Journal of American Science

Websites:
http://www.jofamericanscience.org
http://www.sciencepub.net

Emails:
editor@sciencepub.net
sciencepub@gmail.com

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

In-Vivo and *In –Vitro* Anti-Trypanosomal Activity of *Tithonia diversifolia* Ethanol Leaf Extract on *Typanosoma brucei brucei* Infected Rats

[1]Damilola Alex OMOBOYOWA, [2]O.T. SONIRAN, [1]Kerian Chigozie NGOBIDI and [1]Garba Jeremiah DANLDI

[1]Biochemistry Research Unit, [2]Biology Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Ebonyi State, Nigeria
Corresponding Author E-mail: damlexb@yahoo.com
+2347032665874

Abstract: The ethanol leaf extract of *Tithonia diversifolia* was studied *in-vitro* and *in-vivo* for activity against *trypanosoma brucei brucei* in albino rats. Phytochemical study and acute toxicity test were also carried out for the plant extract. The phytochemical results reveal the presence of Terpenoids, steroids, reducing sugar, alkaloids, phenol and flavonoids in high concentration. The lethality dose (LD_{50}) result of the plants extract is found to be equal to or greater than 5000mg/kg body weight. The ethanol extracts showed appreciably high *in-vitro* and *in-vivo* anti-trypanosomal activities compared to the reference drug. Motility of *trypanosoma brucei* was stopped by the ethanol extract of the leaf after 40 minutes at concentration of 4 mg/kg body weight. The packed cell volume (PCV) showed non- significant ($P>0.05$) increase in the rats infected with *T. brucei brucei* treated with varying doses of *T. diversifolia* compared with the PCV of rats infected and administered 0.5 ml of distilled water. The *in-vitro* and *in-vivo* anti- trypanosomial activity exhibited by the extracts might be attributed to the bioactive compounds present in the plant extract.

[Damilola Alex OMOBOYOWA, O.T. SONIRAN, Kerian Chigozie NGOBIDI and Garba Jeremiah DANLDI. ***In-Vivo* and *In –Vitro* Anti-Trypanosomal Activity of *Tithonia diversifolia* Ethanol Leaf Extract on *Typanosoma brucei brucei* Infected Rats.** *J Am Sci* 2020;16(11):96-102]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 9. doi:10.7537/marsjas161120.09.

Key words: *In-vitro*; *In-vivo*; Phytochemical; *Trypanosoma brucei brucei*; Motility

Introduction

Trypanosoma brucei brucei are unicellular parasites transmitted by the tse-tse fly. They are the causative agent of African Animal Trypanosomosis (AAT) (Antia *et al*., 2009). The disease results in acute, sub-acute or chronic disease characterized by intermittent fever, anaemia, occasional diarrhea, rapid loss of condition and often death (Nurulaini *et al.,* 2007). Despite development of attempts at control, trypanosomiasis is still one of the limiting factors to livestock industry in sub-Saharan Africa (Kamuanga, 2003). Currently, chemotherapeutics agents constitute the principal method of control, as development of vaccines against AAT is still in progress (Olukunle *et al*., 2010). Trypanosome infections are known to cause immunosuppression responsible for the host's inability to eliminate the trypanosomes even after administration of trypanocidal drugs (Godwin *et al.,* 1972; Osma *et al.,* 1992) Diminazene aceturate and isomethamidium chloride are the most currently used trypanocides, used both for prophylactics and curative purposes for the control of the disease in cattle (Olukunle *et al*., 2010). Unfortunately the parasite have developed resistance to these drugs (Schrevel *et al*., 1996; Anene *et al*., 2000; Geerts *et al.,* 2001) Therefore, there is urgent need for intensification of research into medicinal plants claimed to be effective in the management trypanosomiasis.

