Centrepoint Journal (Science Edition)
Volume 24, No.1, pages 121-144
http//www.unilorin.edu.ng/centrepoin
CPJ 2018008/24108
2141-3819/2017 $5.00 + 0.00
©2018 University of Ilorin

Toxicological study and anti-trypanosomal potentials of aqueous extracts of *Psidium guajava* leaf and *Moringa oleifera* stem bark in *Trypanosoma brucei*-infected wistar rats

*[1]Sulaiman A. Faoziyat, [2]Olatunde Ahmed, [3]Iyiola O. A., [4]Adisa M. Jimoh, [1]Quadri A. L., [1]Oyegoke, R. A.,[1]Onajobi T. Esther, [1]Olaniya D. David, [1]Dosumu Kehinde, and [1]Akinyele Tunde

[1]*Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria*

[2]*Department of Biochemistry, Abubakar Tafawa Balewa University, Bauchi, Nigeria*

[3]*Department of Zoology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria*

[4]*Department of Chemistry, Ibrahim Badamosi Babangida University, Lapai, Niger State.*

[*]*Author for correspondence: Tel: +2348051505889 E-mail: faoziyat20022002@yahoo.com*

Abstract

African Trypanosomiasis is one of the diseases threatening more than sixty million lives on daily basis. In the search for potential new drugs, the trypanocidal activity of the aqueous extracts of *Psidium guajava* leaf and *Moringa oleifera* stem barks were investigated in *Trypanosoma brucei*-infected rats. The albino rats (18) were randomly assigned into five (A-F) groups, each of which contains three rats. Group A (Positive control-not infected), B (Negative control-infected) groups C and D received Iml each of the extracts, which is equivalent to 200mg/kg body weight of aqueous extracts of *P. guajava* leaf and *M. oleifera* stem bark respectively while groups E and F received 1ml each of Inositol Hexaphosphate (IP6) and Deferoxamine respectively as standard drugs. The administration of the two extracts caused a significant alteration in the average body weight,

percentage liver and kidney body weight ratios, AST, albumin and urea concentrations in the serum of *T. brucei*-infected rats when compared with the control rats. Moreover, the two extracts cause no significant alteration in the kidney weight, while no significant difference was recorded for ALP and ALT activities in the kidney, serum and heart of the groups. The aqueous extract of *P. guajava* leaf reduced the level of parasitemia with extension in lifespan, comparable to that of Deferoxamine and Inositol hexaphosphate while the aqueous stem bark extract of *M. oleifera* could not. This shows that the *P. guajava* leaf extract has anti-trypanosomal activity. Furthermore, both aqueous extracts *of P. guajava* leaf and *M. oleifera* stem bark causes mild changes in the biochemical parameters at the studied dosage (200mg/kg rat body weight). Of the two natural plants used, *P. guajava* leaf is less toxic and produced the best pharmacological effect as such; it can be used in the management of trypanosomiasis more than *M. oleifera*.

Keywords: *Psidium guajava*, *Moringa oleifera*, parasitemia, biochemical markers, trypanosomiasis

Introduction

The World Health Organization (WHO) estimates that 80 percent of the population of African countries presently use traditional medicine to help meet their health care needs. Available Pharmaceuticals are prohibitively expensive for most of the world's population, half of which lived on less than $2 U.S. per day (Edgar *et al.,* 2002). In comparison, herbal medicines can be grown from seeds or gathered from nature at little or no cost. Herbal medicine has become a popular form of healthcare; even though several differences exist between herbal and conventional pharmacological treatments, herbal medicine needs to be tested for efficacy, as well as their toxicity, using conventional trial methodology, and several specific herbal extracts have been demonstrated to be efficacious and safe at certain concentrations for specific disease conditions such as trypanosomiasis, malaria, thypoid, etc.

Human African trypanosomiasis (HAT), a vector borne neuro-inflammatory disease caused by protozoan of the species *Trypanosoma brucei* is still a considerable burden for life quality and economy in 36 sub-Saharan African countries, with 15 – 20 million persons at risk (Paul *et al.,* 2012). Upon invasion of the host, these parasites produce numerous

changes in the cellular and biochemical constituents of blood and other tissues (Igbokwe and Mohammed, 1992; Taiwo *et al.,* 2003).

Some of these plants extract cure various diseases, which include human trypanosomal infection (Akanji *et al.,* 2009). *Psidium guajava* is one which has an enormous wealth of medicinal value. Every single part of the plant from the root to the fruits was to aid in everything from childbirth to skin rashes. It for long has been known for its antibacterial, hypoglycaemic, anti-inflammatory, antipyretic, spasmolytic and central nervous system depressant activities (Akanji *et al.,* 2009). *Moringa oleifera* also known as horseradish tree, on the other hand, is a pan-tropical perennial softwood tree with timber of low quality, but which for centuries has been advocated for nutritional, medicinal and industrial uses. The plant has been reported for its antitumor, hypotensive, antioxidant, radio-protective, anti-inflammatory and diuretic properties. The two plants have also been used individually by native people across Africa to manage trypanosomiasis. Therefore this work was designed to investigate the toxicity and anti-trypanosomal activity of aqueous extracts of *Psidium guajava* leaf and *Moringa oleifera* stem barks in *T. brucei* infected wistar rats.

