Some Hepatic Function indices in *Trypanosoma brucei brucei*-infected Rats treated with Aqueous Extract of *Mitracarpus scaber*

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Abstract: Disturbances in hepatic functions are hallmarks of African trypanosomiasis, and *Mitracarpus scaber* has been reported to normalize these alterations. In this present study, some hepatic function indices in *Trypanosoma brucei brucei*-infected rats treated with aqueous extract of *Mitracarpus scaber* were investigated for five days. Twenty whista albino rats were grouped into four (A, B, C, and D) of five each. Groups A, B and C were infected with *Trypanosoma brucei brucei,* while group D was not infected. Group A was treated with diminazene aceturate (3.5mg/Kg body weight) while group B was treated with aqueous extract of *Mitracarpus scaber* (450mg/Kg body weight). Group C was not treated (positive control) but was given phosphate buffer saline only as group D (negative control). The results obtained showed that *Trypanosoma brucei brucei* significantly increased ($p<0.05$) the activity of Alkaline phosphatase (ALP) and Alanine amino transferase (ALT). Treatment with aqueous extract of *Mitracarpus scaber* showed significant reduction ($p<0.05$) in the activity of the Alkaline phosphatase (ALP), and reduction in Alanine amino transferase (ALT) previously increased by the parasites. Both increase and reduction of conjugated and total bilirubin as a result of the infection and treatment respectively were not significant at $p<0.05$. These findings are discussed in relation to toxicological evaluation of medical plants and chemotherapy of African Trypanosomiasis.

Keywords: *Trypanosoma brucei brucei,* Infection, Hepatic function, *Mitracarpus scaber*.

INTRODUCTION

African trypanosomiasis is one among the few human infectious disease that is 100% fatal if left untreated (seed, 2000). According to the World Health Organization (WHO), there are 300,000 to 500,000 cases of Human African Trypanosomiasis (HAT) with 36 countries on the continent endemic for the disease, putting about 60 million people at risk. Trypanosomiasis causes an estimated 20% decrease in calving, 25% decrease in milk production and 3 million livestock death per year (seed, 2000).

Trypanocidal are very expensive and many of which are also associated with problems of resistance by some strains of trypanosomes, while some are toxic which could lead to death like in the case of Melarsoprol (Seed, 2000).Therefore, there is need for research to discover new trypanocidal from plant origin that is non-toxic, affordable and readily available especially in developing countries. Recent reports have shown that a lot of tropical plants contain clinically efficacious constituents against diseases caused by protozoa (Shittu, Umar and Usman, 2013). *Mitracarpus scaber* is one of these plants that have been shown to posses both *in vitro* and *in vivo* trypanocidal activity (Nok (2002). *Mitracarpus scaber* is from the family Rubiaceae, popularly known as Madder family belonging to the order Gentianales, recently called Rubiale order (Abere *et al.,* 2007). It is known as 'Irawoile' by Yorubas (Gbile, 1984), 'obuobwa' by Ibos and 'Gududal' by Sokoto Fulanis or Guga Masu by the Hausas in Nigeria (Jegede *et al.*, 2005). It grows as a weed on fallow farmlands and has been found among the tropical countries like Senegal, Gambia, Mali, Nigeria (Southern and Northern parts) and Liberia (Jegede *et al.*, 2005). *Mitracarpus scaber* is a perennial annual herb of about 30cm tall or much smaller and possesses rough leaves (Abere *et al.,* 2007).The leaf is used in traditional medicine practice in Africa for the treatment of headaches, toothaches, sore throat, skin diseases, wounds, antidote to arrow poison, antidiarrhea and antidysentery, antibacterial and antimycotic, amenorrhea, dyspepsia, hepatic diseases, venereal diseases as well as leprosy (Germano *et al.,* 1999; Jegede *et al.,* 2005; Abere *et al.*, 2007a,b)

There is the need therefore for the evaluation of scientifically relevant plant species commonly used in herbal treatment of diseases both for the sake of safety and as alternative to the already existing trypanocidals (Okunji *et al.,* 2000; Abere *et al.,* 2007; Markus *et al.,* 2014). Already, reports have shown that *Mitracarpus scaber* leaf extract has significant

antihepatotoxic potentials on carbon tetrachloride induced acute liver damage in rat (Germano *et al.,* 1999). However, there is need for an evaluation of the plants with parasitized animals.

MATERIALS AND METHODS

Plant Sample

Fresh *Mitracarpus scaber* (plant) were obtained from Zaria, Kaduna State Nigeria and the plant was indentified at the herbarium of the Department of Biological Science, Ahmadu Bello University Zaria. The voucher number of the plant is 00962.