Plants are of immense benefit to man and their uses as food and medicine is as old as the history of man. From time memorials plants have proved useful as a source of relief to man from most of the diseases that have being known to plague man (Adebayo *et al.,* 2009). *Tithonia diversifolia* is specie of flowering plant in the tree marigold, Mexican turnsole, Mexican sunflower, that is native to eastern Mexico and Central America but has a nearly tropical distribution as introduced specie (Adebayo *et al.,* 2009). It is either annual or perennial depending on the area; it has shown great potential in raising the soil fertility in soils depleted in nutrients. It has shown its potential in benefiting poor African farmers. This plant is a weed that grows quickly and has become an option as an affordable alternative to expensive synthetic fertilizer (Adebayo *et al.,* 2009). *Tithonia diversifolia* leaf is administered in several forms: oral decoction of the leaves for treatment of hepatitis, diabetes, malaria, pain, chemoprevention and anti-helicobacter pylori

(Kuroda *et al*., 2007; Adebayo *et al.,* 2009). The findings of Olukunle *et al* (2010) indicate that aqueous extract of *Tithonia diversifolia* leaf holds promise as an anti-trypanosomial agent despite its inability to clear parasitaemia completely. The present study is undertaken to investigate phytochemical composition and medicinal properties of the plant relating to the *in-vitro* and *in-vivo* anti-trypanocidal Potential and its effect on the haematological indices of *Trypanosomal brucei brucei* infected rats.

Materials And Methods

Plant material

The plant material used for this study *(Tithonia diversifolia* leaf) was collected from university of Nigeria, Nsukka environment and authenticated by a taxonomist at the botany Department, university of Nigeria, Nsukka, Enugu state, Nigeria. The plant was air dried, ground to powder, extracted with ethanol and freeze dried.

Extraction Procedure

The ethanol extract of *T. diversifolia* leaves was prepared by macerating 400 g of the powder red leaves in 1200 ml of 95% ethanol for 48 hrs and was filtered through Whatman No 4 filter paper. The filtrate was evaporated to dryness *in vacuo* on a rotary evaporator. The yield was calculated and recorded.

Experimental Design

A total of twenty male albino mice weighing between 180-220 g were used for the study, the rats were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. They were acclimatized for seven days in the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic animal house given regular feed and water *ad libitum*. The rats were divided into five different groups with four animals per group (n=4):

Group I: Normal Negative control (un-infected)

Group II: Positive control (infected + 0.2 ml of distilled water)

Group III: Standard control (infected+ 3.5 mg/kg b. w. of diminazene aceturate).

Group IV: Infected + 200 mg/kg b. w of the ethanol extract

Group V: Infected +400 mg/kg b. w of the ethanol extract**.**

Test organism and determination of parasitaemia:

Trypanosoma brucei brucei were obtained from the Department of Veterinary Pathology of the University of Nigeria, Nsukka. The parasites were maintained in the laboratory by continuous passage in rats intraperitoneally. Blood from the tail was used for the estimation of parasitaemia in wet mount. The trypanosome count was determined by examination of the wet mount microscopically at x40 magnification using the "rapid matching" method of Herbert & Lumsden (1976). This method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2).

Acute toxicity and lethality (LD_{50}) test

The acute toxicity and lethality of ethanol extract of the *T. diversifolia* was determined using the modified method of Lorke (1983). The test was divided into two stages. In stage one, nine (9) randomly selected adult mice were divided into three groups, three per group (n=3) and received 10, 100 and 1000 mg/kg body weight of the methanol extract and the signs of toxicity and number of death for a period of 24-hours were recorded. After 24 - hour observation, the doses for the second phase were determined based on the outcome of the first phase. Since there was zero death, a fresh batch of animals were used following the same procedure in phase I but with higher dose ranges of 1900, 2600 and 5000 mg/kg body weight of the extract. The animals were also observed for 24-hours for signs of toxicity and possible number of death. The LD_{50} was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose (Lorke, 1983).

Phytochemical Test

Basic quantitative phytochemical screening of the ethanol extract of the *T. diversifolia* leaf was carried out by testing for the concentration of the following plant constituents: flavonoids, tannins, saponins, steroids, alkaloids, reducing sugar, cyanogenic glycosides and soluble carbohydrate. The phytochemical analysis of the sample was carried out using procedures outlined by Harborne, (1989) and Pearson, (1976).

In Vivo Test of Extract for Anti Trypanosomal Activity

Swiss Albino rats were used for the study. The rats were divided into groups. Four rats was used for each of the treatment (n= 4). All the rats were infected with *T. brucei brucei* as except the group one rats.