Materials and Methods

Chemicals and reagents

The assay kits for urea, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were products of RANDOX Laboratory Limited, Cumlin, UK. All other reagents used were of analytical grade and supplied by Sigma-Aldrich Inc., St. Louis, USA.

Collection and Authentication of the Plant Materials

Fresh samples of the leaves of *Psidium guajava* and stem barks of *Moringa oleifera* were obtained from a forest in Ilorin, Kwara State, Nigeria. The plants were botanically authenticated by at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria where voucher specimens were deposited. Their voucher numbers are UILH/003/973 and UILH/004/1008 respectively.

Preparation of Aqueous Extracts of *Psidium guajava* and *Moringa oleifera* Stem Bark

The stem barks of *Moringa oleifera* and leaves of *Psidium guajava* were washed and air-dried to a constant weight separately. Dried plant materials were each pulverized using an electric blender. 500g and 200g of the powdery plant materials of *Psidium guajava* and *Moringa oleifera* respectively were weighed and soaked in 5000 mls and 2000 mls of distilled water respectively and allowed to macerate for forty-eight hours (with intermittent shaking at eight hours interval). Each sample solution was filtered with Whatman No 1 filter paper to obtain the filtrate, which was later concentrated using a water bath at 40^{o}C to obtain the two extracts which were later re-dissolved in distilled water to give the required concentration of 200 mg/kg body weight each, as used in this study.

Experimental Animals

Eighteen (18) healthy adult male albino rats weighing approximately 138.5 ± 11.5g were obtained from the Animal House of the Biochemistry Department of the University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in well ventilated plastic cages with saw dust as beddings. They were fed on standard rat feed and were allowed access to water *ad libitum*. The animals were allowed to acclimatize for a period of one week before the commencement of the experiment. The animals were used in accordance with the regulations set by the University of Ilorin Research Ethical Committee.

Animal Grouping

The experimental rats (*Rattus norvergicus*) were separated into six groups (A-F) of three male rats as shown below:

Group A: Positive control (Not infected not treated) administered with 1 ml distilled water/day

Group B: Negative control (Infected not treated) administered with 1 ml distilled water/day

Group C: infected and treated with 1ml of 200mg/kg body weight *Psidium guajava* leaf extract/day

Group D: infected and treated with 1ml of 200mg/kg body weight *Moringa oleifera* stem bark extract/day

Group E: infected and treated with 1ml of (5mg/kg body weight) of Inositol Hexaphosphate (IP6)/day (natural source)

Group F: infected and treated with 1ml of (5mg/kg body weight) of Deferoxamine/day (synthetic source)

The administrations were carried out orally using cannula till the end of the experiment.

Trypanosome stock

A stabilate of pleomorphic *trypanosome brucei*, strain (*Trypanosoma brucei brucei*) was obtained from Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITR), Kaduna and maintained by serial passage into other rats in the animal house of the Department of Biochemistry, University of Ilorin, by serial passage into rats.

Parasite inoculation into donor rats

Blood was collected by jugular puncture from a heavily infected rat and immediately diluted with phosphate buffer solution (PBS). The diluted infected blood contains about 2-5 parasites per field (microscopic field).1ml of this blood was passage into two new healthy rats intraperitoneally to serve as the donor to all subsequent experimental rats. Parasitaemia was monitored in them every morning by microscopic examination of the blood samples taken from the tail of the infected rats.

Infection of experimental animals

Blood was collected by jugular puncture with a syringe from a heavily infected rat and immediately diluted with phosphate buffer solution (PBS) to serve as inoculums. Healthy rats in each test group other than the control group were each infected intraperitoneally with 1ml of the inoculums containing about 100 trypanosome cells. The experimental animals were checked for infection from the third day post infection by checking their parasitaemia level. The animals were confirmed infected on the fourth day by the presence of about 3-5 parasites per field (microscopic field). The extracts were thereafter administered every

morning and parasiteamia was monitored and recorded every three days by microscope examination till the end of the experiment (11^{th} day).

Blood collection, isolation and homogenization of tissues of interest

Collection of sample for biochemical analyses was as described by (Sulaiman and Adeyemi, 2010). Briefly, the rats were weighed and sacrificed by anaesthetizing in a jar containing cotton wool soaked with diethyl ether. When they became unconscious, blood samples were collected by jugular puncture into EDTA sample bottles. The blood samples were centrifuged and 1ml of the serum was collected into appropriately labelled sample bottles containing 4ml of 0.9% saline solution. The rats were then dissected and organs of interest (kidneys, heart and liver) were collected, weighed and preserved in 5ml of 0.25M sucrose solution in preparation for homogenization and centrifugation.

Statistical Analysis

Data were expressed as mean of three replicates ± standard error of mean (S.E.M). The data were subjected to analysis of variance and Turkey's multiple range tests using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $P < 0.05$.

Results and Discussion

Effect of aqueous extracts of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the Parasitemia of *T. brucei* infected wistar rats

Table 1 shows the average daily parasitemia in *T. brucei* infected wistar rats. The positive control rats were not infected, hence there were no parasite count recorded in them. In the negative control rats, a progressive increment in the parasitemia level was documented from the 4th day till the 8th day of infection before their death in the 9th day. Also, there were progressive increments in the parasitemia level of *Moringa oleifera* and treated rats before their eventual death on the 7th day of infection, which is also the 4th day of treatment. The parasitemia count of rats treated with *Psidium guajava* increased up till day 6 of infection and then decreased before their death on the 10th day.