Preparation of Crude Plant Extract

The method of extraction used was the cold extraction method described by Nok, (2002). Exactly 200g of fresh *Mitracarpus scaber* leaves was weighed using weighing balance and was then homogenized in 600ml chilled ethanol with continuous shaking for three days. The extract was then filtered using muslin cloth into a clean beaker. The filtrate was again filtered with double filter paper to obtain a clear solution. It was then condensed using the rotary evaporator and water bath. The concentrate was then partitioned between ethyl acetate and water. The ethyl acetate fraction was then collected and condensed further in the rotary evaporator and water bath. After evaporation to dryness, it was collected into a clean container and stored in refrigerator at 4^0C until it was required for use during treatment. Aqueous solution of phosphate buffer saline pH 7.2 was used to dissolve the extract for the treatment.

Parasite

Trypanosoma brucei brucei was used. The parasite was obtained from the Department of Biochemistry, Faculty of Science, Kaduna State University, Kaduna, Nigeria.

Isolation of Parasites

The method of isolation used was the method described by Lamham and Godfrey, (1970). The parasites were isolated using column containing DEAE-Cellulose. Blood from infected rats was collected through cardiac puncture after the rat had been anesthetized with chloroform and sacrificed. Column was then prepared by weighing 10g of DEAE-cellulose and dissolved in phosphate buffer saline glucose (1% glucose), pH 8. The DEAE-cellulose was then poured into a separating funnel which gave two layers (DEAE-cellulose layer and the buffer layer). The blood containing the parasite was then put into the column drop-wise. Due to the pH of the column, only the parasite (*Trypanosoma brucei brucei*) passed through the column and was collected in a clean beaker together with the buffer.

Experimental Animals

Healthy whista albino rats of either sex were used for the experiments. The rats were purchased from the Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were maintained on water and feed (diet prepared by chick grower's mash, Pfizer Company, Nigeria) *ad libitum.* The animals were housed under similar condition at 27 ± 2^0C, with 12 hour light/dark cycle and weighed between 122g to 326g before the commencement of the experiment. The experiments were conducted in compliance with the international acceptable principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997).

Animal Grouping

Twenty (20) rats were used in this experiment. The rats were divided into four (4) groups of five each as follows:
Group A- Infected treated with standard drug
Group B- Infected treated with extract
Group C- Infected not treated (Positive control)
Group D- Not infected not treated (Negative control)

Infection of Rats

Isolated parasites, numbering 2,500 in 0.2ml phosphate buffer saline (PBS) were administered to each of 15 rats of the three groups A, B and C, having 5 rats per group. Group D (negative control) was not infected with the parasites.

Parasitemia level and count

Blood smear prepared by spreading blood over a slide obtained from the tail of an infected rat was viewed and counted under the microscope at x40 magnification to determine the parasitemia level. At high parasitemia, the rats were sacrificed and the blood collected for the isolation of the parasites as described above. The isolated parasites were counted using Haemocytometer to determine the number of parasites present per ml.

Treatment

The treatment began on the six day after infection when the parasitemia level was high. Group A was treated with standard drug (diminazene aceturate) 3.5mg/kg body weight for one day. Group B was treated with aqueous extract of *Mitracarpus scaber* 450mg/kg body weight for five days. The two controlled groups (group C and group D, the positive control and negative control respectively) were given only the phosphate buffer saline. All administration was intraperitoneal. The animals were observed frequently for the five days of treatment.

Collection of blood for analysis

A day after the treatment, all the animals were anesthetized with chloroform and sacrificed. The blood was collected through cardiac puncture for analysis.

Estimation of Alanine amino transferase (ALT), Alkaline Phosphatase (ALP) and Serum Bilirubin

Alanine amino transferase (ALT) activity was estimated by the method of Reitman and Frankel (1957). Serum alkaline phosphatase activity was determined using the method of King and Armstrong (1934). Serum total and direct bilirubin was determined by the method of Jendrassik and Grof (1938).

Statistical Analysis

All values are presented in Mean±SE, from 5 animals in each group. The data were analyzed by one way analysis of variance (ANOVA). Values of $p<0.05$ were considered statistically significant.

