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Research Article

Effects of Repeated Doses of Acetone Extract of Siam Leaves (*Chromolena odorata*) on Spermiogram, Sperm Morphology, Haemogram of Albino Rat (Wistar Strain)

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Abstract: This study was aimed at determining the effects of the acetone extract of the *Chromoleana odorata* leaves on male fertility by evaluating some andrological and haematological parameters of the Wistar rat. A total of 20 sexually male Albino haematological Wistar rats were randomly divided into 4 groups. The rats in group A (Control) were administered with 1ml of distilled water, while rats in groups B, C, and D were administered with the extract of 100, 200 and 500 mg per Kg body weight, respectively, once daily for 28days. The result revealed that the rats in the control group (distilled water) result had significantly higher ($p<0.05$) sperm concentration ($72.00 \pm 2.83 + 10^6$cell/ml) compared to group B ($65.10 \pm 2.63 + 10^6$ cell/ml), group C ($53.40 \pm 3.45 + 10^6$ cell/ml) and group D $42.75 \pm 2.39 + 10^6$ cell/ml treated with 500mg/kg with significant decrease in sperm concentration with increase in extract dosage. The rat in group A (control) had significantly higher ($p<0.05$) sperm viability percentage (96.45 ± 0.60) which when compared to group B which has the highest percentage, (92.43 ± 0.55), then a little decrease in group C (80.45 ± 3.75) and group D which has the lowest percentage (77.94 ± 2.56). The sperm motility at the control group (A) (98.53 ± 4.32) is high compared to the other group B, C and D, (95.48 ± 3.83), (83.73 ± 2.94), (75.75 ± 3.63) respectively. The pH value of the semen which vary from the control group (7.14 ± 0.16) to group B which is a little high (7.20 ± 0.13) and group C 7.19 ± 0.15, then group D which has the highest pH value 7.23 ± 0.17.There is significant increase in abnormal sperm morphology with the corresponding increase in the dosage of the extract compared across the groups. The results showed that there was a significant decrease ($p<0.05$) in the levels of Packed cell volume (PCV), Haemoglobin (Hb),white blood cell(WBC), red blood cell(RBC), Mean Corpuscular Haemoglobin Concentration (MCHC), lymphocyte, monocytes, platelets and eosinophil counts. Therefore with the pattern of differences variation observed in the percentage liveability, mortality, sperm concentration and morphological abnormality, it was concluded that long term orally uses of tramadol at both dosage affects the rat sperm indices, therefore should be discourage in a breeder farm and Artificial insemination as it can result in infertility and/or sterility on the herd or flock.

Keywords: *Chromolaena odorata*, Sperm, Albino rat, Haematology.

INTRODUCTION

Ethno-veterinary survey enables us to know the medicinal plant that can be used to treat parasitic disease (Nalule *et al.,* 2003). Medicinal plants had been used in various places for treating cattle diseases such as Kenya. In South Africa, the ethno-veterinary practice including medicinal plants have been shown to be used to treat cough, wounds, skin diseases, mild diarrhea and reproductive disorders (McGaw and Eloff 2008) . The medicinal plants used have been shown to have antibacterial activity (McGaw and Eloff 2008). In Pakistan, 77 ethno-veterinary practice comprising 49 medicinal plants have been used for treatment of parasitic diseases like tick and lice infestation, mange, myiasis and helminthiasis (Farooq *et al.,* 2008). Ethno-veterinary practice has varying aspects such as economic, socio-cultural, biochemical, environmental magico-religious (Wanzala *et al.,* 2005).

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro *et al.,* 2000). Plants for decades have been a valuable source of natural products for maintaining human health, especially with in -depth investigation for their natural therapeutic potentials. According to World

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Health Organization (Santos *et al.*, 1995) several varieties of drugs can be derived from medicinal plants. There is a continuous and urgent need to discover new antimicrobial compounds with diverse mechanisms of action and chemical structures that can be used against novel and re-emerging infectious diseases (Rojas *et al.*, 2003). Medicinal plants and herbs are of great importance to the health of individual and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant species has been studied for their application. However, in the recent past, an increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers (Zhang, 1976). The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional plant as potential sources of new antimicrobial agents (Bonjor *et al.*, 2004). Therefore, researchers are increasingly turning their attention to complementary medicine looking for new ways to develop better drugs against microbial infections (Benkeblia, 2004). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized as secondary metabolites of plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen *et al.*, 1987) as well as tannin (Saxena *et al.*, 1994). Medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Daniel, 1999). Secondary metabolite (also called specialized metabolites) is a term for pathways and small molecule products of metabolism that are not absolutely required for the survival of the organism, many of which are antibiotics and pigments. Plants synthesize varieties of phytochemicals such as alkaloids, phenolics, terpenoids, glycosides etc.The quest for plants with medicinal properties continues to receive attention as scientist survey plants, particularly of ethno-botanical significance, for a complete range of biological activities, which range from antibiotic to antitumor. Thus, plants have provided western medicine with an abundance of drugs and treatment for a variety of health problems (Lewis and Elvin-Lewis, 1977); (Bruneton, 1999). One of the great advantages of these medicinal plants is that they are easily available and have moderate side effects (Mehta, 1982). The use of traditional medicines holds a great promise as a easily available source as effective medicinal agents to cure a wide range of ailments in animal particularly in tropical developing countries. In this context, the people consume several plants or plant derived formulations to cure helmintic infections (Satyavati, 1990) and treatment of wounds (Raina *et al.*, 2008) among the therapeutic important of the plant. Also the crude protein content of leaves as prompt some researcher to use the plant as feed inclusion not minding the reproductive toxic effect. Therefore the utilization of the plant extract as a feed inclusion and various reported therapeutic uses call for concern.

