

Full Length Research Paper

## Evaluation of *in vivo* antitrypanosomal activity of selected medicinal plant extracts

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Accepted 14 September, 2009

This study was based on the observation that traditional practitioners in Kenya use plant based extracts for the treatment of parasitic diseases. This necessitated the need to investigate the potential of such plants. Four plants (*Kigelia africana*, *Artemisia annua*, *Bidens pilosa* and *Azadirachta indica*) were selected for investigation against African human trypanosomiasis. The methanol, dichloromethane and aqueous extracts of these plants were administered intraperitoneally to Swiss white mice that had previously been inoculated with *Trypanosoma brucei rhodesiense* KETRI 3798. The parasitaemia, packed cell volume and body weight in each mouse was monitored for 60 days. This was done in parallel with control mice, which had been given water and ethanol (Negative control) and standard drugs; Melarsoprol and Suramin (positive control) respectively. Among the extracts tested, the dichloromethane extract prepared from the fruits of *Kigelia Africana*, tested at a dose of 2000 mg/kg was effective, curing 60% of the animals treated. The other extracts did not show significant anti trypanosomal activity. The treated positive controls (Melarsoprol and Suramin at dose of 3.6 and 5 mg/kg respectively), showed 100% survival and cleared parasites. These results show that *K. africana* has great potential as anti trypanosomiasis agent, which could be developed into an alternative drug to complement treatment of trypanosomiasis.

**Key words:** Antitrypanosomal activity, *Kigelia africana*, Packed Cell Volume, Human African trypanosomiasis.

### INTRODUCTION

African trypanosomes are protozoan parasites responsible for Human African trypanosomiasis (HAT) and nagana in livestock and are transmitted by the bite of an infected tsetse fly. *Trypanosoma b. brucei*, the causative agent of nagana, is closely related to *Trypanosoma b. rhodesiense* causative agent of HAT which occurs in East and Southern Africa and *Trypanosoma b. gambiense* found in West and Central Africa.

Sleeping sickness currently affects about a half million people in Sub-Saharan Africa and an estimated 60 million people are at risk of contracting this disease which is fatal if untreated (WHO 1988; Barrett, 1999).

The treatment of this condition has been the major challenge. The registered trypanocides are frequently toxic require lengthy parenteral administration, lack efficacy and are unaffordable for most of the patients (Legros et al., 2002). Therefore, there is need for new, safe, effective and cheaper alternative drugs to complement the existing drugs.

In countries where sleeping sickness occur, plants have been used traditionally for centuries and are still widely used to treat this illness and other parasitic diseases. It is estimated that two-third of the world population rely on traditional medical remedies due to the limited availability and affordability of pharmaceutical products (Tagboto and Townson, 2001). Furthermore, several well-known drugs, such as quinine and artemisinin used as antiprotozoan agents; have their origin in plants (Kirby, 1996; Camacho et al., 2000; Tagboto and

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Townson, 2001). Therefore, in this study, the *in vivo* antitrypanosomal activities of crude extracts from the four plants were investigated. The plants are commonly used in Africa as traditional medicine for wide spectrum of other medical indications including schistosomiasis and protozoan diseases such as malaria (Neuwinger, 2000)

## MATERIALS AND METHODS

### Laboratory animals

Swiss white male Mice (20 – 30 g) aged between 6 - 8 weeks were used. They were acquired from Kenya Agricultural Research Institute- Trypanosomiasis Research Centre (KARI-TRC) colony. The animals were kept in an animal room where the temperature was maintained at  $22 \pm 3^{\circ}\text{C}$  under a 12 h light –dark cycle. They were provided with mice cubes and water throughout the experimental period including the 2 weeks of acclimatization.

### Trypanosomes

*Trypanosoma brucei rhodesiense* isolate was obtained from KARI-TRC trypanosome bank. The isolate KETRI 3798, which was isolated from infected patient in 1977, was used in the study. Two mice were inoculated each by 0.2 ml of the stabilate intraperitoneally. The mice were bled on the tail vein three times a week and examined by the wet film method for the presence of trypanosomes by light microscopy

### Plant materials

*Azadirachta indica*, *Bidens pilosa*, *Artemesia annua* and *Kigelia africana* were collected from various locations in Kenya (Kasarani, Kajiado, Voi and Alkurto) and identified at the Department of Botany, Jomo Kenyatta University of Agriculture and Technology. They were selected on the basis of their frequent use in the treatment of parasitic diseases by traditional practitioners.