RESULT AND DISCUSSION

Past studies have shown that a lot of tropical plants contain clinically efficacious constituents against diseases caused by protozoa (Shittu, Umar and Usman, 2013). *Mitracarpus scaber* is one of these plants that have been shown to possess both *in vitro* and *in vivo* trypanocidal activity (Nok, 2002).The trypanocidal activities of certain plants have been attributed to the alkaloids and other constituents they contain (Shittu, Umar and Usman, 2013). The need, however, for the evaluation of scientifically relevant plant species commonly used in herbal treatment of diseases both for safety and as alternative to the already existing trypanocidals is very crucial, as there has been more recognition of the potential risks associated with herbal medicinal products (Okunji *et al.,* 2000; Abere *et al.,* 2007; Jordan, Cunningham and Marles, 2010; Markus *et al.,* 2014)

Analysis of serum enzymes have been reported to be of value and are early warning signs for certain diseased conditions (Shittu, Umar and Usman, 2013). Fig. 1 shows the effect of aqueous extract of *Mitrascarpus scaber* on Alkalin Phosphatase (ALP) activity in *Trypanosoma brucei brucei* infected rats. It was observed that infection with *Trypanosoma brucei brucei* significantly increase ($p<0.05$) the ALP activity (from 15±1.1U/L to 47.2±6.1U/L). This significant increase was observed in group C (positive control) which was the infected not treated group compared to group D (negative control), which was the not infected not treated group. High level of ALP in blood has been shown to be marker of obstructive liver disease where the bile duct obstruction induces the synthesis of the enzyme by biliary tract epithelial cells (Vasudevan, Sreekumari and Vaidyanathan, 2011). The resultof this study is in correlation with the findings of other researchers where there was also significant increase ($p<0.05$) of ALP in *Trypanosoma brucei brucei* infected rats compared to the uninfected control group (Shittu, Umar and Usman, 2013). Treatment with the aqueous extract of *Mitrascarpus scaber* as indicated by group B (fig 1) showed significant decrease ($p<0.05$) in ALP level earlier increased by the parasites (group C). In this study, it shows that *Mitrascarpus scaber* has antihepatotoxic potential, corroborating the findings of other researchers (Germano *et al.,* 1999).

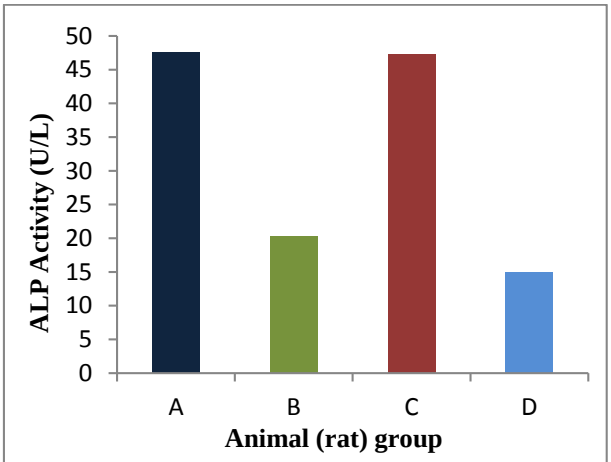

Fig. 1. Effect of Aqueous Extract of *Mitrascarpus scaber* on Alkalin Phosphatase (ALP) Activity in *Trypanosoma brucei brucei* infected rats

Figure 2 shows the effect of *Mitrascarpus scaber* aqueous extract on Alanine amino tranferase (ALT) activity in *Trypanosoma brucei brucei* infected rats. Infection with the parasites (group C) showed significant increase ($p<0.05$) in ALT level (37.8±6.3U/L) when compared to the control group D, negative control (22.6±2.1U/L). This finding come in contrast to the report of some other researches where the transaminases (ALT and AST) activity of serum and liver were only slightly increased in the infected not treated rats when compared with normal rats (Shittu, Umar and Usman, 2013). High level of ALT in the serum has been shown to be a result to hepatocellular damage (Ochei and Kolhatkar, 2000; Vasudevan, Sreekumari and Vaidyanathan, 2011). This means that *Trypanosoma brucei brucei* has damaging effect on liver tissues. Treatment with aqueous extract of *Mitrascarpus scaber* (group B) indicated that the reduction of ALT (from 37.8±6.3 to 25.4±1.3U/L) was not significant at ($p<0.05$) when compared with group C (positive control). This finding is in contrast to an earlier reported work where treatment with 250 mg kg^{-1} extract of *Mitracarpus scaber* significantly reduced the level ($p<0.05$) of ALT previously increased by administration of CCl_4 (Germano *et al.,* 1999). From these results, it is therefore thought that the agent of hepatocellular damage, the extent of damage and the period of administration of treatment may play a big

role on how normalcy of hepatic alterations could be significantly achieved. These could suggest the reason why *Mitracarpus scaber* could not significantly reduce the ALT level as seen in this study.

