

Effect of The Methanol Leaf Extract of *Annona muricata*(MEAM) on The Serum Chemistry of *Trypanosoma brucei brucei* Infected Albino Rats

IKEOGU N.M. , IKEOGU C.F. , EZEH I.O., EKE I. G., ONAH D. N.

Abstract— This study investigated the effect of the methanol leaf extract of *Annona muricata* (MEAM) in the serum chemistry of albino rats experimentally infected with *Trypanosoma brucei brucei*. A total of 20 adult male albino rats weighing between 78g and 100g were used for the study. They were randomly assigned into 4 groups (I – IV) of five rats each. Groups I and II were infected intraperitoneally with 1.0×10^6 trypanosomes suspended in 0.2ml of Phosphate Buffered Saline (PBS) on day 8 of experiment. Group I was treated with 200 mg/kg of MEAM orally every day for 3 days following detectable parasitaemia (4-5days of post infection). Group II was treated with 7.0 mg/kg of diminazene aceturate intramuscularly on day 14 of experiment (6 days post infection). Group III served as the uninfected untreated control while Group IV was uninfected and treated with 200mg/kg MEAM. Parameters such as Parasitaemia, Packed cell volume (PCV), Haemoglobin concentration, Total leucocytes count, Differential leucocytes count, Rectal temperature and serum activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) as well as serum levels of creatinine, Urea, Albumin, Total protein and Globulin were used to assess the anti trypanosomal activity of MEAM. The acute toxicity test and the *in vitro* anti trypanosomal activity of the extract were studied using standard methods prior to commencement of the study. The results of the acute toxicity test showed the LD₅₀ of the MEAM to be 447 mg/kg whereas the LC₅₀ of the extract was recorded at 0.01 *in vitro*. The pre- patent period of infection was 3days. Treatment with diminazene aceturate cleared the Parasitaemia in group II. An overall significant decrease ($P < 0.05$) in PCV and Haemoglobin were observed in the infected groups. Similarly, a significant decrease ($P < 0.05$) in total leucocytes count was also seen in the infected groups. This decrease was mainly due to a significant decrease ($P < 0.05$) in the leucocytes count of the infected groups. There was a significantly higher ($P < 0.05$) mean serum AST and ALT activities in rats of group I than those of group II, III and IV. A significantly higher ($P < 0.05$) mean serum creatinine level was also seen in groups I and IV than in groups II and III, whereas the mean urea level was significantly higher ($P < 0.05$) in group I than in groups II, III and IV. It is therefore concluded that

MEAM showed an *in vitro* anti trypanosomal activity. However, this was observed *in vivo* at the dose and route of administration., MEAM may be nephrotoxic as seen with the elevated levels of creatinine in group IV.

Index Terms— *Annona muricata*, albino rats, *Trypanosoma brucei brucei*, methanol leaf extract, , serum chemistry.

I. INTRODUCTION

Trypanosomiasis is a complex debilitating and often fatal disease caused by infection by one or more of the pathogenic tsetse-transmitted protozoan parasites of the genus, *Trypanosoma*. African trypanosomiasis constitutes a large and diverse group of blood infections caused by pathogenic trypanosomes (WHO, 1986). The most important species responsible for the disease complex commonly known as Nagana in livestock include *Trypanosoma brucei brucei*, *T. congolense*, *T. vivax*, and the suid parasite, *T. simiae* in pigs.

In Africa, Asia, middle East and South America, *T. evansi*, the causative agent of surra is important especially in draft and transport animals and is exclusively transmitted mechanically by biting flies such as *Tabanus* and *Stomoxys* spp (Anene, *et al* 2001). Other pathogenic trypanosome species of ruminants include *T. uniforme*, found in central and East Africa and Angola, *T. diniorphon* found in tropical Africa which parasites sheep, goat, cattle, pigs and horses. *T. suis* parasites pigs in Zaire and Tanzania. These are all tsetse transmitted while *T. equiperdum* is transmitted venereally and is distributed in South Europe, Asia and North Africa (Hoare 1972). On the other hand, *T. gambiense* and *T. rhodesiense* are the most important human pathogens responsible for West and East African sleeping sickness respectively (Anene, *et al* 2001).