Parasitemia was confirmed in the rats after 48 hours of infecting them with *T. brucei*. The plant extract of ethanol base was then administered to the experimental group. Animals (group IV and V at varying dose daily for 7 days (Ene *et al.,* 2009) (i.e. treatment commenced after 48 hours of infections). This is because at this time, the parasites were not numerous and can still be counted. Diaminal standard drug was also administered to the standard control group at a dose of 3.5 mg/kg for one week. The negative control rats were not treated. The administration of the extract and the standard drug was done through the oral route. Parasiteamia was

monitored in all the treatments and the control groups for a period of 14 days (Ene *et al.,* 2009).

***In Vitro* Test of Extract for Anti- Trypanosomal Activity**

Assessment of ethanol leaf extract of *T. diversifolia* for *in-vitro* trypanodal activity was performed in duplicate in 64 well microtitre plates as described by Ene *et al.,* 2009. The extract doses 20.0, 10.0 and 2.0 mg/ml were prepared and neutralized by dissolving 20.0, 10.0 and 2.0 mg of the extract respectively in 1 ml of PBS. Blood (2 ml) of *T. brucei* infected blood was mixed with 0.5 ml of extract solution of 20.0, 10.0 and 2.0 mg/ml to produce effective test concentration of 4.0, 2.0 and 0.4mg/ml respectively. To ensure that the effect monitored was that of the extract alone, a set of control was concluded which contained the parasites suspended in normal saline which was used to reconstitute Diaminal (445 mg diminazene diaceturate, 555 mg phenazone/g). The test mixtures were incubated for 5 minutes in the covered microtitre at 37^0C. then, 1m l of the incubated test mixture were placed on separate microscope slide and covered with cover slip and the parasites observed every 10 minutes for a duration of 60 minutes cessation or drop in motility of the parasites in extract treated blood compared to that of parasites- loaded with control blood without extract was taken as a measure of trypanocidal activity. The parasitemia count was also recorded.

Haemotocrit (HCT)

Haemotocrit (HCT) or pack cell volume (PCV) was determined using the blood collected in heparized vacutainer tubes, sealed with plasticine at each end and then placed in haemotocrit centrifuge at 3,800 rev/min for 5min. the values was read using Hawksley microhaematocrit reader. (Daci and Lawis, 1991).

Statistical Analysis

The data obtained were analyzed using One Way Analysis of Variance. The data were further subjected to LSD post hoc test for multiple comparisons and differences between Means regarded significant at $P<0.05$. The results were expressed as Mean ± SEM.

Results

Yield of the ethanol extract of *T. diversifolia* leaf

The yield of the extract was 20.7 g (3.45%).

Acute toxicity and lethality (LD50) test

Intraperitoneal administration of up to 5000 mg/kg body weight of ethanol extract of *T. diversifolia* leaf to mice caused no death in the two stages of the test. Thus, the intraperitoneal LD_{50} of ethanol extract in mice was estimated to be greater than or equal to 5000 mg/kg body weight.

Phytochemical test

The quantitative phytochemical composition of ethanol leaf extract of *Tithonia diversifolia* showed relatively very high concentration of bioactive compounds such as Terpenoids, steroids, reducing sugar, alkaloids and phenol. The tannins and soluble carbohydrates were observed to be moderately presence while cyanide hydrogen, glycosides and saponnins were detected in low concentration as shown in Table I.

Table 1: Result of the Quantitative phytochemical composition of ethanol extract of *Tithonia diversifolia* leaf

phytochemical Compounds	*Tithonia diversifolia* leaf extract (mg/100g)
Saponins	1.567 ± 0.0014
Tannins	48.120 ± 0.0064
Alkaloids	236.728 ± 0.120
Steroids	172.842 ± 0.00042
Terpenoids	122.989 ± 0.0021
Cardiac glycoside	2.493 ± 0.0071
Soluble carbohydratye	12.178 ± 0.000
Cyanide hydrogen	0.0865 ± 0.0035
Reducing sugar	271.743 ± 0.00495
Phenol	138.568 ± 0.0035

Data represented in Mean ± SEM

***In Vivo* Effect of Ethanol Leaf extract of *Tithonia diversifolia* on Parasitaemia Count of *Trypanosoma brucei brucei* Infected Rats.**