### Preparation of plant materials for extraction

The materials were carefully washed under running tap water (to remove dust and any other foreign materials) and left to drain off. The plant materials (barks and fruits) were chopped into small bits by a sharp machete and leaves were reduced by a knife. They were then spread on laboratory benches and left to air dry for three weeks.

The leaves were ground into powder using a blender while the barks and fruits were ground into powder form using a mortar and thistle. The weights of each plant material in powder form was determined and recorded.

### Ethical approval

Approval for use of the animal was obtained from Institutional Animal Care and Use Committee (IACUC), Trypanosomiasis Research Centre (TRC-KARI).

### Preparation of aqueous extract

The following powdered plants parts were selected for use; *Azadirachta indica* (stem barks), *Kigelia africana* (fruits), *Artemesia*

*annua* (leaves) and *Bidens pilosa* (leaves). One hundred grams (100 g) of each of the above was weighed out and soaked separately in 1000 ml of distilled water in a conical flask. The content, were warmed in a water bath for 2 h at  $60^{\circ}\text{C}$ , then left to stand at room temperature for 10 h, undisturbed. They were subsequently filtered off with sterile filter paper (Whatman No. 1) into a clean conical flask and the filtrate was freeze dried to powder, weighed and recorded.

### Preparation of dichloromethane extract

One hundred grams (100 g) of each powdered plant were weighed out and soaked separately in 1000 ml of dichloromethane into a conical flask with a rubber cork. The contents were kept for 5 days away from direct sunlight, undisturbed, then filtered through sterile filter paper into a clean conical flask. The filtrate was transferred into sample holder of rotary vacuum evaporator where the dichloromethane solvent was evaporated at its boiling temp of  $38.5 - 42^{\circ}\text{C}$ . The standard extract obtained was then weighed, recorded and stored in refrigerator at  $4^{\circ}\text{C}$  until required for use.

### Preparation of methanol extract

The residues obtained after filtering dichloromethane filtrate (as described in previous section) were re-soaked separately in 1000 ml of methanol in a conical flask with a rubber cork for 36 h away from direct sunlight, undisturbed, then filtered through sterile filter paper into a clean conical flask. The filtrate was transferred into sample holder of rotary vacuum evaporator where the methanol solvent was evaporated at its boiling temp of  $65^{\circ}\text{C}$ . The standard extracts obtained were then stored in refrigerator at  $4^{\circ}\text{C}$  until required for use.

### Controls

The negative controls used were water and ethanol 10%(v/v) (Chem-Rectic, Nairobi, Kenya) or water while the positive ones were Melarsoprol and Suramin (Kobian, Kenya limited).

### Determination of PCV

This is the fraction of whole blood volume that consists of red blood cells (RBCs). The blood was obtained by bleeding tail vein of mice. The blood was collected in heparinised capillary tubes which were sealed immediately. The capillary tubes with the blood were then centrifuged in a micro-centrifuge for 5 min at 10000 rpm. After centrifugation, the height of the red blood cell column was measured by use of haematocrit reader and compared to the total height of the column of the whole blood. The percentage of the total blood volume occupied by RBC mass is the Haematocrit which depends mostly on the number of the RBCs.

### Determination of *in vivo* efficacy of the plant extracts

The mice were divided into 17 groups, each comprising of 5 mice. They were infected with the trypanosomes as described earlier. The treatment of each group was begun 24 h post-infection. The plant extracts were administered intraperitoneally, two times in a day (9 a.m and 4.30 p.m) for 3 days at the doses mentioned below.