There are six compounds used in the control of trypanosomiasis namely; diminazene aceturate, Homidium, Isometamidium, Pyrimethidine, Quinapyrimine sulphate and Suramin (Radostits *et al*, 2006). After the introduction of isometamidium in 1961 (Berg *et al*, 1961), the development of new trypanocidal drugs has made little progress. Besides the resistance of *T. brucei brucei* to diminazene aceturate has been identified as a significant problem in trypanosomiasis-endemic areas (Anene, *et al* 2001, 2006). Hence the need to develop new anti trypanocidal drugs especially from medicinal plants. Medicinal plants are a rich source of substances that are claimed to induce para immunity, the non-specific immune modulation of granulocytes, macrophages, natural killer cells and complement function in mammalian models (Kumar *et al*, 2012) *Annona muricata* a plant belonging to the family of

IKEOGU N.M., Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria

IKEOGU C.F., Department of Fisheries and Aquaculture, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

EZEH I.O., Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria

EKE I. G., Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria

ONAH D. N., Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria

Annonaceae has a widespread pan tropical distribution and has been widely known as corossol or sour sop. The leaves and seeds of *Annona muricata* contain a great number of acetogenins which display some interesting biological or pharmacological activities such as anti tumoral, cytotoxicity, antiparasitic and pesticidal properties (Christophe *et al* 1997). The aim of this study is to assess the effect of the methanol extract of *Annona muricata* (MEAM) on the serum chemistry of *T.brucei brucei* infected albino rats, having already shown in our previous study that the methanol leaf extract of *Annona muricata* shows anti trypanosomal activity in vitro.

II. MATERIALS AND METHOD

The rectal temperatures of the rats were taken daily. This was done by inserting a digital thermometer into the rectum and tilting the bulb against the rectal wall. After a beep which signifies the end of measurement, the value is then recorded in degree Celsius (^oC)

AST and ALT kits were used for the study. AST and ALT kits were obtained from RANDOX laboratories Ltd, 55 Diamond Road Crumin, Co. Antrim, United Kingdom, BT29 4QY, through product distributors in Nigeria.

1. Three test tubes for sample, sample blank and reagent blank were set up.
2. 0.1 ml of sample was put into the tube labelled sample.
3. 0.1 ml of distilled water was put into the tube for reagent blank.
4. 0.5 ml of Reagent 1 was put into each of the three tubes.
5. The content of each tube was mixed thoroughly and incubated in a water bath for 30 minutes at 37^oC
6. At the end of the incubation, 0.5 ml of Reagent 2 was added into each of the tubes. 0.1 ml of sample (control group) was added into the tube for sample blank.
7. The content of each tube was mixed and allowed to stand exactly for 20 minutes at 25^oC.
8. At the end of 20 minutes, 5.0 ml of sodium hydroxide (0.4mol/1) was put into each of the tubes.
9. After 5 minutes, the absorbance of the sample was read against that of reagent and sample blank using a digital colorimeter at wavelength of 546nm.

A.FOR AST

$$X \text{ (u/l)} = \frac{Y - 0.02}{0.0019}$$

Y = absorbance of sample

X = AST IN u/l

The equation was generated from the graph of absorbance given by RANDOX laboratories.

B. FOR ALT

$$X \text{ (u/l)} = \frac{Y - 0.0149}{0.0053}$$

Y = absorbance of sample.

X = ALT in u/l

The equation was generated from the graph of absorbance given by RANDOX laboratories.

Creatinine kit was obtained from RANDOX Laboratories

Ltd, 55 Diamond Road Crumin, Co. Antrim, United Kingdom, BT29 4QY.

Three test tubes labeled reagent blank, standard and sample were set up.

1. 0.1ml of distilled water was put into the tube labeled reagent blank.
2. 0.1ml of standard solution was put into the tube labeled standard.
3. 0.1ml of sample was put into the tube labeled sample.
4. 1ml of working reagent (mixture of equal volumes of picric acid and sodium hydroxide-35mmol/l and 0.32 mol/l respectively) was put into each of the tubes and the absorbance of the sample, reagent blank and standard read 30 seconds and 2 minutes later using a digital colorimeter at a wavelength of 492 nm.