MATERIALS AND METHOD

Experimental site

The experiment was carried out at Teaching and Research Farm, Oyo State College Of Agriculture and Technology Igbo-ora, in the derived savanna zone of Nigeria. The college is located on longitude 70.15 north and 30.30 east of the equator with average annual rainfall 1278mm and average temperature of 270c (Sanusi 2011).

Experimental animals' management

A total of twenty (20) matured male albino rats (wistar strain) of about 175±25g body weight were used for this study. These rats was acclimatized for two weeks in the cage house. The animals were fed ad *libitum* with concentrate feed of TOP FEEDS® Lagos, Nigeria and water was also given *ad libitum.*

Experimental Design

Rats were allowed to acclimatize for two weeks, after which they were divided into four groups (A, B, C and D) of five rats per group. Group (A) serve as control, given distill water, while other groups (B, C and D) serve as experimental groups and were treated with graded oral doses of 100mg/kg, 200mg/kg, and 500mg/kg of acetonic extract of *Chromolaena Odorata* in group B,C and D respectively and they were treated for 14days.

Restraint for Administration of Extracts

The rats were grasped by the loose skin of the neck with the tail held between the palm and little finger then the chirring was administered.

Plant Collection and Extraction

The *Chromolaena odorata* is collected in OYSCATECH premises and identification was done by expert botanist. After which the leave is air-dry, then grind into powdery form. Extraction was done by

- Soaking the leave at ratio 1g:10ml (powdery form of *Chromolaena odorata* to acetone) for 48hour (2days)
- Filtration was done using hartman's filter paper
- Drying of the filtrate was done using Rotary Evaporator. The pasty extract was collected and weighed

Sperm collection and analysis

After euthanasia, sperm was collected from the epididymis as describe by Akusu *et al.*,(1985) and Oyeyemi and Ubiogoro (2005)

- Sperm motility was assess by the method described by Zemjanis (1977)
- Sperm concentration was also assessed

- pH of the sperm was also assessed
- Morphological abnormalities and percentage viability assay

These was determined from a total count of 400 spermatozoa in smears obtained with Wells and Awa stains

Haemogram profiling; Haematology of the blood samples were carried out in order to detect the variation in the RBC and the WBC counts. Total white blood corpuscles (WBC), total red blood corpuscles (RBC) and platelet counts were made by using an improved Neubauer's haemocytometer .The haematocrit values, Haemoglobin concentration, Mean Corpuscles Volume (M.C.V.), Mean Corpuscles Haemoglobin (M.C.H.) and Mean Corpuscles Haemoglobin Concentration (M.C.H.C) were recorded. The packed cell volume (PCV) was determined as described by Schalm *et al.,* (1975) in which the packed cell volume is expressed as a percentage volume of blood, which is occupied by red blood cells.

Data Analysis;

The data obtained was evaluated by one way ANOVA by the use of Graph Pad Instat version 3 software. All results were expressed as mean ±.S.E.M (standard error of means) P<0.05 was accepted as significant in this study.

RESULT AND DISCUSSION

Observing the rats in the control group (distilled water) result has the highest sperm concentration 72.00 ± 2.83 + 10*6 cell/ml compared to that of group B 65.10 ± 2.63 + 10*6 cell/ml, treated with 100mg/kg, also higher compared to group C 53.40 ± 3.45 + 10*6 cell/ml, treated with 200mg/kg, also higher compared to the concentration of group D 42.75 ± 2.39 + 10*6 cell/ml treated with 500mg/kg. when observing the sperm viability, the group A (control) was a little high in percentage (96.45 ± 0.60) which when compared to group B which has the highest percentage, (98.43 ± 0.55), then a little decrease in group C (80.45 ± 3.75) and group D which has the lowest percentage (77.94 ± 2.56).

The sperm motility at the control group (A) 98.53 ± 4.32 is high compared to the other group B, C and D, 95.48 ± 3.83, 83.73 ± 2.94, 75.75 ± 3.63 respectively. The pH value of the semen which vary from the control group (7.14 ± 0.16) to group B which is a little high (7.20 ± 0.13) and group C 7.19 ± 0.15, then group D which has the highest pH value 7.23 ± 0.17. The sperm characteristics and morphology of Albino wistar rat treated with acetonic extract of *Chromolaena odorata* was shown in the table 1.