The extracts (freshly prepared) were administered by intraperitoneal means at the doses of (1000, 2000 and 5000 mg/kg) at v/w ratio 1/100 of mouse weight. Preliminary experiments had indicated this as the best range of concentrations. Mice were checked daily

**Table 1.** Activity of four plant extracts at varying doses against *T. rhodesiense* KETRI 3798 in Swiss white mice.

| Treatment                  | Survival time (days) |       | No of mice treated (n) | No of mice cured | Cured rate (%) |
|----------------------------|----------------------|-------|------------------------|------------------|----------------|
|                            | Mean                 | SD    |                        |                  |                |
| Drug /dose                 |                      |       |                        |                  |                |
| <i>K. africana</i> extract |                      |       |                        |                  |                |
| DCM                        |                      |       |                        |                  |                |
| 1000 mg/kg                 | 41.8                 | ±16.7 | 5                      | 2                | 40             |
| 2000 mg/kg                 | 50.8                 | ±12.0 | 15                     | 9                | 60             |
| 5000 mg/kg                 | 18.4                 | ±4.8  |                        | 0                | 0              |
| Methanol extract)          |                      |       |                        |                  |                |
| 1000 mg/kg                 | 17.6                 | ±5.4  | 5                      | 0                | 0              |
| 2000 mg/kg                 | 17.6                 | ±5.2  | 5                      | 0                | 0              |
| 5000 mg/kg                 | 23                   | ±3.7  | 5                      | 0                | 0              |
| <i>A. indica</i> extract   |                      |       |                        |                  |                |
| DCM                        |                      |       |                        |                  |                |
| 1000 mg/kg                 | 18                   | ±2.0  | 5                      | 0                | 0              |
| 2000 mg/kg                 | 23.4                 | ±3.4  | 5                      | 0                | 0              |
| 5000 mg/kg                 | 27                   | ±8.9  | 5                      | 0                | 0              |
| <i>A. annual</i>           |                      |       |                        |                  |                |
| Methanol                   |                      |       |                        |                  |                |
| 1000 mg/kg                 | 18                   | ±4.9  | 5                      | 0                | 0              |
| 2000 mg/kg                 | 19.2                 | ±3.0  | 5                      | 0                | 0              |
| 5000 mg/kg                 | 15.6                 | ±3.3  | 5                      | 0                | 0              |
| Positive controls          |                      |       |                        |                  |                |
| Melarsoprol(3.6 mg/kg)     | Cured                | N/A   | 5                      | 5                | 100            |
| Suramin(5 mg/kg)           | Cured                | N/A   | 5                      | 5                | 100            |
| Negative control           |                      |       |                        |                  |                |
| Ethanol/Water              | 11.6                 | ±2.5  | 5                      | 0                | 0              |

during 5 days after the first treatment to estimate the number of trypanosomes in their tail blood in a wet blood film. The absolute number of parasites per milliliter of blood was taken as a log using the rapid matching method for estimating the host's parasitaemia. At higher levels of parasites, this was achieved by matching microscopic fields of wet blood film against charts and, when fewer parasites were present, by counting the number of trypanosomes in 5, 10 or 20 such microscopic fields. For the assessment of antitrypanosomal effect of the extracts, the level of parasitaemia (expressed as log of absolute number of parasites per millimeter of blood) in the animal was compared to that of the control animals. Animals were checked three times in a week for parasites in tail vein blood for 60 days. Animals that survived to the end of the experiment, with no parasite in their blood sample were considered as cured. During this period PCV and weights of mice were also determined and recorded.

