EFFECTS OF *Maytenus senegalensis* (L) AND *Cassia alata* (L) EXTRACTS ON THE BLOOD PARAMETERS OF ALBINO RATS INFECTED WITH Schistosomes cercariae

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ABSTRACT

An investigation was carried out to assess the effects of the roots, leaves and stem-bark of Maytenus senegalensis and the seed and leaves of Cassia alata extracts of methanol and aqueous water at 3, 6 and 9g/kg body weight on the blood parameters of albino rats previously infected with schistosome cercariae using the Danish Bilharzias' Laboratory method. The results showed that the aqueous water 3g/kg and 6g/kg body weight extracts of the leaves with values of 12.8 x 10^3µ/l and 15.7 x 10^3µ/l of white blood cells respectively were above the normal values of (0.96 – 11.06 x 10^3µ/). Other values of Maytenus senegalensis and those of Cassia alata of blood parameters remain within the normal values. It was observed that these water extracts increased the white blood cell count. Therefore the utilization of water extract in the treatment is not recommended.

Keywords: *Maytenus senegalensis*; *Cassia alata;* Blood parameters; Rat; *Schistosomes cercariae*

INTRODUCTION

Schistosoma spp are tiny blood tremtodes and causative agents of schistosomiasis. More than 800 million people are at the risk of contacting the infection worldwide and more than 200 million are infected (WHO, 2019). Mortality is due to bladder cancer, renal failure, liver fibrosis and portal hypertension among other challenges. Plants are the basis of traditional medicine system that have been in existence for thousands of years and continue into modern times (Jellin *et al*. 2000). Penicillin is the most well-known drug of plant origin. In addition to plants, there has been growing interest in the role of animals and microbes as sources of medicine (Duke, 2002). Schistosomiasis affects the health and the economy of infected population by reducing their ability to work (WHO, 2010) and infected people are generally poor and may not have the resources to afford the cost of modern treament and generally rely on traditional medicine (Mohammed *et al*. 2007).

Praziquantel (PZQ) is currently the drug of choice in the treatment of both intestinal and

urinary schistosomiasis but had being facing serious resistant challenges (Stelma *et al*.1995). Many plant species have been used throughout the world in traditional medicine for the treatment of both veterinary and human helminthes The use of these plants by humans in curing severals ailments poses undoubtedly some problems since doses of such of preparations could not be determined. It is also necessary to investigate their therapeuthic effects as well as the level of their toxicity to humans. It is for this purpose that *Maytenus senegalensis* and *Cassia alata* had been selected.

The aim of this work is the screening two medicinal plants as anti-schistosomal agents namely *Cassia alata* and *Maytenus senegalensis*. The specific objectives are (i) To screen and evaluate the anti-schistosomal potency of these plants used by traditional herbalists in the treatment of schistosomiasis, using laboratory rats as indicators. (ii) To evaluate the effect of the extracts on haematological parameters of infected treated animals.

MATERIALS AND METHODS

Plant Materials

The two plants *Maytenus senegalensis* and *Cassia alata*, were collected from the field and were identified based on the characteristics of the leaves, flowers, fruits, stem- bark, using appropriate botanical keys as described by Stanfield and Hopkins (1966), Hutchinson and Dalziel (1968). Information on plants screened was obtained from traditional healers. After collection, useful parts of each of the plants (leaves, seed and roots) were washed and dried under shade. Methanol and aqueous water were used for extraction of active ingredients. Infection of rats was done using the paddling method as described by Danish Bilharziosis method as (Madsen, 1985).

Formulation and Administration of Praziquantel of Plants Extracts

Praziquantel was purchased and administered orally as single dose of 600mg/kg of body weight as recommended by Van Lieshout *et al*. (1994).

$$\text{Weight of PZQ(mg)} = \frac{600\text{mg x Weight of Animal (mg)}}{1000\text{mg}} \quad (1)$$

The quantity of plant extracts was similarly weighted following the same procedure as described in the oral administration of Praziquantel above. Thereafter a single oral dose of various concentrations was administered to each rat using stomach gavage using the same formula:

$$\text{Weight of Plant extract(mg)} = \frac{\text{(A)mg x Weight of Animal (mg)}}{1000\text{mg}} \quad (2)$$

Key: A = represents the dose: 3, 6 or 9g/kg body weight

Statistical Analysis

The analyses were performed using the Statistical Package for Social Sciences software (SPSS 17.0, Chicago, IL, USA). Continuous variables were expressed as means ± standard deviation (SD) of the median and range. P-values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

General Blood Count in *Maytenus senegalensis*

Changes in haematological parameters were observed in rats treated with the various formulations of the plant extracts as well as the control drug Praziquantel®. The parameters observed included White Blood Cell (WBC), Red Blood Cell (RBC), the platelets and lymphocytes counts as presented in Table 1.