Discussion: The death of the rats treated with aqueous stem bark extract of *M. oleifera* on day 4 post-treatment, depicts that the extract had no trypanocidal effect, the rats in this group astonishingly died before the death of the negative control group, which suggests some toxic effects of the extracts on them . This result is not in accordance with the one reported by Atawodi and Shehu (2010). The results obtained from treatment with aqueous extract of *P. guajava* leaf indicates that the leaf's extract could be effective in the management of African Trypanosomiasis, since there was considerable extension of life span of infected animals compared with the negative control. There was also appreciable decline in parasitaemia for all animals treated with the aqueous extract. This result confirms the many pharmacological uses of *P. guajava* leaf extract (Kamath *et al*., 2008). The death of the treated animals even at such a low parasitaemia level may not have been unconnected with the release of extracellular factors by the trypanosomes (Ekanem *et al.,* 2006; Sulaiman *et al.,* 2010, 2012)..

Studies have shown that these extracellular factors have pathological effects on the host rats (Nwagwu *et al.,* 1987; Boutignon *et aI.,* 1990; Ekanem, 1989; Ekanem *et aI*., 1994, 1996). Upon invasion of the mammalian system trypanosomes proliferate rapidly to establish its population in infected host (Poltera, 1985; Pentreath and Kennedy, 2004).

The antibodies produced by the host against the parasite are not effective because of the ability of the parasite to produce a large repertoire of antigens. The host defence mechanism is only partially specific and often lagging behind the progress of the disease in terms of antigen-antibody interaction (Sternberg, 2004). Eventually there is a breakdown of the host immune system coupled with parasite invasion of the central nervous system, leading to coma and death. Total removal of the parasites from the system and simultaneous boosting of the host's immune system, could be very relevant in the control of African sleeping sickness (Hoet *et al*., 2004; Chibale, 2005). *P. guajava* therefore has trypanocidal properties as well as the ability to extend the life span of *T. brucei* infected rats.

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the average body weight of *T. brucei* infected wistar rats

Table 2 shows the effects of *P. guajava* and *M. oleifera* extracts on average weights *of T. brucei* infected rats over three weeks of experiment.

In the first week, the negative control group (139.7) and other groups (*P. guajava*: 138.8; *M. oleifera*: 135.4; IP6: 132.6 and deferoxamine: 113.4) were significantly reduced ($P<0.05$) in weight compared with the positive control group (151.8). However, the average rat weights of Deferoxamine group (113.4) was significantly lower ($p<0.05$) compared with the control group

By the second week, which was the infection and treatment week, increase in weight were recorded in all the groups except in the positive control group, which remain the same (151.8 / 151.3). Across the treated groups however, there was no significant difference ($P>0.05$) (144.2, 138.0, 139.3) in the weight of rats and other groupsexcept in the Deferoxamine rats (113.4) whose average weights remain low..

In the third week, average weight of rats in the control group (150.5) remain significantly higher ($P<0.05$) compared with the negative control group (135.3) and other groups (118.7, 118.0, 131.9 and 117.9), with deferoxamine (117.9) still having the least weight.

Discussion: The increase in the body weight of *M. oleifera* treated rats from week 1 to week 2 could be due to the androgenic properties of *Moringa* leaves which possess anabolic activity. The leaves contain high concentrations of crude protein, essential vitamins, calcium and iron (Odeyinka *et al.,* 2007; Gidamis *et al.,* 2003). In the third week, all the rats' weight decreased significantly except in the control group, showing that the parasitic infection had greater effects on the weight than the extracts and standard drugs could overcome. Studies have linked changes in body or organ weight to toxicity of administered chemical or toxicant (Orisakwe *et al.,* 2003; Adeyemi and Sulaiman, 2014).

Effect of aqueous extract of *Psidium guajava leaf and Moringa oleifera* stem bark on the weight of organs of *T. brucei* infected wistar rats

Table 3 shows the effects of *P. guajava and M. oleifera* extracts on weight of organs. For the liver, there was no significant difference ($P>0.05$) in its weight in IP6 group when compared with that of the control (5.96). Liver weight in groups B (7.19), C (6.86) D (5.39) and F (7.27) were slightly higher than found in the control. For the kidney weight, there was no significant difference ($P>0.05$) between the control group (1.27), negative control group (1.33) and the other groups (1.28, 1.29, 1.18 and 1.27).

For the heart weight, there was no significant difference (P>0.05) between the control group (0.56) and the IP6 treated group (0.58). However, there was significant increase (p<0.05) in the other groups (0.86, 0.79, 0.68, 0.62) compared with the control. The highest weight was recorded was in the negative control group (0.86).

Discussion: The presence of an effect or a major increase on the computed weight of isolated organs suggests that the extracts and drugs caused a form of swelling, atrophy or hypertrophy on the organs in comparison to the control (Amresh *et al.,* 2008).

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the percentage organ-body weight ratios of *T. brucei* infected wistar rats

Table 4 shows the effects of *P. guajava* and *M. oleifera* extracts on Organ Body-Weight ratio. For percentage liver-body weight ratio, there was a significant increase (P<0.05) in all the groups compared with the control group. Deferoxamine treated group recorded the highest liver body-weight ratio. For percentage heart-weight and kidney-body weight ratio, there was a significant increase (P<0.05) in other groups compared with the control group, with the highest value recorded in the *P. guajava* group for both heart (0.62) and kidney (1.06).

