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Full Length Research Paper

Possible anti-trypanosomal effect of aqueous leaf extracts of *Mangifera indica* on plasma proteins of *Trypanosoma congolense*-infected rats

Ngozi O. Awa-Imaga

Department of Biochemistry, Faculty of Basic Medical Science, College of Medicine, University of Lagos, P. M. B. 12003, Idi-Araba, Lagos State. E-mail: ngoziawaimaga@yahoo.com.

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This study investigates the effects of leaves of *Mangifera indica* L. (Anacardiaceae), commonly known as mango, in attenuating immunopathogenic infections caused by *Trypanosoma* spp. The crude extract is used by local folk in Africa as an ethnomedical health management remedy for parasitic diseases, including its use in malaria and trypanosomiasis. In this study, 500 mg/kg concentration of mango leaf extract was tested on rats infected with *T. congolense* strain of the disease trypanosomiasis to deduce the *in vivo* efficacy and toxicity levels of the aqueous herbal extract compared with uninfected control rats. This was done by measuring its effects on concentration of whole plasma proteins and fractionated plasma proteins, determined using SDS-polyacrylamide gel electrophoresis and total protein biuret assay. Results indicated that the extract showed relatively no toxic effect on the plasma proteins of rats at the 500 mg/kg concentration. Low albumin levels were observed with increased globulin level in infected-treated rats compared to infected-untreated and uninfected controls, which suggests that plasma protein synthesis may be stimulated by the mango leaf aqueous extract. Electrophoretic plasma protein patterns were similar across all groups but more distinctive in the treated groups. These results suggest that the extracts may have a palliative effect on the *T. congolense* - infected rats if extract is administered at the onset of infection.

Key words: Mango*, Mangifera indica,* trypanosomiasis, immunoglobulins.

INTRODUCTION

Trypanosomes, of the genus *Trypanosoma*, are parasitic protozoa affecting man and animals resulting in the disease, Trypanosomiasis, also called sleeping sickness, borne by the purely African genus *Glossina* (tsetse flies). They have been found in the blood plasma of a great variety of vertebrates. Many of them appear to produce no symptoms but a few are of great pathogenic importance and continue to pose grave economic and social problems in Africa (Nigeria inclusive) and other affected areas of the world. *Trypanosoma congolense* of the subgenus nannomonas is responsible for the form of African trypanosomiasis (nagana) in domestic animals such as bovines, equines, sheep, goats, camels and pigs, as well as dogs. In the blood of rats and mice the trypanosomes multiply rapidly, producing high parasitemia which may kill the hosts in a few days. The manifestations of the disease vary according to the strain and host of the parasite, being generally characterized by fever, anaemia and cachexia. Thus, it has a pathogenic effect on the host.

In the recent past, drastic measures were taken to cut down the epidemic in the affected areas of Africa with more tsetse fly nets put up in several forests and habitats. Fumigation of swampy areas and modern drugs has also been used to treat cases of human and animal trypanosomiasis. These drugs however have their toxic effects and consequently harm the body and some do not prevent relapses. Some drugs are effective only in the latter stages of infection when the nervous system is involved, while others are effective only in the initial stages of infection. Moreover these drugs are very expensive and are only now within reach due to aids from the World Health Organization (WHO). But with phytotherapy research work springing up all over Africa highlighting our richly endowed forests, the need to screen local medicinal plants for anti-trypanosomal cannot be over-emphasized. Some of these plants are already being used as cures for fevers, infertility,

diseases and so on, so if a herbal cure for sleeping sickness is found, suffering victims can have the drugs faster and cheaper without side effect-threats as is the case with synthetic drugs like Suramin and Eflornithine treatment (TDR Progress, 1991–1992).

Many studies on pharmacological effects of fermented wheat and garlic bulb crude extracts have indicated their usefulness in treatment of African sleeping sickness (Yusuf and Ekanem, 2010). The aqueous stem and leaf extract of Mangifera is a known medicinal formulation widely reported for its anti-inflammatory, analgesic, gastroprotective and immunomodulatory effects in *vivo*. (Leiro et al., 2004). The crude extract is used by local folk in Africa as an ethnomedical health management remedy for parasitic diseases, including its use in malaria and trypanosomiasis (ethnomedical reports). This research involved the screening of aqueous leaf extracts of *Mangifera indica* (mango) for its medicinal effect on trypanosomiasis by measuring the level of plasma proteins, which is a measure of the functional state of the liver and an indicator of pathogenicity. The effect of this treatment on plasma proteins of infected rats was analyzed along with preliminary tolerance and toxicity levels of the leaf aqueous extract. Findings from this research may help kick-start further research on the efficacy and therapeutic suitability of mango leaf extract as an alternative treatment for trypanosomaisis and also adduce a suitable extract concentration for its use as a herbal formulation.

MATERIALS AND METHODS

Chemicals and biochemicals

All chemicals were purchased from the Sigma chemical company USA and used without further purification.

