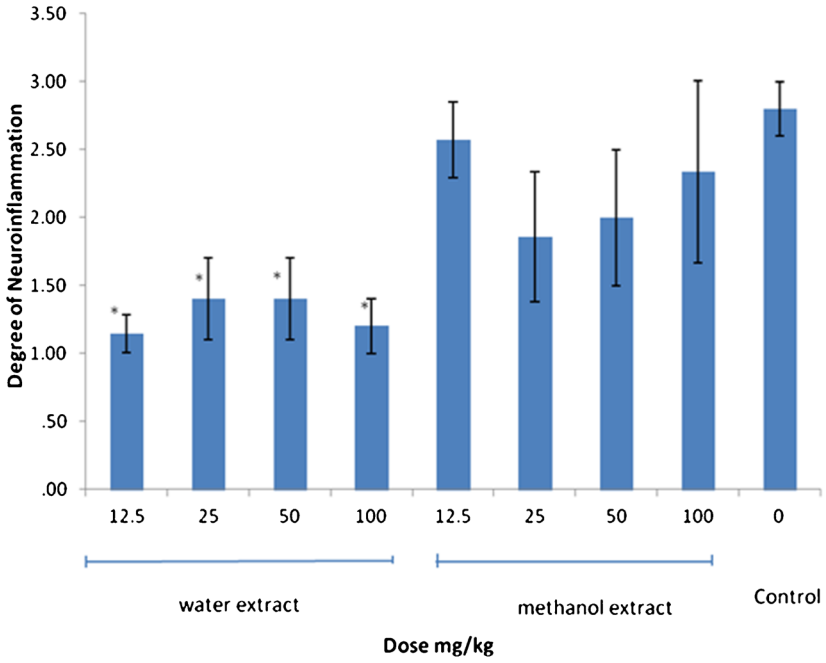

Fig. 3 Graph showing the degree of inflammation of various dosage groups and controls of mice infected with *Trypanosoma brucei brucei* (GUTat 3.1). Water extract groups show statistically significant difference (*asterisk*) compared to control. Means ± SD: 2.8±0.2, 1.14±0.14, 1.40±0.3, 1.40±0.3, 1.20±0.2, 2.57±0.28, 1.86±0.48, 2.2±0.5 and 2.33±0.67; *n*=6, 7, 5, 5, 5, 7, 7, 5 and 6 respectively

Dose mg/kg	0	12.5	25	50	100	12.5	25	50	100
Extract		water	water	water	water	metha-nol	metha-nol	metha-nol	metha-nol
Mean± SD	2.8± 0.02	1.14 ± 0.14	1.4 ± 0.3	1.4 ± 0.3	1.2 ± 0.2	2.57± 0.28	1.86 ± 0.48	2.2± 0.5	2.33 ± 0.67
N	6	7	5	5	5	7	7	5	6

of the parasites were observed. In early parasitaemic wave, slender forms were more prominent while blunt forms were prominent during the wave peak (Fig. 2). At post mortem, the parasites were observed within the third ventricle, on the choroid plexus, and in the cerebral parenchyma (Fig. 2). In the cerebral parenchyma, the parasites were conspicuously noted to have a halo around them.

Neuroinflammation

GFAP immunohistochemistry, and routine hematoxylin and eosin staining, showed astrocytosis and meningoencephalitis, which were greatly reduced in aqueous *EA* extract treatment. Infected non- treated mice had severe astrocytosis, perivascular cuffing and meningoencephalitis which were reduced in the *EA* extract treated mice (Fig. 3).

There was infiltration by lymphocytes into the cerebrum. The inflammatory cells aggregated around blood vessels forming perivascular cuffs. Within the parenchyma, the infiltrating inflammatory cells varied in shape and distribution, ranging from unifocal, multifocal, nodular or streak (Fig. 4). In infected-non treated mice, the inflammatory cells completely obliterated perivascular spaces in the hippocampus, brain parenchyma and meninges where as in infected- *EA* treated mice, there was reduction or absence of inflammatory cells in perivascular spaces and cerebral parenchyma (Fig. 4). However, only the aqueous extract has a statistical significance prevention of neuroinflammation. This prevention was similar in all the dose groups. On the other hand, all the methanol extract dose groups did not have any statistical difference compared to control.