Discussion

Organ-body weight ratio is a useful marker of cellular swelling, atrophy or hypertrophy (Amresh *et al.,* 2008). Therefore, the presence of an effect on the computed organ-body weight ratios suggests that the extracts and the drugs used caused a form of swelling, atrophy and hypertrophy on the organs. In addition, changes in the levels of biochemical indices following the administration of chemical compounds, including plant extracts, could be an earlier event preceding gross morphological changes in the organ(s). This however is not always the case in some other instances (Aboyade *et al.,* 2009).

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the activity of aspartate aminotransferase (AST) of *T. brucei* infected wistar rats

Table 5 shows the AST Activity in the serum and selected tissues of rats administered aqueous extracts of *P. guajava* and *M. oleifera*. The AST activity was significantly higher ($P<0.05$) in the serum of negative control (245.70) rats when compared with the control (137.00). However, there was a significant reduction ($P<0.05$) in the AST activities in the serum of rats treated with the extracts (94.49 and 50.00), rats treated with IP6 (107.00) and Deferoxamine (97.53) when compared with the control (137.00).

The AST activity was significantly lower ($P<0.05$) in the liver of negative control rats (100.0) when compared with the control (153.5). Also, there were significant reductions in the AST activities of the liver of the experimental rats (109.1, 115.2, 98.3 and 103.5) compared with the control. The AST activity was significantly lower ($P<0.05$) in the heart of negative control rats (229.0) when compared with the control (275.2). Also, there were significant reductions ($P<0.05$) in the AST activities of the heart of other groups (107.1, 32.17, 81.59 and 56.81) compared with the control (275.2).

Discussion: The measurement of the activity of some enzymes could be used to evaluate for cellular toxicity arising from exposure to chemical compounds including drugs (Adeyemi and Akanji, 2011). AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchyma cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart and skeletal muscle, while AST is found in the liver, heart, skeletal muscle, kidneys, brain and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST. The activity of AST in the serum is lower in contrast to Oluduro and Aderiye (2009), but the activity in the liver is lower which is in agreement with Oluduro and Aderiye (2009).

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the activity of alanine aminotransferase (ALT) of *T. brucei* infected wistar rats

Table 6 shows the ALT Activity in serum, liver and heart of rats administered aqueous extracts of *P. guajava* and *M. oleifera*. In the serum, there was a significant increase ($P<0.05$) in the ALT activity of the negative control rats (87.86), *P. guajava* group (41.43) and Deferoxamine group (39.70) when compared with the control (23.33). Although, *M. oleifera* (21.55) and IP6 (24.05) treated rats have no significant difference ($P>0.05$) in ALT activities when compared with the control.

In the liver, there was a significant reduction ($P<0.05$) in the ALT activities of the groups compared with the control (111.30). However, there was no significant difference ($P>0.05$) in ALT activity in the *P. guajava* (100.40), *M. oleifera* (103.90) treated rats and the negative control rats (97.80) compared with the control group. The IP6 (86.96) and deferoxamine (82.09) treated rats also showed no significant difference between each other ($P>0.05$).

In the heart, there was no significant difference ($P>0.05$) in the ALT activity of the IP6 (49.88) and *M. oleifera* group (63.82) when compared with the control (57.44). However, the other treated groups (73.30 and 79.64) and the negative control group (98.99) showed significant increase ($P<0.05$) in ALT activity when compared with the control group (57.44).

Discussion: ALT is found in the plasma and in various tissues, but is moat common in the liver. Significantly elevated level of ALT often suggests the existence of other medical problems such as bile duct problem, liver damage, diabetes and congestive heart failure. There is no significant difference between the ALT activity in the serum of the positive control and that of the group treated with *Moringa oleifera* extract. This is in contrast to Oluduro and Aderiye (2009) report which says there was an increase in the ALT activity in the serum. The activity of ALT in the liver was reduced and is in agreement with Oluduro and Aderiye (2009).

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the activity of alkaline phosphate (ALP) of *T. brucei* infected wistar rats

Table 7 shows the alkaline phosphatase activity in the serum and some tissues of rats administered aqueous extracts of *P. guajava* and *M. oleifera*. In the serum, there was significant decrease ($p<0.05$) in ALP activity of the negative control (6.284), *P. guajava* (5.636) and *M. oleifera* group (3.329) when compared with the control (8.468). The IP6 group (7.685) and Deferoxamine group (9.263), however showed no significant difference ($P>0.05$) with control (8.468).

In the liver, the most significant reduction ($P<0.05$) in the ALP activity was recorded in the liver of the negative control group (10.939) compared with the other groups. A significant increase ($P<0.05$) in the IP6 treated rats (19.312) compared with the control group (14.924). There was however no significant difference ($P>0.05$) between the Deferoxamine group (15.269) and the control group (14.924). In the kidney, there was no significant difference ($P>0.05$) in the ALP activities between the various groups and the control group (21.515).

