CHANGES IN PARASITEMIA AND GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE ACTIVITY IN T. CONGOLENSE INFECTED RATS FOLLOWING ORAL ADMINISTRATION OF AQUEOUS EXTRACTS OF GARLIC, AIDON AND MANGO LEAF.

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ABSTRACT

This study investigated whether water extracts of Allium satirum (garlic), Tetrapleura tetraptera (aidon) and Mangifera indica (mango) have trypanocidal effects against T. Congolese. Studies also investigated their effect on glucose - 6 - phosphate dehydrogenase (G-6-PD). Result showed that oral administration of the three extracts significantly reduced the parasite load in the infected and treated ones. These extracts also decreased the activity of G-6-PD in the different groups relative to the controls, which were not [illegible] These observations affirm our earlier reports on the beneficial effects of these plant extracts in other trypanosomal species. These findings also underline the need for further studies to identify and develop the active components in these plants as potential trypanocides.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G-6-PD) is a key regulatory enzyme of the pentose phosphate pathway. One of its major metabolic functions is the generation of NADPH used in some vital biosynthetic processes. For example, NADPH is a cofactor used by glutathione reductase to maintain glutathione in the reduced state. High levels of NADPH are required by red cells to maintain sufficient glutathione in the reduced state for the preservation of the cell membrane integrity (1). Besides, glutathione is involved in the regulation of diverse cellular processes such as enzyme activation and synthesis, detoxification of Xenobiotic substances and reactive oxygen metabolism (2). Considering the essential role of G-6-PD in these metabolic functions, we are curious to know if it can be used as a marker in following the progress or otherwise of trypanosomiasis in trypanosome infected rats after treatment with some medicinal plant extracts, more so that hemolysis is a characteristic feature of trypanosomiasis (3). The level of G-6-PD is low in plasma but it is used here on the premise that its concentration in plasma will increase directly with the rise in parasitemia and the subsequent hemolysis.

Trypanocidal activity of garlic is well documented (9-11) as part of its numerous therapeutic effects (2, 4 - 11, 13). We have recently confirmed this property in our laboratory (12). Furthermore, it has been reported that garlic derivatives markedly influence the steady state concentration of reduced glutathione as well as the activities of enzymes that control its metabolism (1,14-17). Tetrapleura tetraptera (aidon) has been associated mainly with molluscidal activities (8-20). Recently, also studies in our laboratory revealed that aidon has trypanocidal properties against T. brucei infection in rats (21). In the case of Mangifera indica (mango), the leaves along with other plants are used traditionally against infectious diseases and other ailments (22-23). A preliminary data from an on-going study in our laboratory suggests that water extract of M. indica possibly has a palliative effect on T. vivax infected mice. This account is based on the improvement in the haemoglobin level reduced total protein and - globulin fractions of infected treated animals compared with the infected untreated controls.

In the present study, we have extended our investigations on these medicinal plants to T. congolense infection in experimental rats. The work is designed to find out if the water extracts of the plants (garlic, aidon and mango) would influence:

1. The parasite load in the blood of the infected animals.
2. The plasma G-6-PD activity of infected and treated rats compared to those which were infected but not treated as well as those that were uninfected but treated with the extracts.

MATERIALS AND METHODS

Experimental Animals - Male and Female albino rats (weighing between 126 - 140g) were collected from the animal house of College of Medicine of the University of Lagos. They were left to acclimatize in the animal room of the research laboratory of the Department of Biochemistry for a week before the commencement of the experiments.

The Parasites: The T. congolense used for this study was obtained from the Faculty of Veterinary Medicine, University of Ibadan and maintained in our laboratory by serial passage from one infected animal to another clean rat.