#### Statistical analysis

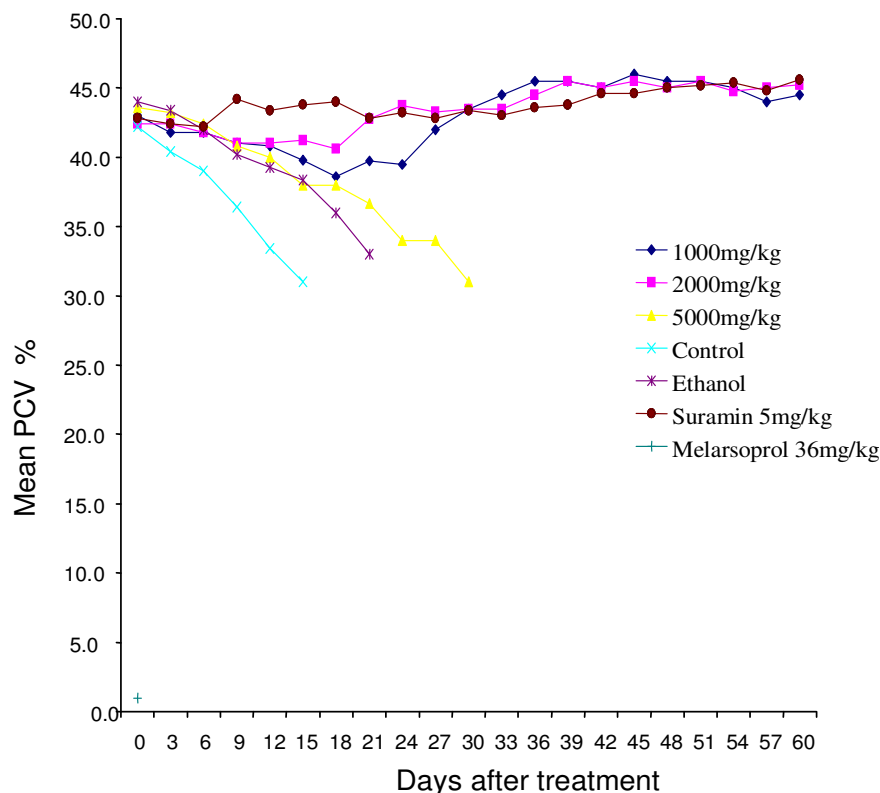
To assess the therapeutic effects, the parasitological data of treated and control animals were statistically analyzed using Student's t-test (Minlab computer program). P-Values < 0.05 were considered

as significant, those > 0.05 as not significant.

## RESULTS

### *In vivo* evaluation of trypanocidal activity

The results of *in vivo* mice studies showed that only one extract of *Kigelia africana* was able to stop trypanosomes from establishing and multiplying in the animals. The best results were obtained with DCM extract at an optimum dose of 2000mg/kg, which cured 60% of the animals treated (Table 1). This activity compared very well with that of standard drug, where the treated positive controls (Melarsoprol and Suramin at dose of 3.6 and 5 mg/kg respectively) relative to the negative controls, showed 100% survival and cleared parasites ( $p < 0.005$ ). The extracts from the other three plants did not show significant trypanocidal activity relative to ethanol and water ( $p > 0.05$ ). In fact the mice that were treated with the extracts



**Figure 1.** The trend of mean PCV of mice treated with *Kigelia africana* extract.

lived for less than 23 days with 0% survival and high parasitaemia levels (results for *A. annual* and *A. Indica* are shown as representative data). Therefore the reported result focuses mostly on the *K. africana* DCM extract that was tested at different doses.

It is noteworthy that in preliminary stage of this study, toxicity test of these extracts were carried out using mice. Pathological examinations of the tissues particularly liver, kidney and heart on gross and microscopic basis indicated that there were no detectable abnormalities of the tissues in the animals treated.

The results on activity of dichloromethane and methanol extracts of *K. africana*, *A. indica*, and *A. annual* tested at three doses (1000, 2000 and 5000 mg/kg), Negative and positive controls on mice infected with *T.b. rhodesiense* KETRI 3798. The DCM extract of *K. africana* at 2000 mg/kg cured 60% of the treated animals while extracts *A. indica* and *A. annual* did not show anti-trypanosomal activity. The treated positive controls (Melarsoprol and Suramin at dose of 3.6 and 5 mg/kg respectively) relative to the negative controls, showed 100% survival. The negative controls (ethanol and water) did not show trypanocidal activity.

The results on parasitaemia showed that mice treated with 2000 mg/kg of DCM extract on average had low parasitemia level since the majority of mice did not have trypanosomes after post treatment and they survived up

to and beyond 60 days. The mice in the same group that showed high levels of parasitaemia survived for at least 39 days, showing that they resisted the parasites for appreciable period of time. On the other hand, the mice treated with 1000 mg/kg had higher parasitaemia levels than that of 2000 mg/kg and only 40% of infected mice survived. It was further observed that the mice treated with 5000 mg/kg had the highest parasites and none of the animals was cured. They survived for only 24 days. Negative controls (water and ethanol) survived for 30 and 27 days respectively and parasitaemia levels were high. Melarsoprol and Suramin stopped the parasites from establishing and the animals survived up to and beyond 60 days.