Table 1: Haematological value in rats treated with various *M. senegalensis* extracts and control

Pants	Parts	Doses (g/kg)	Methanol extract				Water extract			
			Parameters				Parameters			
			WBC (x 10^3/µl)	RBC (x10^6/µl)	PLAT (x10^4/µl)	LYM (%)	WBC (x10^3/µl	RBC (x10^6/µl)	PLAT (x10^4/µl)	LYM (%)
Maytenus senegalensis	Roots	3	6.4	8.57	833	64.6	6.5	8.54	809	64.3
	Leaves		11.1	9.40	1036	71.2	12.8	9.31	1033	71.1
	Stem-back		9.9	9.16	871	70.2	9.9	8.82	823	70.8
	Total		27.4	27.23	2740	206.0	29.2	26.67	2765	206.4
	Roots	6	9.6	8.87	871	79.0	11.0	9.93	774	73.4
	Leaves		10.4	9.20	949	72.9	15.7	9.23	800	71.2
	Stem-back		10.0	8.99	896	73.2	9.5	8.94	838	74.6
	Total		30.0	26.86	27.16	225.1	36.2	28.20	2412	219.2
	Roots	9	8.1	8.82	890	57.2	7.3	9.25	875	60.4
	Leaves		10.1	9.35	872	69.9	10.5	9.42	820	68.4
	Stem-back		10.1	8.97	859	75.0	10.9	8.94	920	74.5
	Total		28.3	27.14	2621	202.1	28.7	27.61	2615	203.3
Control	PZQ		10.4	9.02	851	74.3	10.4	9.02	851	74.3
	Non infected		9.4	8.60	947	76.3	9.4	8.60	947	76.3
	Infected not treated		18.4	7.10	1216	87.1	18.4	7.10	1216	87.1

KEYS: WBC= White Blood Cell, RBC= Red Blood Cell, PLAT= Platelet, LYM= Lymphocyte

White Blood Cell Count in *Maytenus senegalensis*

Results for the white blood cell count for rats treated with different doses of both methanol and water extracts of *M. senegalensis* as well as that of the control drug Praziquantel® is presented on Table 1.

From the data presented the least white blood cell count of 6.4x10^3 /µl was recorded in the 3g/kg body weight category of *M. senegalensis* in the methanol extracts of the roots. This increased gradually to 9.9x10^3/µl in both mehanol and water extracts of the stem-bark with the highest value of 12.8x10^3/µl recorded in the water extract of

the leaves of the plant. The Praziquantel® treated group had white blood cell count of $10.4x10^3$ /µl. For rats in the non-infected and infected non-treated groups, values count of $9.4x10^3$/µl and $18.4x10^3$/µl respectively were obtained. All these values are within the normal range of 0.96 – 11.06 $x10^3$/µl. Analysis of variation of white blood cell count of the rats treated with the extracts and using ANOVA revealed that the difference is not significant at $P \leq 0.05$ with calculated value of Fcal. = 1.181 and tabulated value Ftab. = 4.945. For rats treated with the 6g/kg body weight dosage of *M. senegalensis*, a similar trend of the 3g/kg body weight dosage is followed with fluctating vaues of the white blood cell count in either the roots, the leaves or the stem-bark of the methanol and water extracts. The highest white blood cell count of $15.7x10^3$/µl was recorded in the water extracts of *M. senegalensis* with the lowest value $9.5x10^3$/µl also obtained in rats treated with stem-bark extracts. The Praziquatel® treated groups had white blood count of $10.4x10^3$/µl while rats infected with *Schistosoma* but non-treated with either extracts and Praziquantel® treated had the highest values of white blood cell count of $18.4x10^3$/µl. All these white blood cell count fall within the range of 0.96 – 11.06 $x10^3$/µl. Similar analysis of the values by use of ANOVA also revealed that differences are not significant statistically at $P \leq 0.05$ with calculated value of Fcal.= 8.267, tabulated value Ftab.= 10.973 and $\alpha = 0.05$

Several reports on blood chemistry on use of plant extracts suggested that an increase in white blood cell counts only in case of infections and the severity of the increase is proportional to the severity of the infection, Amir Al-hroob (2010), Abdel-Ghaffar, *et al.* (2005) and Frances, (2002). Such were observed as in the cases of 3g/kg and 6g/kg body weight of leavesin the water extracts, indicating that these extracts were toxic