C. FOR CREATININE

$$\frac{\text{Change } (\Delta) \text{ in absorbance of sample}}{\text{standard concentration (mg/dl)}} \times$$

Change (Δ) in absorbance of standard

Given that:

$$\text{Standard concentration} = 1.97\text{mg/dl}$$

D. SERUM UREA

Urea kit was obtained from RANDOX laboratories Ltd, 55 Diamond Road Crumin, Co. Antrim, United Kingdom, BT29 4QY,

Three test tubes labeled blank, standard and sample were set up.

1. 0.01ml of sample was put into the tube labeled sample.
2. 0.01ml of standard solution was put into the tube labeled standard.
3. 0.01ml of distilled water was put into the tube labeled blank.
4. 0.01ml of reagent 1 was put into each of the tubes.
5. The contents of the tubes were mixed properly and incubated at 37^oC for 10 minutes.
6. 2.50 ml of reagent 2 and 3 each were added into each of the tubes at the end of the incubation.
7. The tubes were again incubated at 37^oC for 15 minutes after being properly mixed.
8. At the end of the incubation, the absorbance of the blank, standard and sample were read using a digital colorimeter at a wave length of 546 nm

E. FOR UREA

$$\frac{\text{Absorbance of sample}}{\text{(mg/dl)}} \times \text{standard concentration}$$

Absorbance of standard

Given that:

$$80.5\text{mg/dl}$$

urea.

$$\text{standard concentration} = 0.467\text{mg of BUN} = 1\text{mg of urea}$$

F. TOTAL PROTEIN

The kit used for the study was from RANDOX Laboratories Ltd, 55 Diamond Road Crumin, Co. Antrim, United Kingdom, BT29 4QY.

1. Label some clean test tubes for sample 1, 2,

- 3,.....standard 1 and 2 and blank.
2. Add 1.2ml of Reagent A to all test tubes (both samples, standards and blank).
3. Add 0.024ml of the serum samples to the labeled sample test tubes according to their label. To the test tubes labeled standard 1 and 2, add 0.024ml of the standard (Reagent B). Mix the contents of the test tubes by shaking.
4. Leave to stand at room temperature for 10 minutes.
5. Read the absorbance of the contents of all the test tubes at 540nm using a spectrophotometer or a colorimeter against the Blank.

G. FOR TOTAL PROTEIN

$$\text{Total protein(g/dl)} = \frac{\text{Absorbance of Sample} \times 5}{\text{Absorbance of standard 1}}$$

The kit used for the study was obtained from RANDOX Laboratories Ltd, 55 Diamond Road Crumin, Co. Antrim, United Kingdom, BT29 4QY. The test was carried as follows:

1. Label some clean test tubes for sample 1, 2, 3 Standards 1 and 2, and blank.
2. Add 2.5 ml of Reagent A to all the test tubes (both samples, standard and blank).
3. Add 0.01 ml of the serum sample to the labeled sample test tubes according to their label. To the test tubes labeled standard 1 and 2, add 0.01ml of the standard Reagent B.
4. Leave to stand at room temperature for 5minutes.
5. Read the absorbance of the contents of the entire test at 630nm using spectrophotometer or 620nm using colorimeter against the blank.

H. FOR ALBUMIN

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of Sample} \times 5}{\text{Absorbance of standard 1}}$$

The value of globulin is expressed as the difference between Total protein and Albumin.

Data collected were subjected to one-way analysis of variance (ANOVA). Duncan's Multiple Range Test was used to separate the means at post hoc. Probability values of $P < 0.05$ were considered significant.

III.RESULTS

Fig 1 shows the mean rectal temperature. Following infection there was a significantly higher ($P < 0.05$) mean rectal temperature in groups 1 and 2 than in groups 3 and 4. There was a significantly lower ($P < 0.05$) mean rectal temperature in group 2 (following treatment with 7.0 mg/kg of diminazene aceturate) than in group 1 (despite treatment with 200mg/kg of MEAM). There was no significant difference ($P < 0.05$) in the mean rectal temperature of rats in groups 3 and 4 throughout the experiment.

Fig 2 shows the mean AST and ALT levels. There was a significantly higher ($P < 0.05$) mean serum ALT and AST activity in group 1 than in group 2. However, there was no significant difference ($P < 0.05$) in the mean serum ALT and AST activity of rats in group 2, 3 and 4.