The experiment on packed cell volume (PCV) analysis gave results that were consistent with the observations made on parasitaemia. As shown in Figure 1, the PCV of mice treated with 2000 mg/kg was on average above 43% which was fairly within the reference values of 42 - 52 for males. The PCV of animal treated with 1000 mg/kg was 42% and there is no significant difference between 1000 and 2000 mg/kg ( $p = 0.004$ ). The mice treated with 5000 mg/kg relative to negative controls, their PCVs were below the reference values (42-52%). The animals treated with Melarsoprol and Suramin their PCVs were within the accepted limits (43 - 44%) (Figure 1).

The PCV of mice treated with dichloromethane extract

of *Kigelia africana* at three doses (1000, 2000 and 5000 mg/kg), negative control (ethanol) and positive controls (suramin 5 mg/kg and melarsoprol 3.6 mg/kg) were monitored post treatment for 60 days.

Change in body weight of the treated animals was monitored during the period of the experiment. It was observed that animals treated with 2000 mg/kg of the plant extract on average maintained their body weight post treatment while the animals treated with 1000 mg/kg showed reduced body weights. There was significant difference between 2000 and 1000 mg/kg ( $p \leq 0.172$ ). For the negative controls (ethanol and water, the animals lost a lot of body weights and survived for only 27 days. In contrast, the animals treated with Melarsoprol and Suramin slightly increased their body weight and maintained their weight after 27 days.

## DISCUSSION

Literature surveys and field studies have shown that plants are used in traditional medicine in Africa to treat trypanosomes in humans and animals (Freiburghaus et al., 1996; Youan et al., 1997). This was one of the basis for the scientific evaluation of anti trypanosomal activity of the extracts of the four selected plants in the current study.

The results of this study showed that only one of the four extracts tested was able to stop trypanosomes from establishing and multiplying in the animals. The dichloromethane extract of *K. africana* fruits tested at three doses was effective at 2000 mg/kg, curing 60% of the animals treated. This activity is quite significant compared with those of standard drugs suramin and melarsoprol which showed 100% cure rate. However, the administration of the extract at a dose of 5000 mg/kg formed bolus at the point of injection. This observation may suggest that the drug failed to reach the target sites or organ possibly due to low absorption and explains the observed low PCVs that were below the reference values (42 - 52%). The results of our study have shown that *K. africana* has great potential as anti trypanosomiasis agent. This is the first report on the effect of *K. african* on Human African Trypanosomiasis (HAT).

The findings in this study supports other studies that have reported biological activity of *K. africana* extracts in consistent with its traditional use. For instance, the ethanolic stem extract have been shown to have antibacterial activity (Grace et al., 2002). Root extract of the closely related species *K. pinnata* have also been reported to have antibacterial activity (Akunyili et al., 1991 and 1993). In addition, other reports have demonstrated that the ethanolic extracts of the *K. africana* have analgesic and anti-inflammatory activities (Owolabi and Omogbai, 2007), as well as having a potential central nervous system stimulant effect that can be explored for therapeutic purposes (Owolabi et al., 2008). These reports and others have equivocally demonstrated the huge

medicinal potential that this plant species possesses.

The observed activity of *K. africana* extract in our study is speculated to be due to the increase of oxygen consumption and stimulation of hydrogen peroxide production in the protozoan cell. Protozoans do not have the same biochemical mechanism as mammalian cells for dealing with excess peroxide and consequent oxygen free radicals. In fact, this process is used as a target in the search for novel antiprotozoal compounds. It is important to note that the fact that the dichloromethane extract showed no toxicity when it was administered to the mice, indicates it is safe for use in humans.

## Conclusion

The results of our study showed that it is only crude extract of *K. africana* out of the four plants screened showed anti-trypanosomal activity in mice against *T.b. rhodesiense* KETRI 3798. And that the dichloromethane extract of *K. africana* had antitrypanosomal activity that compared very well with that of standard drugs.

Further work is still in progress to purify and isolate the bio-active compound that could lead to the development of safer and cost effective alternative drug for human trypanosomiasis.

## ACKNOWLEDGEMENTS

We wish to thank Jomo Kenyatta University of Agriculture and Technology (JKUAT) for funding the project and also we express our appreciations to Trypanosomiasis Research Centre (TRC-KARI) for providing their laboratory facilities and technical assistance.

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