Discussion: Alkaline phosphatase (ALP) is a 'marker' enzyme of damage for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan *et al.*, 2004). It is frequently used to assess the integrity of the plasma membrane (Akanji *et al.*, 1993). ALP is present in a number of tissues including liver, bone, intestine and placenta. ALP concentration in the liver is significantly lower than the control which is in agreement with Oluduro and Aderiye (2009) and is lower in the serum which is in contrast to Oluduro and Aderiye (2009).

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the level of protein in *T. brucei* infected wistar rats

Table 8 shows the protein concentration in the serum and liver of rats administered aqueous extracts of *P. guajava* and *M. oleifera*. In the serum, there was significant reduction ($p<0.05$) in the protein concentration of the negative control group (20.44) and other treated groups (17.36, 8.46, 3.85, 18.24) in comparison with the control group.

In the liver, there was significant reduction (P<0.05) in the protein concentration of the negative control group (23.12) in comparison with the control group (45.15), the significant reduction (P<0.05) was also recorded by the *M. oleifera* (26.69) treated rats. However, there was no significant difference (P>0.05) between the *P. guajava* (47.43), IP6 (42.22) and Deferoxamine treated rats (51.99) when compared with the control group (45.15).

Discussion: Total protein is composed of albumin and globulin and reflects the balance of protein biosynthesis and catabolism (Sulaiman *et al.,* 2012; Ekanem *et al.,* 2006; Salau *et al*., 2012). Plasma proteins are synthesized predominantly in the liver; immunoglobulin's are synthesized by mononuclear cells of lymph nodes, spleen and bone marrow. The two general causes of extreme low level of protein in the serum of the animals shown by the therapeutic group of *M. oleifera* and *P. guajava* indicates hypoproteinemia

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the level of albumin in *T. brucei* infected wistar rats

Table 9 shows the Albumin Concentration in the serum and liver of rats administered aqueous extracts of *P. guajava* and *M. oleifera*. In the serum, there was a significant increase (P<0.05) in the albumin concentration of the negative control rats (1.789) compared with the positive control (1.458). *P. guajava* (1.111) and *M. oleifera* (0.655) treated rats showed a significant reduction (P<0.05) when compared with the control. The IP6 treated rats (1.496) had no significant difference (P>0.05) with the positive control (1.496) while no significant difference (P>0.05) between deferoxamine treated rats (1.799) and the negative control (1.789).

In the liver, there was a significant decrease (P<0.05) in the albumin concentration of the negative control rats (0.443) compared with the control (1.592). *P. guajava* (0.976) and *M. oleifera* (0.812) treated rats showed a significant reduction (P<0.05) in the albumin concentration when compared with the control (1.592), whereas the IP6 treated rats had the highest liver albumin concentration compared with the control. There was also no significant difference between the deferoxamine treated rats (1.305) and the control (1.592).

Discussion: Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. The human version is human serum albumin, and it normally constitutes about 50% of human plasma protein. Serum albumins are important in regulating blood volume by maintaining the oncotic pressure of the blood compartment. Low level of albumin (hypoalbumin) shown by the group treated with *Moringa oleifera* in the serum and in the liver is an unfavourable state indicating that administration of the extract for prolonged time impairs albumin synthesis in the liver and causes malabsorption of albumin (Sulaiman *et al.,* 2012; Ekanem *et al.,* 2006; Grant *et al.,* 1987).

Effect of aqueous extract of *Psidium guajava* leaf and *Moringa oleifera* stem bark on the level of urea in *T. brucei* infected wistar rats

Table 10 shows urea concentration in the serum and kidney of rats administered aqueous extracts of *P. guajava* and *M. oleifera*. In the serum, there was a significant increase ($P<0.05$) in the urea concentration of negative control (8.411) while *P. guajava* (3.664) groups showed a significant reduction when compared with the control (6.885). However, there was no significant difference ($P>0.05$) between the *M. oleifera* group, IP6 group (6.270) and deferoxamine group (6.201) when compared with the control. In the kidney, there was a significant decrease ($P<0.05$) in the urea concentration of negative control (21.150), and all the other groups when compared with the control.

Discussion

Urea is a nitrogen-containing substance normally cleared from the blood by the kidney into the urine. Urea serves an important role in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. In comparison with the positive control rats, there was a reduction in the level of urea in the serum and in the kidney in groups administered with *P. guajava* and *M. oleifera* in both therapeutic and prophylaxis administration condition. This denotes that the kidney role in the absorption and excretion of urea has been impaired (Ekanem *et al*., 2005; **Sulaiman *et al.,*** 2010).

Table 1: Effect of *psidium guajava* and *Moringa oleifera* on the parasitemia of *trypanosoma brucei* infected rats

	Days										
Groups	1	2	3	4	5	6	7	8	9	10	11
A (Control)	0	0	0	0	0	0	0	0	0	0	0
B (Negative control)	0	0	0	2.33	6.33	7.50	9.00	11.00	D	-	-
C (*Psidium guajava*)	0	0	0	3.67	8.00	12.67	10.00	8.00	6.00	D	-
D (*Moringa oleifera*)	0	0	0	5.00	10.33	13.00	D	-	-	-	
E(Inositol hexaphosphate)	0	0	0	5.00	8.33	12.67	11.33	9.67	9.00	5.00	D
F(Deferoxamine)	0	0	0	4.67	9.67	14.00	14.00	10.00	12.00	10.00	D

Values are expressed as mean ± SEM of 3 replicates. D: death

Table 2: Effect of *psidium guajava* and *Moringa oleifera* on the average weight of *trypanosoma brucei* infected rats.