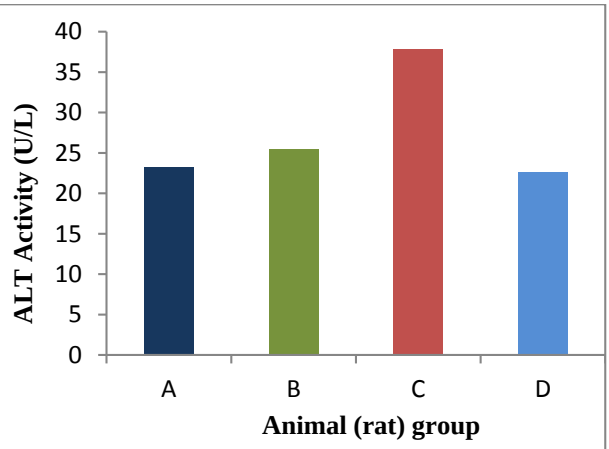

Fig. 2. Effect of Aqueous Extract of *Mitrascarpus scaber* on Alanine amino tranferase (ALT) Activity in *Trypanosoma brucei brucei* infected rats

Figure 3 and 4 shows the effect of aqueous extract of *Mitrascarpus scaber* on conjugated and total Bilirubin in *Trypanosoma brucei brucei* infected rats respectively. It was observed that both the increase and reduction of conjugated and total bilirubin as a result of the infection and treatment respectively were not significant at $p<0.05$. This finding comes in agreement with work of other researchers where they also observed no significant change of bilirubin in both the serum and liver of all the experimental groups (Anosa, 1983; Ekanem and Yusuf, 2007). High plasma bilirubin concentration has been shown to be marker of hepatic dysfunction (Vasudevan, Sreekumari and Vaidyanathan, 2011).

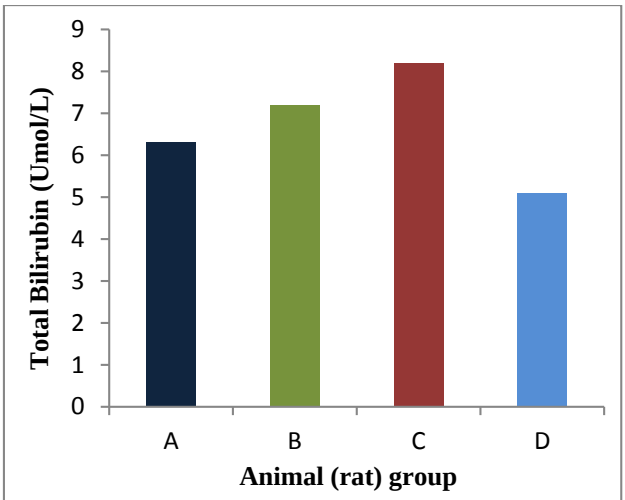

Fig. 3. Effect of Aqueous Extract of *Mitrascarpus scaber* on Conjugated Bilirubin in *Trypanosoma brucei brucei*-infected rats

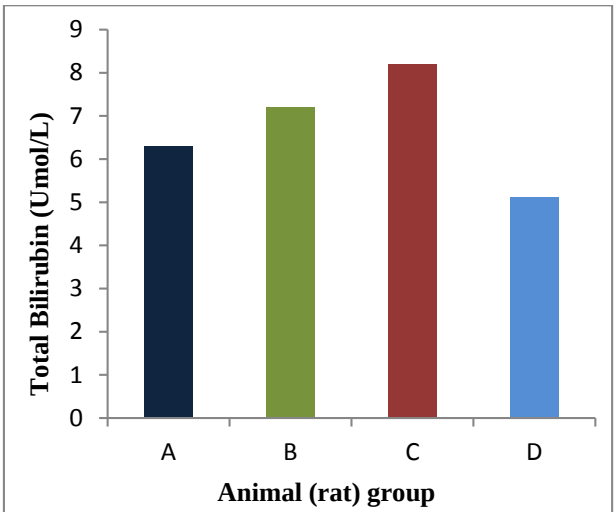

Fig. 4. Effect of Aqueous Extract of *Mitrascarpus scaber* on Conjugated Bilirubin in *Trypanosoma brucei brucei*-infected rats

CONCLUSION

The results of this study showed that aqueous extract of *Mitrascarpusscaber* has antihepatotoxic potentials. This finding therefore corroborates the work of other researchers on the plant, supporting its validity and usage as traditional medicine for the treatment of liver diseases in many parts of Africa. Also, with the scientific reports of it trypanocidal activity, it therefore presents it as a promising non-toxic and cheap source for harnessing against trypanosomiasis.

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