Preparation of the water extracts of garlic, aidon and mango - Allium sativum (garlic, bulb), Tetrapleura tetraptera (aidon, fruit) and Mangifera indica (mango, leaf) were purchased from a local market in Lagos, Nigeria. Each of the plant products was washed and left under the sun to dry. They were then chopped into tiny pieces, placed in the oven at 40°C and left to dry till crispy. They were ground into powder with pestle and mortar. The water extract of garlic was prepared by introducing the powdered material in hot distilled water and stirring continuously for one hour. After cooling, the extract was made to give a final concentration of 500mg/ml. This was filtered and stored at 4°C for subsequent use. Water extracts of aidon and mango were prepared by boiling 20% quantity of the powdered materials in distilled water under reflux for one hour and left to stand over night, thereafter filtered. The extracts were concentrated to dryness. The extracted products were then reconstituted to final concentrations of 500mg/ml. They were stored at 4°C for subsequent use.

EXPERIMENTAL DESIGN:

The experimental animals were randomly distributed into different groups. The study design is summarised in the tables below:

Table 1: Group Characteristics

A1 Consisted of animals infected and treated with aidon.

B1 The animals in this group were infected and treated with garlic extract.

C1 This group of infected animals were treated with a mixture containing equal volumes of the garlic and aidon extracts.

D1 The animals in this group were infected but not treated with any of the extracts.

A2 - C2 The animals in these groups were not infected with the parasites, rather, they were, respectively, treated with the extracts as the animals in groups A1 - C1.

D2 Consisted of "neat" rats which were neither infected nor treated with any of the extracts.

All the groups contained 4 animals each. The experimental rats in groups A1 - C1 were treated with 500mg/ml of the respective extracts daily throughout the duration of the study, with the treatment starting 24 hours post-innoculation.

Table II: Group Characteristics

E: This group was made up of animals treated with mango extract (500mg/ml), two days after innoculation with the parasites.

F: Infected animals were not treated with the mango extract (500mg/ml) till seven days after innoculation with the parasites.

G: Treatment of the infected animals started 14 days post-infection.

H: Consisted of animals which were infected but not treated with mango extract.

I: Contained animals which were not infected but were treated with mango extract.

J: This group was made up of "neat" rats which were neither infected nor treated with the extract.

There were also 4 animals in each of these groups. The donor blood contained 3.3. x 10^3 parasites/ml and each animal was innoculated

intraperitoneally with 0.1ml of blood. All the animals were fed with rat chow (Pfizer Nigeria Limited) and water ad libitum. Treated animals each received orally 1ml of the extracts as in the tables. The texture of the stool of the animals in the course of treatment with the extracts was observed.

Assessment of Parasitemia: Film Preparation: A drop of blood from each infected rat was placed on microscopic slide and dispersed with another slide. The formed film was fixed with the Leishman stain and rinsed with distilled water and then air dried. The film was examined under oil immersion and the number of parasites per 1000 red blood cells were determined.

Determination of Glucose-6 phosphate dehydrogenase Activity - The Glucose-6-phosphate dehyrogenase activity was assayed according to Tietz (28). Briefly, the reaction mixture consisted of 0.05M Tris buffer, pH 7.6, containing 0.005M EDTA and 0.012M NADP. The substrate was 0.042M anhydrous disodium salt of glucose-6-phosphate.

Statistical Analysis - The student t-test was used to evaluate the significance of differences between the experiments.

RESULTS

Fig. 1 is a summary of the percentage (%) parasitemia per 1000 red cells in each of the four groups (E to H) treated with the mango extracts. Parasitemia was evident in all the four groups five (5) days post inoculation. The level of parasitemia in the rats treated two days post infection (group E) was lower than that of those treated 7 days later (group F) but this difference was not statistically significant ($P>0.05$). However, there was statistically significant difference ($P<0.05$) in the respective levels of parasitemia in groups E and F when compared with those of group G (where treatment started 14 days later) and group H (the control animals which were infected but not treated). Even though no statistical difference ($P>0.05$) was obtained between the level of parasitemia in the groups G and H rats, it was observed that the level of parasitemia started dropping when treatment was commenced on the 14th day for group G.