	Average body weight (g)		
Groups	Week 1	Week 2	Week 3
A (Control)	151.8 ± 0.342a	151.3 ± 2.054a	150.5± 2.204a
B (Negative control)	139.7 ± 1.939b	150.3 ± 2.638a	135.3± 0.882b
C (*Psidium guajava*)	138.8 ± 2.479b	144.2 ± 2.197a	118.7± 7.991c
D (*Moringa oleifera*)	135.4 ± 3.523b	138.0 ± 2.259a	118.0± 0.000c
E(Inositol hexaphosphate)	132.6 ± 3.733b	139.3 ± 2.934a	131.9± 2.899b
F(Deferoxamine)	113.4 ± 2.330c	123.9 ± 2.288b	117.9 ± 5.068c

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different ($P<0.05$).

Table 3: Effect *of psidium guajava* and *Moringa oleifera* on some organs weight in *trypanosoma brucei* infected rats

	Organ weight (g)		
Groups	Liver	Heart	Kidney
A (Control)	5.96 ± 0.83b	0.56 ± 0.09d	1.27 ± 0.15a
B (Negative control)	7.19 ± 0.57a	0.86 ± 0.03a	1.33 ± 0.06a
C (*Psidium guajava*)	6.86 ± 0.16a	0.79 ± 0.06b	1.28 ± 0.08a
D (*Moringa oleifera*)	5.39 ± 0.52c	0.68 ± 0.06c	1.29 ± 0.06a
E(Inositol hexaphosphate)	6.42 ± 0.81b	0.58 ± 0.15d	1.18 ± 0.19b
F(*Deferoxamine*)	7.27 ± 0.69a	0.62 ± 0.11c	1.27 ± 0.05a

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different (P<0.05).

Table 4: Effect of *psidium guajava* and *Moringa oleifera* on some percentage organ-body weight ratio *of trypanosoma brucei* infected rats

	% organ-body weight ratio		
Groups	Liver	Heart	Kidney
A (Control)	3.77 ± 0.07c	0.35 ± 0.01c	0.81 ± 0.02c
B (Negative control)	4.98 ± 0.23b	0.58 ± 0.07a	0.93 ± 0.04b
C (*Psidium guajava*)	5.53 ± 0.57a	0.62 ± 0.04a	1.06 ± 0.19a
D (*Moringa oleifera*)	4.10 ± 0.30c	0.50 ± 0.02b	0.99 ± 0.08b
E(Inositol hexaphosphate)	5.53 ± 0.41a	0.48 ± 0.10b	0.98 ± 0.12b
F(*Deferoxamine*)	5.78 ± 0.42a	0.50 ± 0.09b	1.01 ± 0.05b

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different (P<0.05).

Table 5: Effect of *psidium guajava* and *Moringa oleifera* on the activity of aspartate transaminase (AST) in *trypanosoma brucei* infected rats

	Aspartate transaminase activity (U/I)		
Groups	Serum	Liver	Heart
A (Control)	137.00 ± 20.08b	153.50 ± 9.54a	275.20 ± 19.97a
B (Negative control)	245.70 ± 0.67a	100.00 ± 19.53c	229.00 ± 2.01b
C (*Psidium guajava*)	94.49 ± 43.25c	109.10 ± 2.42b	107.10 ± 3.23c
D (*Moringa oleifera*)	50.00 ± 4.57d	115.20 ± 0.66b	32.170 ± 5.02e
E (Inositol hexaphosphate)	107.00 ± 10.79c	98.26 ± 4.02c	81.59 ± 10.67c
F(*Deferoxamine*)	97.53 ± 54.45c	103.50 ± 3.99c	56.81 ± 25.73d

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different ($P<0.05$)

Table 6: Effect of *psidium guajava* and *Moringa oleifera* on the activity of alanine transaminase (ALT) *in trypanosoma brucei* infected rats

	Alanine transaminase activity (U/I)		
Groups	Serum	Liver	Heart
A (Control)	23.33 ± 2.42c	111.30 ± 2.99a	57.44 ± 1.98c
B (Negative control)	87.86 ± 2.52a	97.80 ± 7.20b	98.99 ± 6.77a
C (*Psidium guajava*)	41.43 ± 26.95b	100.40 ± 3.59b	73.30 ± 10.77b
D (*Moringa oleifera*)	21.55 ± 1.70c	103.90 ± 1.52b	63.82 ± 5.14c
E(Inositol hexaphosphate)	24.05 ± 0.77c	86.96 ± 0.10c	49.88 ± 19.28c
F(*Deferoxamine*)	39.70 ± 21.79b	82.09 ± 8.35c	79.64 ± 2.47b

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different (P<0.05)

Table 7: Effect *of psidium guajava* and *Moringa oleifera* on the activity of alkaline phosphatase (ALP) in *trypanosoma brucei* infected rats

	Alkaline phosphatase (U/I X10^3)		
Groups	Serum	Liver	Kidney
A (Control)	8.468 ± 0.695a	14.924 ± 3.039b	21.515 ± 0.044a
B (Negative control)	6.284 ± 0.050b	10.939 ± 2.688c	21.389 ± 0.094a
C (*Psidium guajava*)	5.636 ± 1.726b	18.123 ± 0.455a	22.908 ± 0.646a
D (*Moringa oleifera*)	3.329 ± 1.240c	12.100 ± 3.288c	21.785 ± 0.286a
E (Inositol hexaphosphate)	7.685 ± 3.024a	19.312 ± 0.704a	22.052 ± 0.298a
F(*Deferoxamine*)	9.263 ± 4.110a	15.265 ± 2.649b	22.155 ± 0.307a

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different (P<0.05).