Red Blood Cell Count in *Maytenus senegalensis*

The red blood cell count rats treated with extracts of *M. senegalensis* is also presented in Table 1. The highest red blood cell count in rats treated with 3g/kg body weight was recorded with the methanol extracts of the leaves with values $9.40x10^6$/µl. this decreased gradually to $9.31x10^6$/µl in water extracts of the leaves with least value of $8.54x10^6$/µl in the water extracts of the roots. For rats treated with the 6g/kg body weight, the highest value $9.93x10^6$/µl in water extracts of the roots. The leat value of $8.87x10^6$/µl was recorded in rats treated with methanol root extracts. The red blood cell count for rats treated with 9g/kg body weight ranged from $8.10x10^6$/µl to $8.88x10^6$/µl in methanol and water extracts of the plants.

Rats treated with Praziquantel® and the infected non-treated group had red blood

cell counts of $9.02x10^6$/µl and $97.10x10^6$/µl respectively. Though these red blood cells differ in rats treated with the extracts and Praziquantel® , these differences are not significant statistically at $P \leq 0.05$, when the results were analysed by use of ANOVA with calculated value (Fcal.) of 1.112, tabulated value (Ftab.) of 2.156 and $\alpha = 0.05$ Szeranfin and Jako, (2005), advocated anemia in *S. mansoni* infections is better assessed by an overall descriptive measurement of red blood cells (RBC), their size, uniformity of size, and presence of microcytes. Lack of chronic anemia reported in this work in the infected treated rats could be due to the acute nature of the infection since treatment was given at week 2 after the complition of the life cycle.

The variation in blood parameter in general may be due to the immunilogical status of the infected animals as well as their natural morphological ability to produce more blood cells to replace the ones destroyed by the schistosomes. In this investigation, there were case of low red blood count, but these were not significant and remind the normal range.

The Platelet Count in *Maytenus senegalensis*

Rats treated with 3g/kg body weight with both methanol and water extracts of leaves of *M. senegalensis* had the highest values of $1035x10^4$/µl and $1033x10^4$/µl respectively. These are close to that of infected non-treated rats that had $1216x10^4$/µl. Those treated with extracts from roots and stem-bark administered at the same 3g/kg body weight was $809x10^4$/µl and $871x10^4$/µl respectively. Rats treated with 6g/kg body weight had platelet counts of $774x10^4$/µl in the water extracts of the roots to $949x10^4$/µl in the methanol extracts of the leaves. The trend of the counts is similar in rats treated with 9g/kg body weight either of the extracts ranging between $820x10^4$/µl in water extracts of the leaves to $920x10^4$/µl also in water extracts of the stem-bark. Theses fluctuating platelet values are however not significant by sue of ANOVA at $P \leq 0.05$, calculated value (Fcal.) of 1.617, tabulated value (Ftab.) of 1.713 and $\alpha = 0.05$.

Platelets role is maintainance of the integrity of the endothelium and act as part of the clotting factor during the process of repair of damaged endothelium, where they ensure mechanical strength of the clot and vasoconstriction (Berzigotti *et al*., 2013. In normal albino rats the platelet count varies from 638 - $1253x10^3$/µl depending on the age and sex of the animal according to Giknis and Clifford (2008). All the results for platelets obtained in this current work fall within the normal values. These findings were in agreement with previous separate works in the area by Ezekiel and Onyeyili (2007). A recent study by Köpke-Aguiar *et al*., (2009) showed that the levels of thrombopoietin and reticulated platelets were normal in schistosomiasis patients

even with portal hypertension and that the bone marrow produces platelets normally which led Köpke-Aguiar *et al*. (2009) report that there were no changes in the production of platelets in the bone marrow in schistosomiasis patients.

The Lymphocyte Count in *Maytenus senegalensis*

On lymphocyte counts in Table 1, the values for the same *M. senegalensis* extracts for the various dosages and the control also show varying levels of the results. In rats treated with 3g/kg body weight of the plant extracts, the lymphocyte count varies from 64.3% in the water extracts of the roots to 71.2% in the methanol extracts of the leaves. Those of the 6g/kg body weight, treated rats followed the same pattern with values ranging from 71.2% in the water extracts of the leaves to 79.0% in the methanol extracts of the roots. The 9g/kg body weight treated rats had values generally lower than the 6g/kg body weight groups. Lymphocyte count values of rats in this dosage vary from 57.2% in the methanil extracts of the roots to 74.5% in the water extracts of the stem-bark. Value counts of 74.5% to 87.1% were recorded from rats treated with Praziquantel® and the infected non-treated groups respectively. Analysis of the data by use of ANOVA revealed that these difference were not significant statistically at $P \leq 0.05$, calculated value (Fcal.) of 3.62, tabulated value (Ftab.) of 4.09 and $\alpha = 0.05$.