Collection of mango leaves

Fresh mango leaves were obtained and authenticated from the botanical garden, University of Lagos, Nigeria. The leaves were washed and dried in an oven for 48 h at 37°C. The dried leaves were then ground to powder and stored in brown stoppered bottles. From which samples were taken for the aqueous extraction process.

Aqueous extraction of mango leaves

1 g of the ground mango leaves was boiled in 10 ml of distilled water for 1 h. The solution was then sieved, using a muslin cloth and stored in a refrigerator at 25°C till further use.

Laboratory animals

Albino rats weighing 180 g were obtained from the animal house, College of Medicine of the University of Lagos. They were separated into six Groups: A, B, C, D, E and F and placed on a normal pellet diet.

Parasite inoculum

T. congolense strain was obtained from University College Hospital (U.C.H.), Ibadan and injected intraperitoneally on day 0 into all the test rats with an inoculum size of 1 ml (10 to 15 drops of blood in 70 ml of 0.85% saline). Evaluation of parasitemia was carried out as described by Yusuf and Ekanem, (2010). As soon as parasites were sighted in the blood three days post infection, treatment with extract commenced and this was counted as our experimental Day 1.

Administration of extract

1 ml solution containing 500 mg/kg extract (concentration of extract per body weight), was administered daily using an oral dosing needle to the infected rats and uninfected rats groups as follows; Group A rats: infected treated as from day 1 at the onset of infection for 21 days; Group B: infected treated as from Day 7 post infection for 14 days; Group C: infected treated as from Day 14 post infection for 7 days; Group D: Infected rats left untreated, Group E: uninfected treated with extract and group F uninfected rats, distilled water. Groups D and E were used as positive and negative controls respectively against the treated groups.

Isolation and extraction of plasma from rats

After 21 days of treatment, all surviving rats were euthanized by cervical dislocation under anesthesia and 5 ml venous blood samples collected from each rat into heparinized tubes. The plasma sample obtained was stored in the refrigerator at 4°C according to the methods of Davidson and Henry (1969) and aliquots later used for the analysis.

Total plasma protein determination

The biuret assay was carried out according to the methods of Tiez (1976) and Trease and Evans (1983) as follows. 3 ml of biuret reagent was added to 0.5 ml of plasma. This was mixed and placed in the water bath to warm at 37°C for 10 mm; it was cooled and the extinction read at 540 nm in a spectrophotometer. 3 ml of biuret reagent was also added to a standard protein solution (egg albumin) as control. The formula for concentration of sample was used to calculate the concentration of the plasma proteins.

Calculations

The various concentrations of plasma proteins for the biuret assay were calculated using the following formula:

$$\text{Concentration of sample} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

SDS polyacrylamide-Gel electrophoresis

This was done according to the methods of Kaplan and Savory (1965).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). The students' T-test was used for comparison of the experimental groups. The level of significance was set at $p < 0.05$.

Table 1. Concentration of plasma proteins.

Plasma samples	Concentration (mg/100 ml)
A	290
B	255
C	305
D	220
E	240
F	220

A — Infected rats treated from Day 1 (at the onset of infection) for 21 days; B — Infected, treated after 7 days of infection for 14 days
C — Infected, treated after 14 days of infection for 7 days; D — Infected, untreated; E — Uninfected, treated from Day 1; F — Uninfected, distilled water control - (not treated).

RESULTS

Concentration of plasma proteins

Results from the biuret assay of whole (total) plasma proteins (Table 1) show treated Groups A and E with the highest increase of concentration of proteins and Group D (infected untreated) with the lowest concentration.

The electrophoretic patterns in Figure 1 show that the plasma proteins are all present but are more distinctly seen in the uninfected groups than in the infected groups.

Anti-trypanosomal activity of *Magifera indica* aqueous leaf extract

Results showed that none of group A rats, where treatment started at the onset of infection died, whereas, in the other infected treated groups B, C and infected untreated group D, deaths were recorded.

DISCUSSION

There have been several reports on the pharmacological effects and suitability of medicinal plants as phytotherapies for diseases. Mango stem-bark aqueous extract has been reported to possess anti-inflammatory, analgesic and immunoprotective effects (Garrido et al., 2005; Ojewole, 2005). This study investigates the effects of leaf extracts of *Mangifera indica* L. (Anacardiaceae), commonly known as mango, in attenuating immunopathogenic infections caused by *Trypanosoma* spp.

The findings on low recorded deaths for the infected treated groups suggest that early treatment with mango aqueous leaf extract may delay the infectivity rate of the parasite by perhaps boosting the host immune system and thereby possibly extending the life-span of the host organism.

The ability of the host to produce antibodies (immunoglobulins) may be reflected in the increased concentration of plasma protein as shown in Table 1.

From the standpoint of body economy, the three major categories of protein are tissue (organs) proteins, plasma proteins and haemoglobin. In terms of dynamic protein metabolism, a priority system appears to prevail in protein synthesis and breakdown. The plasma proteins do not reflect a significant decrease until tissue protein has been depleted with clinical evidence of wasting in starvation or protein deprivation. The plasma proteins occupy a central position in protein metabolism; not only do they interact with virtually all the body tissues, but they are intimately related to protein metabolism in the liver. Indeed a myriad of plasma protein components contribute to nitrogen needs to defend against invasion and injury, to maintenance of body pH and osmotic balance and for regulation of cellular activity and function.