Fig 3 shows the mean creatinine and urea levels. The mean serum creatinine level was significantly higher ($P < 0.05$) in rats of group 1 (infected and treated with 200mg/kg of MEAM) and group 4 (uninfected and treated with 200mg/kg of MEAM) than those of group 2 (infected and treated with 7.0 mg/kg of diminazene aceturate) as well as group 3 (uninfected untreated). There was a significantly higher ($P < 0.05$) mean urea level in group 1 (infected and treated with 200mg/kg of MEAM) than in group 2 (infected and treated with 7.0mg/kg of diminazene aceturate). There was no significant difference ($P < 0.05$) in the mean urea level of rats in group 2, group 3 and group 4.

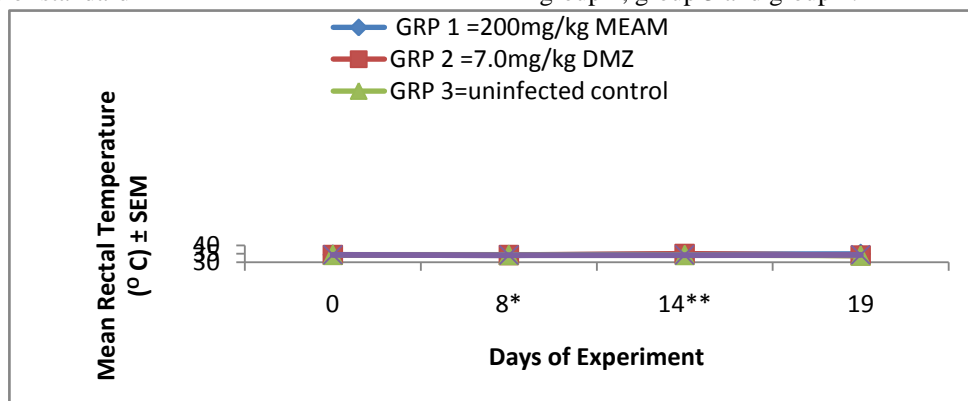


Fig 1: Mean Rectal temperature of rats infected with *T.brucei brucei* and treated with MEAM and Diminazene aceturate
8*- day of infection of Group 1 and Group 2
14**- treatment of Group 2 with Diminazene aceturate/ treatment with 200mg/kg MEAM end.

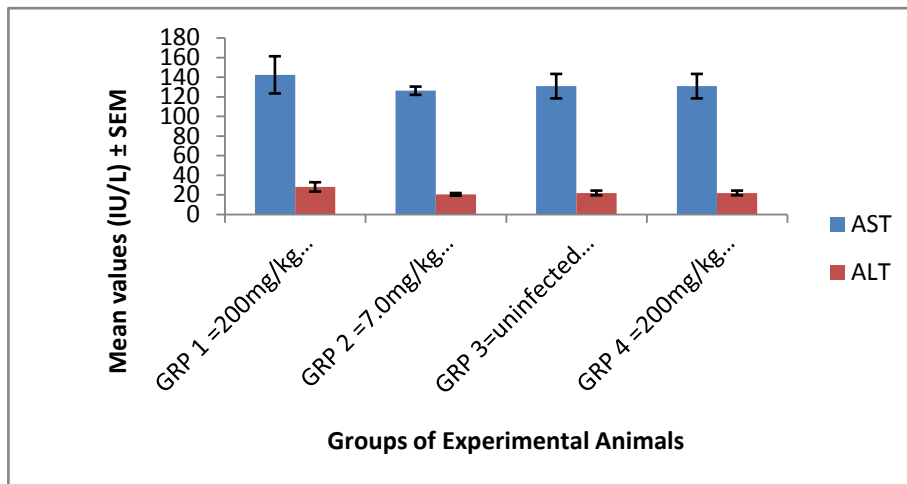


Fig 2: Mean AST and ALT values of rats infected with *T.brucei brucei* and treated with MEAM and diminazene aceturate.