Table 8: Effect of *psidium guajava* and *Moringa oleifera* on the protein concentration of *trypanosoma brucei* infected rats

	Protein concentration (mg/ml)	
Groups	Serum	Liver
A (Control)	31.78 ± 0.00a	45.15 ± 4.05a
B (Negative control)	20.44 ± 0.01b	23.12 ± 1.35d
C (*Psidium guajava*)	17.36 ± 10.73c	47.43 ± 7.82a
D (*Moringa oleifera*)	8.46 ± 5.60d	26.69 ± 8.99c
E(Inositol hexaphosphate)	3.85 ± 1.95e	42.22 ± 2.29a
F(*Deferoxamine*)	18.24 ± 4.78c	51.99 ± 11.41a

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different (P<0.05).

Table 9: *Psidium guajava* and *Moringa oleifera* on the concentration of albumin (ALB) in *trypanosoma brucei* infected rats

	Albumin (g/dl)	
Groups	Serum	Liver
A (Control)	1.458 ± 0.441b	1.592 ± 0.198b
B (Negative control)	1.789 ± 0.032a	0.443 ± 0.171d
C (*Psidium guajava*)	1.108 ± 0.248c	0.976 ± 0.342c
D (*Moringa oleifera*)	0.655 ± 0.032d	0.812 ± 0.184c
E(Inositol hexaphosphate)	1.496 ± 0.187b	2.209 ± 0.683a
F(*Deferoxamine*)	1.799 ± 0.458a	1.305 ± 0.612b

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different ($P<0.05$).

Table 10: Effect of *psidium guajava* and *Moringa oleifera* on the concentration of urea in *trypanosoma brucei* infected rats

	Urea (U/I)	
Groups	Serum	Kidney
A (Control)	6.885 ± 2.307b	26.570 ± 4.140a
B (Negative control)	8.411 ± 0.335a	21.150 ± 0.766b
C (*Psidium guajava*)	3.664 ± 2.524d	21.70 ± 5.235b
D (*Moringa oleifera*)	5.227 ± 1.649c	20.120 ± 2.501b
E(Inositol hexaphosphate)	6.270 ± 2.330c	13.480 ± 0.654c
F(*Deferoxamine*)	6.201 ± 2.553c	14.580 ± 2.821c

Values are expressed as mean ± SEM of 3 replicates. Values in each column with different letters are significantly different ($P<0.05$).

Conclusion

The aqueous extract of *P. guajava* leaf reduced the level of parasitemia with extension in lifespan, comparable to that of Deferoxamine and Inositol hexaphosphate while the aqueous stem bark extract of *M. oleifera* did not. This shows that the *P. guajava* leaf extract has better anti-trypanosomal activity. Furthermore, both aqueous extracts *of P. guajava* leaf and M. *oleifera* stem bark causes mild changes in the biochemical parameters at the studied dosage.

References

Paul, F., Seke Etet and Mahomoodally, M. F. (2012) New Insights in staging and Chemotherapy of African Trypanosomiasis and Possible Contribution of medicinal Plants. *The scientific world Journal*. Volume

Igbokwe IO, Mohammed A. Some plasma biochemical changes in experimental Trypanosoma brucei infection in Sokoto red goats. *Rev Elev Med Pays Trop* 1992;45(3-40): 287-90.

Taiwo VO, Olaniyi MO, Ogunsanmi AO. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental Trypanosome congolense and *T brucei* infections in sheep. *Israel J Vet Med 2003*;58(4), o. Pag.

Akanji MA, Adeyemi OS, Oguntoye SO, Sulyman F (2009). *Psidium guajava* extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. *EXCLI Journal* 8:148-154 – ISSN 1611-2156

Atawodi, S.E. and Shehu, H. (2010). Antitrypanosomal Property of Some Extracts of Different Parts of *Moringa oleifera*, Lam. *Electronic Journal of Biology*. Vol. 6(1): 19-23.

Kamath, J.V., Nair Rahul., Ashok Kumar, C.K. and Mohana ,Lakshmi S. (2008) Psidium guajava L: A review, *International Journal of Green Pharmacy,* 2 (1): 9-12.

Nwagwu M, Okenu DMN, Olusi TA, Molokwu RI. *Trypanosoma brucei* releases proteases extracellularly. *Trans. Roy. Soc. Trop. Med. Hyg* 1987;82:577

Boutignon F, Huet G, Demeyer D, Richet C, Degand P. Study of proteolytic activities released by incubation of trypanosomes (*Trypanosoma brucei brucei*). *Biochim. Biophys. Acta* 1990;1035:369-377.

Ekanem JT. Extracellular fractions derived from Trypanosoma brucei activate erythrocyte Ca2+- ATPase. *Med. Sci. Res* 1989;17:739-740.

Ekanem JT, Akanji MA, Odutuga AA. Host and parasite derived factors during mammalian African trypanosomiasis. *Biokemistri* 1994;4:103-116.