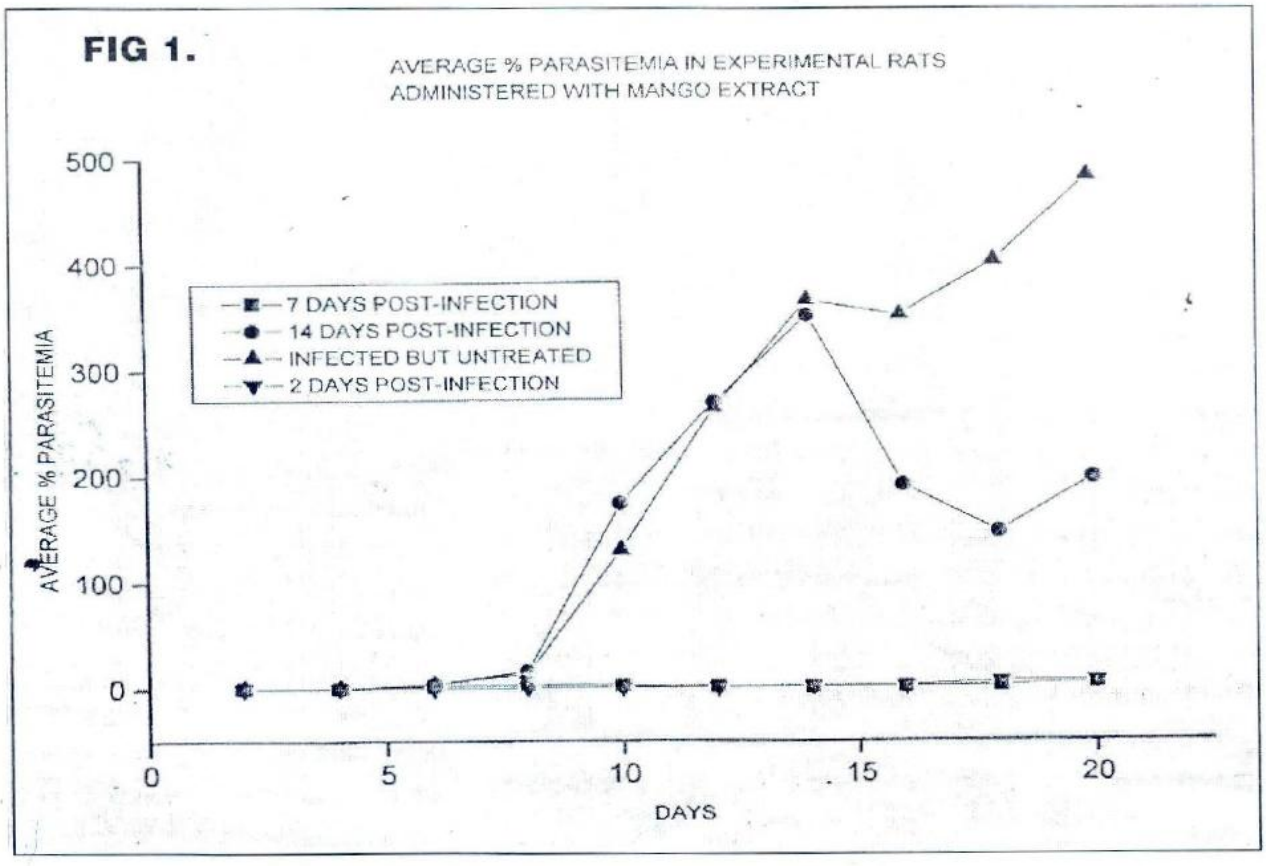

Table 1: % Parasitemia according to extract and duration of treatment

Days of Treatment after inoculation	Type of Extract: Aidon (A1)	Garlic (B1)	Mixture (C1)	Control
1	-	-		
2	-	-		
3	-	-		
4	-	-		
5	0.5	-	1.0	
7	0.8	-	2.2	
9	3.0	6.0	5.5	
12	3.6	10.0	7.0	
14	3.9	11.0	8.5	
16	4.5	15.0	9.2	

Table 2A: G-6-PD activity in uninfected groups of animal according to the given extracts.