After day zero of treatment, the rats infected with *Trypanosoma brucei brucei* and treated with Diminazen aceturate and varying doses of ethanol extract of *Tithonia diversifolia* leaf (200 and 400 mg/kg body weight) showed significant ($P< 0.05$) decrease in parasitaemia count compared with the rats infected and administered 0.5 ml of distilled water. The infected rats treated with 200 and 400 mg/kg body weight of the extract showed non-significant ($P> 0.05$) decrease in parasitaemia count compared with the infected rats, treated with diminazen aceturate

while the rats infected and treated with 400 mg/kg b. w. of the extract showed non- significant (P>0.05) decrease in parasitemia count compared with the infected rats treated with 200mg/kg b. w. of the extract after day zero of treatment. After day 3, 7 and 14 of treatment, the rats infected with *T. brucei brucei* and treated with diminazen aceturate and varying doses of the extract showed significant (P<0.05) decrease in the parasitaemia count compared with the infected rats administered 0.5 ml of distilled water. While the infected rats treated with 200 and 400 mg/kg body weight of the extract showed significant (P<0.05) increase in parasitaemia count compared with the rats infected and administered 3.5 mg/kg b. w of diminazen aceturate and the rats infected and treated with high dose of the extract (400 mg/kg b. w.) showed non-significant (P>0.05) decrease in parasitaemia count compares with the infected rats treated with 3.5 mg/kg b.w of diminazen aceturate after the 3rd, 7th and 14th day of administration of both standard drug (diminazen aceturate) and the extract.

Table 2: *In-vivo* effect of ethanol leaf extract of *Tithonia diversifolia* on parasitemia count of *Trypanosomia brucei brucei* infected rats

	Parasitaemia count (log number/ml)			
Days	**TBDW**	**TBD 3.5 mg/kg b.w**	**TBTD 200 mg/kg b.w**	**TBTD 400 mg/kg b.w**
Day 0 of treatment	80.25 ± 4.55	39.35 ± 5.22*	28.50 ± 9.45*	26.25 ± 8.91*
Day 3 of treatment	20.50 ± 2.60	0.50 ± 0.50*	13.50 ± 2.60	16.75 ± 5.84
Day 7 of treatment	65.25 ± 11.45	0.00 ± 0.00*	27.50 ± 2.40*	20.75 ± 4.87*
Day 14 of treatment	55.75 ± 20.80	0.00 ± 0.00*	15.75 ± 3.90*	10.50* ± 2.53
Percentage parsitaemia suppression (Day 0- 14) (%)	30.55	100	44.74	60

Values represented in Mean ± SEM

*shows significant difference (P<0.05) compared with TBDW group

TBDW: *Trypanosomia brucei brucei* infected rats administered 0.2 ml of distilled water; TBD: *Trypanosomia brucei brucei* infected rats administered 3.5 mg/kg b.w of diminazen aceturate; TBTD 200 mg/kg: *Trypanosoma brucei brucei* infected rats administered 200 mg/kg b. w of *Tithonia diversifolia;* TBTD 400 mg/kg: *Trypanosoma brucei brucei* infected rats administered 400 mg/kg b. w of *Tithonia diversifolia*

In Vitro* Effect of Ethanol Leaf Extract of *Tithonia diversifolia* on Motility of *Trypanosoma brucei brucei

The ethanol extract of leaves at 4 mg/ml caused complete cessation of *T.brucei brucei* motility in 40 mins, while at 2 mg/ml, complete cessation of the parasites were observed in 50 mins. The standard trypanocidal drug, diaminal eliminated motility of the parasites within 10 mins at 4mg/ml concentration even at the lowest concentration of 0.4 mg/ml complete parasites elimination was observed with 50 mins (Table 3).