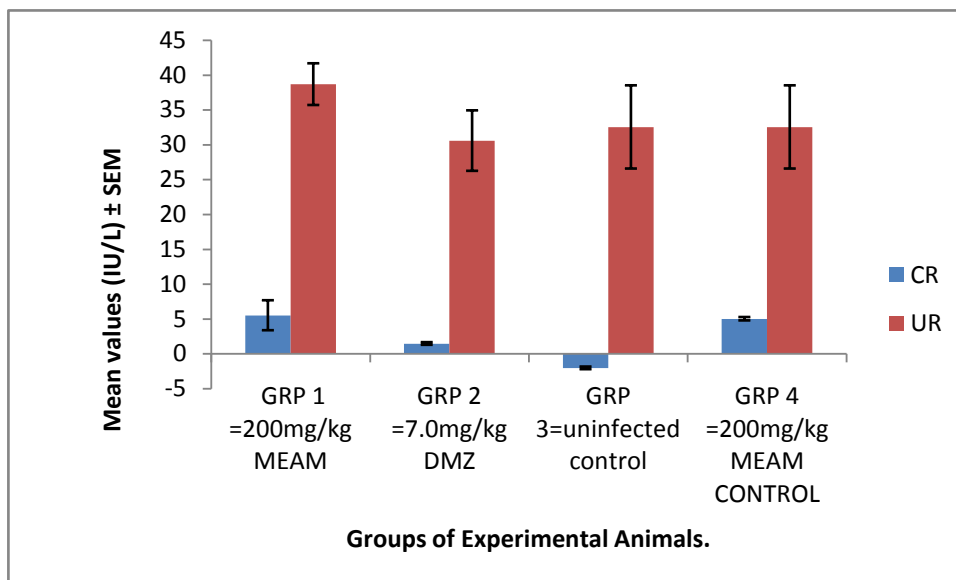


Fig 3: Mean Creatinine and Urea values of rats infected with *T.brucei brucei* and treated with MEAM and diminazene aceturate.

IV. DISCUSSION

Trypanosomosis is characterized by fever and this has been previously reported (Randy, 2013). Fever is characterized by an increase in the body temperature of an animal due to the presence of endogenous or exogenous pyrogens in the blood of the animal (Garmel, 2012). There was an increase in the temperature of the infected animals which is in line with the finding of (Randy, 2013) but this increase in the temperature of infected animals was reversed only in the group treated with diminazene aceturate which suggested the elimination of the trypanosomes. However, this reversal in the temperature of infected animals was not observed in the MEAM treated group.

Trypanosomosis have been reported to have adverse effects in the liver and spleen causing hepatomegaly and splenomegaly respectively due to the activation and expansion of the reticulo-endothelial system during trypanosome infection (Umar *et al.*, 2007). Trypanosomosis causes increase in AST and ALT levels in affected animals

by causing increased hepatocellular leakages of these two enzymes (Aquino *et al.*, 2002). This increase in the AST and ALT levels was also observed in this study in the MEAM treated group thus indicating; the continued multiplication and trypanosome induced hepatocellular damage. However, this increase in the AST and ALT levels were not observed in the diminazene aceturate treated group.

Renal damage because of the use of *Annona muricata* has been reported by (Azim Yahia *et al.*, 2013). In this study the MEAM treated infected and control groups showed a higher creatinine level when compared to the diminazene treated group and the uninfected control group, which is in line with the finding of (Azim Yahia *et al.*, 2013) suggesting that the extract may be toxic to the animals. Elevated serum urea levels have been associated with kidney diseases due to fever and glomerulonephritis which are constant features of African trypanosomosis (Poltera, 1985). This study revealed similar findings where the serum urea levels were highest in the MEAM treated infected control group indicating fever and glomerulo-nephritis.

V. CONCLUSION

This study suggests that the Methanol leaf extract of *Annona muricata* (MEAM) may not possess trypanocidal activity *in vivo*, at the dose and route of administration used. This may have caused the continuous multiplication of the parasites leading to increased liver and kidney damages which were shown by increased liver and kidney enzymes. Furthermore, if *Annona muricata* is shown in the future to possess anti trypanosomalefficacy (at a different dose and route of administration) optimal dosing is required as there were indications of nephrosis in the uninfected MEAM treated group. Finally, further studies should be done on the plant using a different route of administration for example intramuscularly and intraperitoneally to assess the trypanocidal efficacy of the leaf of *Annona muricata*

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