Astrocytosis was especially evident in paraventricular region, evident by increase in number, size and staining intensity (Fig. 5) of astrocytes. The astrocyte count, staining intensity and size, were significantly reduced on treatment with aqueous *EA* extract, which had two fold increase in infected, non-treated mice (Table 3 and Fig. 5).

Another notable pathological change observed was neuronal degeneration which occurred in all brain regions (Fig. 6). This was characterized by shrinkage of the neurons, disintegration of cytoplasm, and pyknotic nuclei. The degeneration was more severe in infected-non treated mice compared to infected, *EA* treated group.

Discussion

Data from this study indicates that *Erythrina abyssinica* extract prevents neuro-inflammation, in a chronic trypanosomiasis

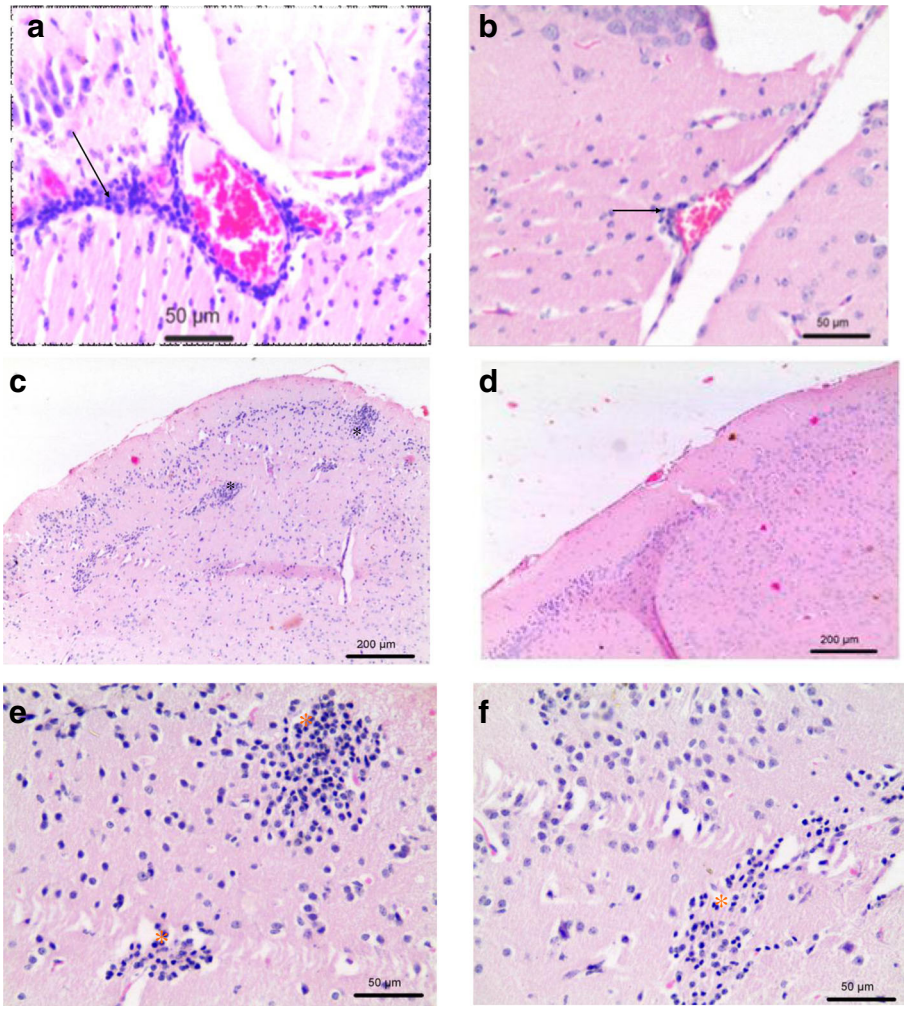

Fig. 4 Micrographs of histopathological sections of mid cerebrum, through the hippocampus, from mice infected with *Trypanosoma brucei brucei* (GUTat 3.1). Lymphocytes (*arrow* and *asterix*) present around blood vessels (**a**), and within cerebral parenchyma (**c**). The infiltration was significantly reduced in infected mice treated with aqueous *Erythrina abyssinica* extract, 50 mg/kg, (**b** and **d**). Note inflammatory cells were multi focal or unifocal in distribution, being nodular or streak shape (**e** and **f**- both are a higher magnification of micrograph c)