Table 3: *In-vitro* effect of ethanol leaf extract of *Tithonia diversifolia* on motility of *Trypanosoma brucei brucei*

	Extract concentration/Time (mins)		
Treatment	**4 mg/ml**	**2 mg/ml**	**0.4 mg/ml**
Infected untreated Control	>60 (VM)	>60 (VM)	>60 (VM)
Infected + Diminazen aceturate	10 (CRM)	40 (CRM)	50 (CRM)
Infected + ethanol leaf extract of *Tithonia diversifolia*	40 (CRM)	50 (CRM)	60 (SRM)

VM: Very motile; CRM: Complete reduction of motility; SRM: Slight reduction of motility

In- Vitro* Effect of Ethanol Leaf Extract of *Tithonia diversifolia* on Parasiteamia Count of *Trypnosoma brucei brucei.

Ethanol leaf extract of *Tithonia divesifolia* showed reduction in parasiteamia count with increase in time. At high concentration of 4 mg/ml the total parasiteamia clearance was observed at 40 mins of the *in-vitro* test, while the parasiteamia count was increased at the end of 60 mins of the extract at concentration of 0.4 mg/ml. the reference drug diminazen showed parasitemia clearance within 40 mins of the experiment at concentrations of 4 mg/ml, 2 mg/ml and 0.4 mg/ml.

Table 4: *In-vitro* effect of ethanol leaf extract of *Tithonia diversifolia* on parasiteamia count of *Trypanosoma brucei brucei*

Treatme nt	Dose	Time (min)					
		0	10	20	40	50	60
Infected untreated Control	-	13	10	12	20	26	24
Infected + Diminazen aceturate	4 mg/ml	5	0	0	1	0	0
	2 mg/ml	10	7	4	0	0	0
	0.4 mg/ml	30	23	28	18	0	0
Infected + ethanol leaf extract of *Tithonia diversifolia*	4 mg/ml	14	3	8	5	0	0
	2 mg/ml	13	6	8	10	5	0
	0.4 mg/ml	20	16	7	10	6	12

Effect of Ethanol leaf Extract of *Tithonia Diversifolia* on Packed Cell Volume of Rats Infected with *T. Brucei Brucei.*

Fig 1 Showed significant (P<0.05) decrease in packed cell volume (PCV) of rats infected with *T. brucei* brucei and administered 0.2ml of distilled water compared with normal control rats. *T. brucei* infected rats treated with 3.5mg/kg body weight of Diminazen showed significant (P<0.05) increase in PCV compared with infected rats administered 0.2 ml of distilled water. the rats infected with *T. brucei brucei* and treated with 200 and 400 mg/kg body weight of *T. diversifolia* ethanol leaf extract showed non-significant (P>0.05) increase in PCV level compared with the PCV value of rats infected and administered 0.2 ml of distilled water.