Study Groups	G-6-PD activity	Statistical significance
D2 "Neat" rats	2.9 ± 0.51	-
B2 (garlic)	2.9 ± 0.35	$p>0.10$
A2 (garlic)	1.87 ± 0.3	$p>0.10$
C2 (garlic and aidon mixture)	4.4 ± 0.45	$p<0.01$

Table 2B: G-6-PD activity of aimal in the Groups infected and treated with the extracts.

Study Groups	G-6-PD activity	Statistical significance
A1 (aidon)	6.25 ± 0.35	$p<0.01$
B1 (garlic)	4.5 ± 0.5	$p>0.01$
C1 (aidon and garlic mixture)	14.8 ± 4.48	$p>0.001$
D1 (infected and untreated)	7.83 ± 1.04	

Table 3: G-6-PD activity following treatment with the mango extract.

Study Groups	G-6-PD activity	Statistical significant
E (treatment started 2 days post-infection)	2.2 ± 0.36	$p>0.05$
F (treatment started 7 days post-infection)	3.0 ± 0.5	$p<0.01$
G (treatment started 14 days post-infection)	4.1 ± 0.14	$p<0.001$
H (infected but not treated)	5.166 ± 0.14	$p<0.001$
I (treatment with only the mango extract)	0.725 ± 0.03	$p<0.001$
J ("neat") rats)	1.57 ± 0.12	

Table 1 shows the percentage parasitemia obtained for the rats in groups A1 to C1 which were infected and treated with aidon, garlic and garlic-aidon mixture, respectively. The parasites appeared in the blood stream of the animals in the respective groups on different days, being 3 days for the aidon-treated group and the control and as much as 7 days for the group treated with the mixture of garlic and aidon. No parasites were observed in the blood of animals

treated with the garlic extract. Even though the mixture delayed the time of appearance of parasitemia in the blood group C1 rats there was upsurge in the parasite-load once infectivity was established and the level of parasitemia in this group remained significantly higher than those in groups A1 and B1 ($P = 0.003$). Also the parasite load observed in the blood of rats in groups A1 and B1, respectively, were significantly lower than that of the control ($P = 0.004$). But there was considerable softening of stool in the groups treated with garlic alone and garlic and aidon mixture.

The data obtained when the G-6-PD activity was determined in groups A2 - D2 (i.e. animals which were not infected but treated with only the extracts, except D2) are presented in table 2a. The Table reveals that the G-6-PD activity of the animals in groups A2 and B2 was not significantly different from that of group D2 but there was significant different between the enzyme activity in group C2 compared with group D2. When the animals became infected (Table 2b), the value of G-6-PD was elevated in group D1 and this was more than twice the level of the enzyme activity of group D2 (table 2a). This elevation was significantly reduced on treatment with garlic (group B1) and to a lesser extent, with aidon (group A1). But a mixture of garlic and aidon caused significant elevation in enzyme activity (group C1).

Table 3 shows the G-6-PD values for the rats treated with the mango extract. The data again reveals that infection with trypanosomes caused significant elevation in the value of G-6-PD activity (group H) when compared with the treated groups (groups E-G), more especially, when treatment was started early (groups E and F). It was also observed that when the rats were treated with the extracts alone (group I), the enzyme activity was reduced significantly ($p<0.001$) compared with the enzyme activity of the "neat" rats (group J).

DISCUSSION

Our recent studies have shown that water-extract of garlic and aidon have trypanocidal effect against T. brucei in laboratory rats (12,21). The objective of the present work seeks to examine if the findings can be extended to another trypanosome species i.e. T. congolense. We also examined possible trypanocidal effect of mango leaf and the relationship with G-6-PD activity. Our results show that the three extracts have significant influence on the onset of T. congolense parasitemia. Oral administration of the three water extracts decreased the parasite load in the blood stream of the experimental rats. These observations are consistent with our earlier findings and the reports of other workers (9-11). It can be deduced that trypanocidal action of garlic, mango and aidon are non-species specific. The next question concerning the biochemical mechanism(s) that is likely to be involved in the trypanocidal observations.