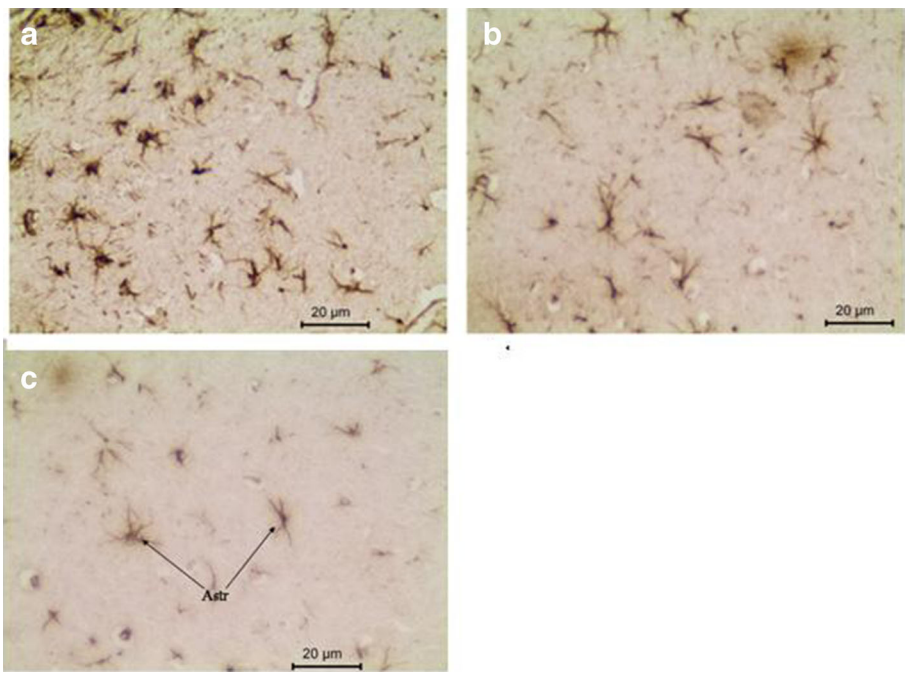

Fig. 5 Micrographs of GFAP immunostained astrocytes in mid cerebrum (through lateral ventricle and hippocampus), of mice infected with *Trypanosoma brucei brucei* (GUTat 3.1) and treated with *Erythrina abyssinica* extracts. **a** Infected mice without treatment showing intense staining of astrocytes (Astr) that have enlarged with prominent cell bodies, increase in both number and staining intensity. **b** Infected mice treated with aqueous *Erythrina abyssinica* extract, 50 mg/kg bwt dose, showing less intense staining and reduced cell count. **c** Non-infected mice showing few astrocytes with less staining intensity

Table 3 Mean numerical density of GFAP stained astrocytes (cells per mm^3) in the cerebrum of mice infected with *Trypanosoma brucei brucei* (GUTat 3.1)

Dose group (mg/kg)	N = animals in the group	Mean numerical density of astrocytes/mm^3
1 ml distilled water	6	26,666±1,414
Water extract		
12.5	7	38,091±1,512*
25	5	34,545±1,140*
50	5	34,545±1,140*
100	5	36,363±837*
Methanol extract		
12.5	7	67,939±1,988
25	7	64,909±1,397
50	5	67,273±3,564
100	6	68,667±1,506
Control		
1 ml distilled water	5	69,886±1,967