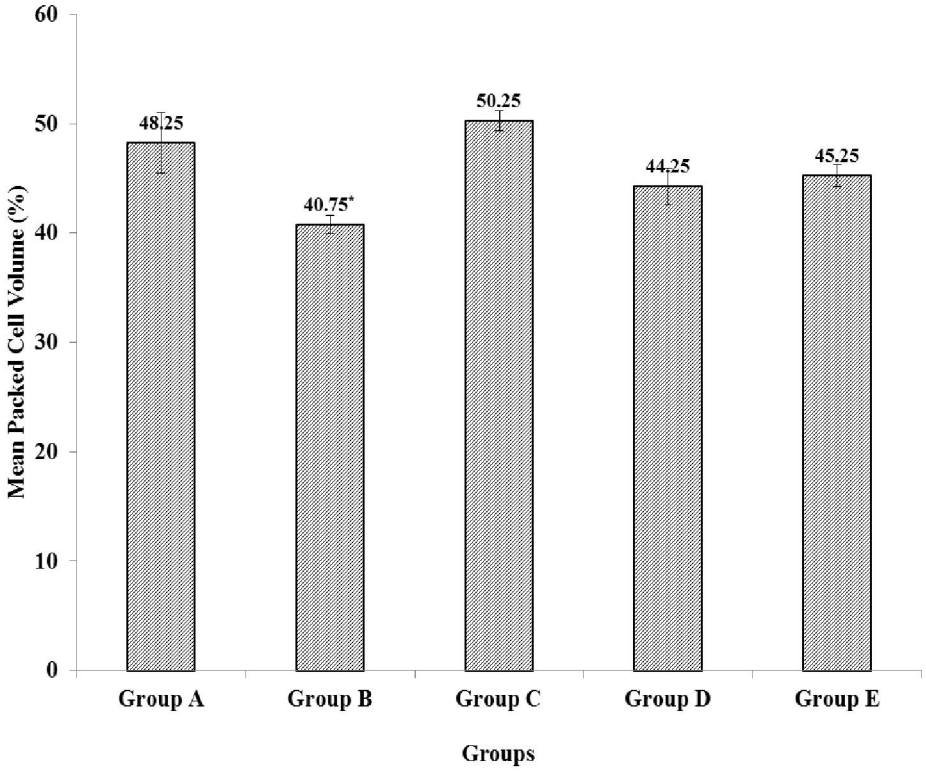

Fig. 1: Effect of Ethanol Extract of *T. diversifolia* on Pack Cell Volume of rats infected with *Trypanosoma brucei brucei*

*shows significant difference (P<0.05) compared with Un-infected, un-treated control

Group A: Normal Control
Group B: Infected + 0.2 ml of distilled water
Group C: Infected + 3.5 mg/kg b. w of diminazen aceturate
Group D: Infected + 200 mg/kg b. w of ethanol leaf extract of *T. diversifolia*
Group E: Infected + 400 mg/kg b. w of ethanol leaf extract of *T. diversifolia*

Discussion

Since, the few trypanocides developed over 40 years ago were expensive and toxic (Amaechi, 2001), it has become necessary to search for new drugs that are safe and efficacious, especially those of plant origin. The plant screened in this present study has folkloric medicinal uses as malaria remedies and treatment of diseases like diabetes, stomach pains etc. (Owoyele *et al.,* 2004). Based on this, the ethanol extract was prepared by macerating the dried leaves in absolute ethanol in order to extract the bioactive compounds that might be responsible for the therapeutic activity. Based on the results of the acute toxicity study, the plant extracts had shown LD_{50} lesser than 5000 mg/kg body weight. Thus, since *T. diversifolia* is believed to have several traditional medicinal uses by different traditional healers, the experimental determination of this good safety margin would justify that the plant is relatively safe at the dosed levels (200 and 400mg/kg body weight) used in this study.

According to the results of the quantitative phytochemical screening, the ethanol extract of *T. diversifolia* showed high concentration alkaloids reducing sugar, steroids, flavonoids and Terpenoids. Soluble carbohydrate, Tannins and saponnins were present in moderate concentration while hydrogen cyanide was very low in concentration. Numerous *in-vivo* studies conducted on the anti-trypanosomal activities of the class of compounds listed above reported the potential of each class of compounds in killing or inhibiting the growth of wide ranges of trypanosomes (Amaechi, 2001). The low PCV value in the infected groups without treatment may be due to acute hemolysis and is as a result of the growing infection. In addition, infection with trypanosomes results in increased susceptibility of red blood cell membrane to oxidative damage. Reactive oxygen species generated by trypanosomes can also attack red blood cells' membranes, induce oxidation and subsequently hemolysis. Phenomenon subjects RBC to massive erythrophagocytos is by an expanded and active mononuclear phagocytic system of the host resulting in anemia (Amaechi, 2001). Thus, scavenging the trypanososmes associated free radicals may ameliorate anemia. The effect of extracts and reference drug in ameliorating anemia is possibly by reducing parasite load, neutralising the toxic metabolites produced by trypanosomes or scavenging the trypanosome associated metabolites (Afewerk *et al.,* 2000).

Conclusion

The *in-vitro* and *in-vivo* evaluation of ethanol leaf extract of *Tithonia diiversifolia* against *Trypanosoma brucei brucei* in albino rats showed that the ethanol extract exhibited appreciably *in-vitro* and *in-vivo* anti trypanosomal activities compares to the reference drug. It is therefore concluded that ethanol leaf extract of *Tithonia diversifolia* possess anti-trypanosomal properties.