Their primary function of lymphocyte is immunologic, including both antibody production and cell-mediated immune responses. Decreased lymphocyte counts or lymphopenia are usually due to an effect of corticosteroids, either endogenous or stress or Cushing disease or therapeutic, and may also accompany neutropenia in some viral infections, especially the parvoviruses according to the reports by Berzigotti *et al*. (2013). The results in this study showed that the values obtained fall below this normal range indicating that there was no variation in value as observed by Abd EL-Mottaleb *et al*. (2008). However, many investigators record some changes in the value of the lymphocytes while others such as Essam and Ashraf, (2014); Bugarski *et al*. (2006) linked some of these variations with changes in white blood cell counts.

General Blood Count in *Cassia alata*:

White Blood Cell Count in *Cassia alata*

The haematological parameters for rats treated with methanol and water extracts of *C. alata* is presented in Table 2. The white blood cell count of rats treated with both methanol an water extracts for the three doses of 3g/kg body weight to 9g/kg body weight ranged from $9.3x10^3/\mu l$ in the water extracts of the seeds of the plant, in the 9g/kg body weight rats to $10.8x10^3/\mu l$ in the seed of water extracts also of the leaves. Other extracts from the leaves and seeds had white blood cell count of $10x10^3/\mu l$ and

above. The white blood cell counts of rats treated with Praziquantel® was $10.4x10^3/\mu l$, slightly higher thatthe $9.4x10^3/\mu l$ for the non-infected rats. The values for rats in the infected non-treated was the highest being $18.4x10/\mu l$. Subjecting the results to analysis using ANOVA revealed that these differences were not significant at $P \leq 0.05$, calculated value (Fcal,) of 2.012, tabulated value (Ftab.) of 3.112 and $\alpha = 0.05$

Table 2: Haematological value in rats treated with various *C. alata* extracts and control

Pants	Parts	Doses (g/kg)	Methanol extract				Water extract			
			Parameters				Parameters			
			WBC ($x\ 10^3/\mu l$)	RBC ($x10^6/\mu l$)	PLAT ($x10^4/\mu l$)	LYM (%)	WBC ($x10^3/\mu l$)	RBC ($x10^6/\mu l$)	PLAT ($x10^4/\mu l$)	LYM (%)
Cassia alata	Leaves	3	10.1	9.29	853	73.0	10.4	8.86	876	73.3
	Seed		10.6	8.90	959	73.4	10.2	9.40	876	71.8
	Total		20.7	18.19	1812	146.4	20.6	18.26	1754	145.1
	Leaves	6	10.1	9.02	837	74.0	10.2	9.15	875	69.9
	Seed		10.0	9.06	999	72.9	10.8	8.82	885	73.2
	Total		20.1	18.08	1836	146.9	21.0	17.97	1760	143.1
	Leaves	9	10.4	8.58	839	71.3	10.5	8.88	908	73.3
	Seed		9.8	8.93	862	73.4	9.3	8.33	854	76.4
	Total		20.2	17.51	1701	144.7	19.8	17.21	1762	149.7
Control	PZQ		10.4	9.02	851	74.3	10.4	9.02	851	74.3
	Non infected		9.4	8.60	947	76.3	9.4	8.60	947	76.3
	Infected not treated		18.4	7.10	1216	87.1	18.4	7.10	1216	87.1

KEYS: WBC= White Blood Cell, RBC= Red Blood Cell, PLAT= Platelet, LYM= Lymphocyte

Several reports on blood chemistry on use of plant extracts suggested that an increase in white blood cell counts only in case of infections and the severity of the increase is proportional to the severity of the infection, Amir Al-hroob (2010), Abdel-Ghaffar, *et al*. (2005. Such cases were not observed in this investigation.