Mean values are given ± Standard deviation. Infected mice treated with *Erythrina abyssinica* aqueous extract had less number of astrocyte reactivity compared to infected non treated mice

*statistical significant $P \leq 0.05$

mouse model. The potential of *Erythrina abyssinica* in reducing inflammation in the central nervous system has not been previously demonstrated. Neuroinflammation is a characteristic feature of chronic trypanosomiasis, and is severely exacerbated following treatment with melarsoprol (Kennedy et al. 2003). The neuroinflammation is 'driven' by activated resident glial cells (astrocytes and microglia), resulting in invasion of circulating immune cells and the production of pro-inflammatory cytokines (Vauzour and Minihane 2012). Acute neuroinflammation is beneficial while chronic neuro-inflammation is most often detrimental to the nervous system (Frank-Cannon et al. 2009).

Neuroinflammation was characterized by infiltration of lymphocytes in perivascular spaces on white matter and meninges, neuronal degeneration and lymphocytic aggregations within the brain parenchyma. These changes are the common pathological feature in late stage trypanosomiasis, first described in ox, and patients dying from the disease (Mott 1905; Haller et al. 1986). In sheep experimentally infected with *TBB* (Ikede and Losos 1972a) meningoencephalomyelitis characterized by perivascular mononuclear cuffs and focal gliosis was observed. Similarly, in experimentally infected cattle there was gliosis in the diencephalic region and infiltration by lymphocytes and macrophages in meninges and Virchow-Robin spaces in the brain and spinal cord (Ikede and Losos 1972b).

These pathological changes have been used in developing experimental animal models for the late disease stage (Kagira et al. 2007; Ngotho et al. 2011). Poltera and others developed a mouse model and studied the neuropathology by histological and immunohistochemical techniques (Poltera et al. 1980). In the study, they reported presence of plasma cells, macrophages, trypanosomes and mononuclear cells in perivascular spaces both in the meninges and the white matter. In a rat model of *TBG,* similar changes were observed including slight demyelination (Chirimwami et al. 1988). These animal models reproduce neurological and histological pathology observed in the human disease (Kennedy 1999), and can be used for further therapeutic studies on chronic trypanosomiasis (Keita et al. 1997). Previously, treatment with corticosteroids and non-steroidal anti-inflammatories (Pepin et al. 1985; Hunter et al. 1992b) ameliorated trypanosome induced neuroinflammation, with similar results observed in a chronic trypanosomiasis mouse model treated with minocycline (Masocha et al. 2006), where the number of parasites, infiltration of T-cell and astrocytosis in the brain were

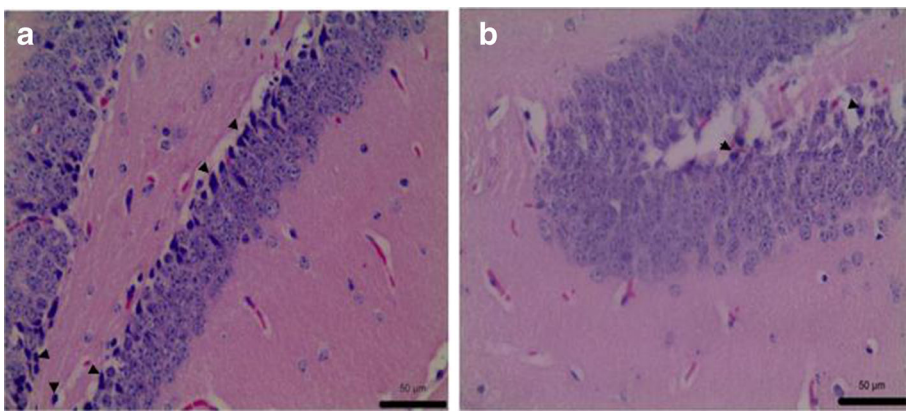

Fig. 6 Micrograph of histopathological sections of mid cerebrum, through the hippocampus, of mice infected with *Trypanosoma brucei brucei* (GUTat 3.1) and treated with *Erythrina abyssinica* extracts, showing neuronal degeneration. **a** Infected mouse treated with 1 ml distilled water showing neuronal degeneration (*arrow head*) in the hippocampus. **b** Infected mice treated with aqueous extract of *Erythrina abyssinica*, 50 mg/kg dose, showing less number of degenerating neurons (*arrow head*)