Ekanem JT, Akanji MA, Odutuga AA. Extracellular proteins of Trypanosoma brucei origin lyse erythrocytes of *rats in vitro. Biokemstri* 1996;6:21-29.

Poltera AA. Pathology of human African trypanosomiasis with reference to experimental African trypanosomiasis and infections of the central nervous system. *Br. Med. Bull* 1985;41:169-174.

Pentreath VW, Kennedy GE. Pathogenesis of human African trypanosomiasis. In The Trypanosomiasis (eds. I. Maudlin, P. H. Holmes and Miles) CABI Publishing 2004;283-301.

Sternberg JM. Human African trypanosomiasis: clinical presentation and immune response. *Parasite Immunol* 2004;26:469-476

Hoet S, Opperdoes F, Brun R, Quetin-Leclercq J. Natural products active against African trypanosomes: a step towards new drugs. *Nat. Prod. Rep* 2004;21:353-364.

Chibale K. Economic drug discovery and rational medicinal chemistry for tropical diseases. *Pure Appl. Chem* 2005;77:1957-1964.

Odeyinka, S.M., Torimiro, D. O., Oyedele, J.O. and Asaolu, V.O. (2007). Farmer's Awareness and Knowledge of Moringa oleifera in Southwestern Nigeria: A Perceptional Analysis. *Asian Journal of plant sciences* 6 (2): 320-325.

Gidamis, A.B., Panga, J.T., Sarwatt, S.V., ChoveB.E., and Shayo, N.B. (2003). Nutrients and anti-nutrients cintents in raw and cooked leaves and mature pods of *Moringa oleifera, Lam Ecol.Food Nutri.*, 42: 113.

Adeyemi OS, Sulaiman FA. (2014). Co-administration of iron sulphate and nitroglycerin promoted oxidative stress and mild tissue damage in Wistar rats. *Comp Clin Pathol*. 2014;23:1525e1533.

Orisakwe OE, Hussaini DC, Afonne OJ. (2003). Testicular effects of sub-chronic administration of Hibiscus sabdariffa calyx aqueous extract in rats. *Reprod Toxicol*. 2003;18:295e298.

Amresh, G.R., Singh, P.N. and Rao, C.V. (2008). Toxicological screening of traditional medicine Laghupatha (Cissampelos pareira) in experimental animals. *Journal of Ethnopharmacology*. 116, 454-460.

Aboyade, O.M., Yakubu, M.T., Grierson, D.S. and Afolayan, A.J. (2009). Studies on the toxicological effect of the aqueous extract of the fresh, dried and boiled berries of Solanum aculeastrum Dunal in male Wistar rats. *Human and Experimental Toxicology*, 28, 12, 765-775.

Adeyemi, O.S. and M.A. Akanji, 2011. Iron and nitric oxide play key role in the development of cardiovascular disorder. *J. Toxicol. Environ. Health Sci*., 3: 249-253.

Wright, P.J. and Plummer, D.T., The use of urinary enzyme measurement to detect renal damages caused by nephrotic compounds. *Biochem. Pharmacol*. 12: 65. (1974).

Shahjahan, M. Sabitha, K.E. Malbika, J. and Shyamala-Devis, C.S., Effect of Solanum trilobatum against carbon tetrachloride induced hepatic damage in albino rats. *Indian. J. Med. Res;* 120: 194-198 (2004).

Akanji, M.A. Olagoke, O.A. and Oloyede, O.B., Effect of chronic consumption of metabisulphite on the integrity of rat liver cellular system. *Toxicology*, 81: 173 – 179 (1993).

Salau, A.K. Yakubu, M.T. Oluleye, D.S. Oloyede, O.B. Akanji, M.A., Toxicological evaluation of aqueous leaf extract of Ficus exasperata on selected tissues of normal Wistar rats. *Centrepoint Journal* (Science Edition). 18 (1), 55 – 66 (2012).

Ekanem, J.T., **Sulaiman**, F.A. and Adeyemi, O. (2005). Therapeutic properties and serum iron in *T. brucei* infected rats treated with Amodiaquine and Mefloquine. *Biokemistri*. 17(2): 115-121. Available online at http://www.bioline.org.br/bk Abstracted online at www.ajol.info/journals/biokem

Sulaiman, F.A., Akanji, M.A. and Ekanem, J.T. (2010). Haematological Status of *T. brucei brucei* infected rats treated with ibuprofen. *Nigerian Journal of Biochemistry and Molecular Biology*. 25(2):23-27. Available online at http://www.njbmb.researchjournals.org

http://www.njbmb.org/Content/Volume%2025%20Number%202%202010.htm

Sulaiman, A. F**.,** Akanji, M.A. and Yakubu, M.T. (2012)**.** Effect of administration of ibuprofen on the levels of parasitaemia, albumin and total protein concentrations in rats infected with *Trypanosoma brucei brucei*. *African Scientists*. 13(1): 57-63. Available online at http://www.niseb.org/afs

Ekanem, J.T., Majolagbe, O.R., **Sulaiman,** F.A. and Muhammad. N.O. (2006). Effect of Honey supplemented diet on the parasitaemia and some enzymes of *Trypanosoma brucei*- infected rats. *African Journal Biotechnology*. 5(17): 1557-1561. Available online at http://www.academicjournals.org/AJB