The rise in the level of whole plasma protein concentration, recorded for leaf extract control (Group E), suggests that the extracts may increase synthesis of proteins for example antibodies, needed to fight infection. The highest increase in the plasma protein level noticed in Group A, suggests that protein synthesis is greatly increased if the extracts are administered at the onset of infection. Thus in the presence of the parasites, the extracts stimulate the host to synthesize more immunoglobulin needed to fight off the infection thus confirming recent studies that removal of the parasites from the system and simultaneously boosting the immune system could be very relevant in the control of African trypanosomaisis (Yusuf and Ekanem, 2010). Results also suggest the extracts might have some palliative effect on the well advanced stages of infection, although not as effective as when started at the onset of infection (as in Group A).

The determination of total proteins is most useful in reference to fractionation of serum proteins by electrophoresis. Since the serum total proteins represents the sum total of many different proteins resolved by electrophoretic separation, elevations or depressions of individual fraction. With advanced under nutrition or prolonged protein deprivation, the serum total protein concentration may be decreased. Electrophoresis yields adequate separation and estimation of various serum proteins most frequently in patterns of configuration consistent with several diseases. These patterns usually involve alterations in the concentration of one or more of the fractions, that is, albumin, alpha and beta globulins, and gamma globulin. It is the pattern of serum protein changes rather than alterations in concentrations of the individual serum protein fractions that has provided the most medical diagnostic usefulness. The electrophoretic patterns in Figure 1, also suggest that the extracts were not harmful to the body, when taken at 500 mg/kg body weight dosage as seen in the normal plasma protein levels of the control.

Conclusion

The extracts can be said to have a palliative effect on the

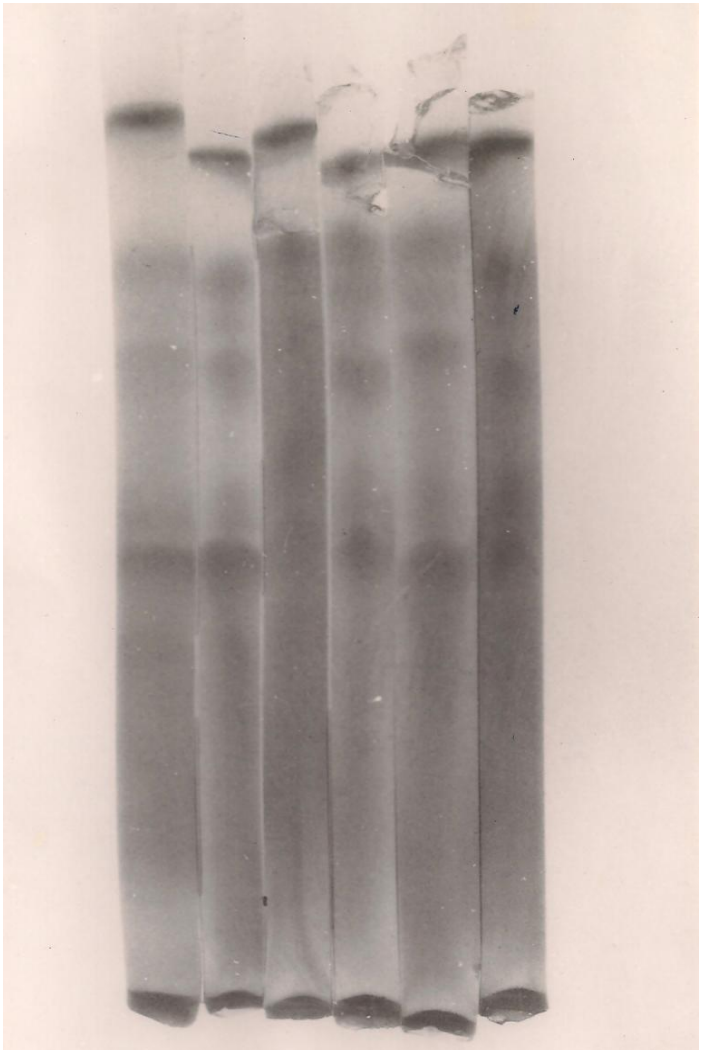

Figure 1. Electrophoretic pattern of plasma proteins. F, E, D, C, B, A. A — Infected rats treated from Day 1 (at the onset of infection) for 21 days; B — Infected, treated after 7 days of infection for 14 days; C — Infected, treated after 14 days of infection for 7 days; D — Infected, untreated; E — Uninfected, treated from Day 1; F — Uninfected, distilled water control - (not treated).

T. *congolense* infected rats and stimulate proliferation of plasma proteins when administered at the early stage of infection.

Further work on more detailed dose-response efficacy and safety experiments using the mango leaf extract is currently on in our laboratories.

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