reduced. Kynurenine pathway inhibitor reduced severity of neuroinflammatory effect in the chronic trypanosomiasis mouse model (Rodgers et al. 2009). The neuropeptide substance P is involved in the generation of the inflammatory response seen in chronic trypanosomiasis mouse model, whose effects are reduced on treatment with substance P antagonist (Kennedy et al. 1997).

Using the mouse model in this study, aqueous *EA* crude extract prevent neuroinflammation, characterized by reduced lymphocytic infiltration, as well as diminished perivascular cuffing and astrocytosis. Other studies on crude extracts from the genus erythrina support the findings of this study. Batista et al. (2011) tested *Erythrina velutina* on carrageenan-induced pedal edema, which showed reduced inflammation. Similarly, *Erythrina senegalensis* had anti-inflammatory activity in egg albumin induced paw edema (Saidu et al. 2000). In a 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation, *Erythrina crista-galli* (Batista et al. 2011) and *Erythrina addisoniae* (Talla et al. 2003) crude extracts had a positive reduction on inflammation. In addition, *Erythrina addisoniae* had similar results on phospholipase A (2)-induced paw edema (Talla et al. 2003). Cyclo-oxygenase pathway is important for synthesis of prostaglandins, mediators of inflammation (Williams et al. 1999). Crude extracts from *Erythrina caffra*, *Erythrina humeana*, *Erythrina latissima*, *Erythrina lysistemon* and *Erythrina zeyheri* were shown to have inhibitory activity on the cyclo-oxygenase pathway (Pillay et al. 2001). The aqueous extract of *EA* was more effective than methanol extract, despite the fact that both water and methanol are polar solvents, often used to extract flavonoids, as most flavonoid glycosides are polar (Marston and Hostettmann 2006). Interestingly, it is known that the composition of the extract does vary with the solvent, whether water or methanol, because certain flavanoids are difficult to dissolve in methanol (Marston and Hostettmann 2006). The aqueous extract may therefore have more active compounds than methanol extract.

Despite *EA* preventing neuroinflammation in the current study, the mechanism of action and responsible molecules cannot be explicitly explained. However, the effect may be attributed to flavonoids, whose presence was confirmed in preliminary phytochemical tests. Flavonoids are polyphenolic compounds (Kovacsova et al. 2010), being the most significant compounds for the antioxidant properties of plant raw materials (Kratchanova et al. 2010). Selection of a particular food plant, plant tissue or herb for its potential health benefits, rely on flavonoid composition (Hatti et al. 2009). Flavonoids are known to act as anti-inflammatories (Spencer et al. 2012). Specifically, two flavonones, Sigmoidin A and Sigmoidin B, isolated from *Erythrina sigmoidea* had anti-inflammatory activity (Njamen et al. 2004) through inhibition of 5-lipoxygenase and phospholipase A. Further, three flavonoids; abyssinone V, a new isoflavonone, and erycristagallin from *Erythrina variegate* (Hegde et al. 1996) inhibited phospholipase A, an attractive therapeutic target for the design of novel anti-inflammatory agents (Hegde et al. 1996). The results of Hegde et al. especially support the observation in this study as abyssinone V and erycrystagallin have also been isolated in *EA* (Yenesew et al. 2009).

Conclusion

This study has provided evidence that *Erythrina abyssinica* prevents neuroinflammation in chronic trypanosomiasis mouse model. However further studies are needed to ascertain the exact mode of action. In addition, the different bioactive metabolites need to be isolated separately and tested in neuroinflammation model. This will ascertain whether the observed effect in this study is due to single molecule or is due to synergism.

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