References

1. Adebayo, J.O., Balogun, E.A. and Oyeleke, S.A. (2009). Toxicity study of the aqueous extract of *Tithonia diversifolia* leave using selected biochemical parameters in rats. Phlog. Res., 1:143- 147.
2. Afewerk, Y., Clausen, P.H., Abebe, G., Tilahun, G. and Mehlitz D. (2000). Multiple- drug resistant Trypanosoma congolense population in village cattle of Metekel District, North- west Ethiopia, *Acta Tropical*, 76:231- 238.
3. Amaechi, N. (2001). Toxicity of antiprotozoan drug diminazene aceturate in Rats. *Journal of Sustainable Agriculture and Environment*. 3:365- 370.
4. Anene, B. M., Ezeokonkwo, R. C., Mmesirionye, T. I., Tettey, J. N. A., Brock, J. M., Barrett, M. P. and Dekoning, H. P. (2006). A Diminazene-Resistant Strain of *Trypanosoma brucei brucei* isolated from a Dog is Cross Resistant to Pentamidine in Experimentally infected Albino rats. *Parasitology*. 132: 127-133.
5. Antia, R. E., Olayemi, J. O., Aina, O. O. and Ajaiyeoba, E. O. (2009). *In-vitro* and *in-vivo* animal model antitrypanosomal evaluation of ten medicinal plant extracts from southwest Nigeria. *African Journal of Biotechnology* 8(7): 1437-1440.
6. Ene, A.C., Atawodi, S.E., Ameh, D.A., Nnamani, C.N. and Apeh, Y.E.O. (2009). Antitrypanosomal effects of petroleum either, chloroform and methanol extracts of *Artemisi maciverae lim*. *Indian Journal of Experimental Biology*. 47: 981- 986.
7. Geerts, S., and Holmes, P. H. (1998). Drug management and parasite resistance in Bovine Trypanosomiasis in Africa. *Food and Agricultural Organisation Document Repository*: p: 31.
8. Godwin, L. G., Green, D. G., Guy, M. W, and Voller, A. (1972) Immunosuppression during trypanosomiasis. *British Journal of Experimental Pathology* 53: 40-43.
9. Harborne, J. B. (1984). Phytochemical methods. A guide to modern technique of plant analysis. Chapman and Hall. London. Pp: 54-60.
10. Herbert, W. J. and Lumsden, W. A. (1976). *Trypanosome brucei:* A rapid matching method

for estimating the host's parasitaemia. *Experimental Parasitology,* 40: 427-431.
11. Kamuanga, M. (2003). Socio-economic and cultural factors in the research and control of Trypanosomiasis:1-10. Information division FAO, Rome.
12. Kuroda, M., Yokosuka, A., Kobayashi, R., Jitsuno, M., Kando, H., Nosaka, K., Ishii, H., Yamori, T. and Mimaki, Y. (2007). Sesquiterpenoids and flavonoids from the aerial parts of *Tithonia diversifolia* and their cytotoxic activity. *Chemical Pharmaceutical Bull*etin 55: 1240-1244.
13. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol*. 54: 275-287.
14. Nurulaini, R., Jamnah, O., Adnan, M., Zaini, C. M., Khadijah, S., Rafiah, A. I. and Chandrawathani, P. (2007). Mortality of domesticated java deer attributed to Surra. *Tropical Biomedicine* 24(2): 67-70.
15. Olukunle, J. O., Abatan, M. O., Soniran, O. T., Takeet, M. I., Idowu O. A., Akande, F. A., Biobaku, K. T. and Jacobs, E. B. (2010). *In-vivo* anti-trypanosomal evaluation of some medicinal plant extracts from Ogun State, Nigeria. *Science World Journal*, 5(1): 17-19.
16. Osma, A. S., Jennings, F. W. and Holmes, P. H. (1992). The rapid development of drug-resistance by *T. evansi* in immunosuppressed mice. *Acta Tropica.,* 50: 249-255.
17. Owoyele, V. B., Wuraola, C. O., Soladoya, A. O. and Olaleye, S. B. (2004). Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* lead extracts. *Journal of Ethnopharmacology*, 90: 17- 321.
18. Pearson, D. (1976). The chemical analyses of food. 7th Edition. London, Churchill Living Stone. Pp: 3-4.
19. Schrevel, J., Milliériouz, V., Sinov, V., Frappier, F., Santus, S. and Greither, P. (1996). New trends in chemotherapy of Human and Animal Blood Parasites. *Parasitology Research* 82(93):283-284.

11/22/2020