Red Blood Cell Count in *Cassia alata*

For red blood cell counts of rats treated with the same extracts of *C. alata*, results of the test is also presented in Table 2. The values generally fall between $8.58x10^6/\mu l$ and $9.40x10^6/\mu l$ for rats treated with 9g/kg body weight of leaves extracts and 3g/kg body weight of water extracts of seed of the plant. Rats treated with Praziquantel® had red blood cell counts of $9.02x10^6/\mu l$ with the least red blood cell count of $7.10x10^6/\mu l$ recorded in the infected non-treated rats. Analysis the results using ANOVA showed that these differences are not significant statistically at $p \leq 0.05$, calculated value Fcal.) of 8.752, tabulated value (Ftab,) of 9.9413, and $\alpha = 0.05$. In this investigation, there were case of low red blood count, but these were not significant and remind the normal range.

Platelet Count in *Cassia alata*

The reults for platelet counts for rats treated with extracts of *C. alata* is shown in Table 2. For rats treated with 3g/kg body weight, the values of the platelet count varied from $853x10^4/\mu l$ for rats in the methanol extracts to $878x10^4/\mu l$ for rats in the water extracts. For those treated with 6g/kg body weight, the highest value of $999x10^4/\mu l$ was recorded in the methanol of the seed of the plant with the lowest value of $837x10^4/\mu l$ in the water extracts of the leaves. Those of

rats treated with 9g/kg body weight remind below $900x10^4$/µl except that of the water extracts of the seeds that had value of $902x10^4$/µl. The value of platelet counts in infected non-treated rats reminded the highest at $1216x10^4$/µl while those in control drug Praziquantel® was $851x10^4$/µl. Analysis of the data by use of ANOVA revealed that these differences are not statistically significant at $P \leq 0.05$, calculated value (Fcal.) of 1.000, tabulated value (Ftab.) of 1.741, and $\alpha = 0.05$.

Platelets are pale blue granular fragments, much smaller than red blood cells (RBCs) shed from multinucleate megakaryocytes in the bone marrow. They maintain the integrity of the endothelium and act as part of the clotting factor during the process of repair of damaged endothelium, where they ensure mechanical strength of the clot and vasoconstriction (Berzigotti *et al.,* 2013 and Frances, 2002). In normal albino rats the platelet count varies from 638 - $1253x10^3$/µl depending on the age and sex of the animal according to Giknis and Clifford (2008), increased platelet counts thrombocytosis occur as a reaction to consumption of toxic drugs, after injury, when large juvenile platelets may also appear; after splenectomy, as splenic stores are liberated to the circulation.

Lymphocyte Count in *Cassia alata*

The percentage of lymphocyte counts are shown in Table 2. The least value of 69% was recorded in rats treated with 6g/kg body weight with water extracts from the leaves of *C. alata* with the other values for both extracts remainning above 70% with the highest value of 76.3% recorded in the extracts from seeds of the groups of rats treated with 9g/kg body weight. The Praziquantel®treated rats had percentage lymphocyte counts of 74.3% followed by 76.3% in the non-infected control groups reaching a value of 87.1% in the infected non-treated rats. ANOVA revealed that these differences are not significant statistically at $P \leq 0.05$, calculated value (Fcal.) of 6.035, tabulated value of 7.552 and $\alpha = 0.0$.

Decreased lymphocyte counts or lymphopenia are usually due to an effect of corticosteroids, either endogenous or stress or Cushing disease or therapeutic, and may also accompany neutropenia in some viral infections, especially the parvoviruses according to the reports by Berzigotti *et al*. (2013) and Frances, (2002The results in this study showed that the values obtained fall below this normal range indicating that there was no variation in value as observed by Abd EL-Mottaleb *et al*. (2008) and Sharma *et al*. (2011). However, many investigators record some changes in the value of the lymphocytes while others such as Essam and Ashraf, (2014); Bugarski *et al*. (2006) linked some of these variations with changes in white blood cell counts.

CONCLUSION

The crude methanol and water extracts from the roots, leaves, stem-bark of *Maytenus senegalensis* as well as that of the leaves and seed of *Cassia alata* showed varying levels of antischistosomal activities on schistosome infected winstar albino rats. Oral administration of the extracts of these plants reduced the number of granulomas and the number of worm load. The results obtained from the haematological and biochemical parameters the treated rats showed that the 6g/kg body weight had a good potential although, the extracts from the roots of *M. senegalensis* at the dose of 9g/kg body weight and above should not be administered as antischistosomal agents due to its haemolytic effects and had side effects on the lungs. The extracts from the stem-bark of *Maytenus senegalensis*had side

effects on the lungs. The extracts from the leaves of *Cassia alata* can cause ulcers and tumours of the intestines of rats. The extracts from the seed of *Cassia alata* can cause hepatomegaly in rats. Therefore any part of *C. alata* should not be used as antischistosomal drug despite some good result because of side effects.

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