It has been reported that ajoene [(E, Z) - 4, 5, 9-trithiadodeca-1, 6, 11 - triene-9-oxide] derived from garlic has antitrypanosomal activity, an effect exerted by interfering with the synthesis of membrane lipids by the parasites (9). The considerable softening of stool in the groups treated with garlic and the mixture of garlic and aidon cannot be adequately explained by the available data. However, a previous study had reported that doses of garlic between 300 - 600mg/kg/24, given for 21 days, manifested toxic effects in male and female rats (24). In this study, we used 500mg/ml/24hr. for 16 days, which is rather in the high range.

The molluscicidal properties of aidon (18, 19) have been associated with the presence of triterperiod saponins and some coumarin compounds. We are not sure if the same compounds are responsible for its antitrypanosomal effect. Meanwhile further studies are in progress in our laboratory to identify the trypanocidal principles in both plant extracts.

The antiproliferative effects of these extracts against T. congolense in the experimental animals are in agreement with the pattern of changes observed in the activity of G-6-PD recorded for the different groups. When we treated the animals with only the extracts without infection, no significant changers were observed in the

activity of the enzyme in the respective groups treated with garlic and aidon. Infection of the animals with trypanosomes without treatment caused significant increase in enzyme activity. But when infection was followed by treatment, G-6-PD activity was appreciably reduced by all the three extracts, viz-garlic, aidon and mango.

Following tissue damage by trypanosomes, increases in enzyme activities have been claimed previously (27). Therefore the observed increase in G-6-PD activity in this study is consistent with previously reported increases in enzymes such as lactate dehydrogenase, malate dehydrogenase, succinate dehydrogenase and leucine amino peptidase, which were associated with liver damage (27). Increase in the plasma glucose-6-phosphate dehydrogenase activity would not be unexpected under the stress of trypanosomiasis. As already stated earlier, it is well known that hemolytic anaemia is a characteristic feature of trypanosomiasis. Since G-6-PD is an important enzyme of the red cells, it is reasonable to suggest that the raised G-6-PD activity following parasitemia may be the outcome of G-6-PD released from the destroyed red cells into the plasma. Therefore, the reduced enzyme activity observed with treatment of the animals with the extracts, along with the reduced parasitemia in the blood stream of the treated animals may be indices of the positive effects of the extracts on the experimental rats. This interpretation is supported by the fact that the untreated animals did not give similar response, rather, they had both high enzyme activity and parasitemia throughout the study period. Further studies are, however, necessary to confirm this suggestion.

The results obtained with the mixture of garlic and aidon are worthy of note. They were able to prolong the prepatent period but were not able to suppress the proliferation of the parasites once infectivity was established. The mixture also increased the enzyme activity above that of the infected and untreated group. Even when uninfected animals were treated only with the mixture, the enzyme activity was increased almost twice the value of the "neat" rats. This is in contrast to the observed effects of garlic or aidon alone as well as the mango extract which even decreased the enzyme activity when the animals were given only the extract without infection with the parasites. Even though the reasons for these observations are not clear at the moment, the adverse implication can be noted, i.e., in the use of natural products in unstandardized mixtures which could turn out to be counter productive in health management. The present work, therefore, highlights the need for detailed studies on the mechanism of action of natural plant products which are used widely by a large percentage of our population who find them cheap and easily available.

LEGENDS

Fig. 1: Fig. 1 shows the pattern fo changes in % parasitemia in rats treated with the mango extract (500mg/ml/24 hr. for 16 days. Treatment with the extracts started on different days after innoculation of the rats with the parasite - viz: 2 days post-innoculation, 7 days and 14 days, respectively.

Table1: Per cent parasitemia recorded in the groups treated with aidon garlic, garlic and aidon mixture, for a period of sixteen days. The control is the group infected but not treated with any of the extracts.

Table 2a: G-6-PD activity in the uninfected groups of animals which were treated with the different extracts. The "neat" rats were neither infected nor treated.

Table 2b: G-6-PD activity of animals in the groups

infected and treated with aidon, garlic and aidon mixlture.

Table 3: G-6-PD activity following treatment with the mango extract. Treatment started on different days post-innoculation. Full experimental procedures for all the tables and the figure are explained in the text.

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