The sperm concentration, viability (live/dead ratio), motility morphology and semen pH was significantly (p<0.05) difference when compare within the group in an increase in dose-related phenomenon. Although there is no significant different (p>0.05) in comparing between the control group and 100mg/kg dose group in all the sperm characteristic and morphology. the haematological parameters of male albino rats for 21 days are shown in Tables 1, and 2. Administration of the plant extract at various doses (100, 200 and 500mg/kg body weight) produce significant change (P<0.05) in the RBC and factors relating to it (Hb, PCV, MCV, MCH and MCHC). Extract administration also produced significant decrease (P<0.05) in WBC counts. Interestingly, administration of the plant extract produced significant alterations in the platelets, neutrophils and lymphocytes. The plant extract used while administration at higher doses resulted in significant decrease (P<0.05) in the blood parameters. The percentage lymphocytes increased significantly (P<0.05) across the experimental group for all the doses investigated.

Table1: Sperm characteristic of male albino wistar treated with acetonic extract of *Chromoleana odorata* (mean ± sem)

Parameters	Control	100mg/kg	200mg/kg	500mg/kg
Sperm concentration 10^6cell/ml	72.00±2.83	65.10±2.63*	53.40±3.45*	42.75±2.39*
Sperm viability (%)	96.45±0.60	98.43±0.55	80.45±3.75*	77.94±2.56
Sperm motility (%)	98.53±4.32	95.48±3.383	83.73±2.94*	75.75±3.63*
pH of semen	7.14±0.16*	7.20±0.13*	7.19±0.15*	7.23±0.17
Sperm Morphology	90.64±2.14	86.52±4.38	75.18±4.38*	68.73±1.87*

Table 2: Effects of acetonic extract of *Chromolaena odorata* son haematological parameter of albino rats

PARAMETER	CONTROL	100MG/KG	200MG/KG	500MG/KG
PCV (%)	48.3±0.3	44±0.6	46±0.55	33.0±0.67*
RBC (10^6/mm^3)	8.5±0.7	8.8±0.4	7.6±1.4	5.2±0.7*
WBC 10^3/mm^3	10.3±0.1	9.4±0.3	5.6±00*	4.56±0.12*
Haemoglobin (g/dl)	16.3±0.5	15.1±0.2	13.7±0.6	11.4±0.4*
M.C.V	56.8	50.0	60.0	63.5
M.C.H	19.2	17.2	18.0	21.9
M.C.H.C	33.7	34.3	29.8	34.5

Table3: Differential Leucocyte count of acetonic extract of *Chromolaena odorata* son haematological parameter of albino rats

NEUTROPHIL	18.26±0.19	18.20±0.43	18.02±0.37	18.27±0.48
Lymphocyte	73.4±0.15	73.25±0.14	70.34±0.11*	66.25±0.19*
Monocytes	5.58±0.60	5.50±0.4	7.63±0.41*	7.83±0.12*
Eosnophils	2.93±0.60	3.05±0.70	4.20±0.32	4.38±0.08
Basophils	0.5±0.32	0.45±0.15	0.70±0.63	0.60±0.17

Plants have been used over the years not only for their nutritional value but for their therapeutic potential. Therefore, the increasing consumption of crude extracts or active constituents could warrant investigation into their safety or toxicological implications on fertility and reproduction. In this study, the sperm count was observed to have reduced significantly ($P<0.05$) which is an indication that the acetone extract of *Chromolena odorata* reduced or inhibited spermatogenesis. This is similar to what was observed in some medicinal plants with detrimental effects on male fertility such as *Carica*papaya and *Quassia amara*. *C. papaya* was reported by Chinoy and Padman (1996) to have anti fertility effect by reduction of testicular mass, sperm count and sperm motility when the benzene extract of the seeds was administered to male albino rats. The chloroform extract of the bark of *Q. amara* has been shown to decrease sperm count, motility and viability in albino rats (Parveen *et al.*, 2003), while on the contrary, aqueous extracts of root, leaf, or whole plant of *Withania somnifera* Dunai is known to increase sperm count (Abdel-Magnied *et al.*, 2001). Phthalate esters, which were suspected to have originated from medications given to some patients, have been implicated in the decline of sperm count (Hauser *et al.*, 2004).

Abundance of spermatozoa in the seminiferous tubules, which is an indication of spermatogenesis, is maintained quantitatively by both testosterone and FSH. Therefore, reduction in sperm count and density and the increased number of malformed sperm cells could be attributed to the reduced testosterone content by the extracts. Furthermore, the reduction in the seminiferous tubular sperm cell motility may be connected to the reduced glycogen and sialic acid contents because sperm cells deprived of an adequate supply of energy and increased frictional force will be sluggish. Reduced numbers of spermatozoa, insufficient motility, and/or increased number of malformed spermatozoa are the leading causes of disturbed fertility or infertility in animals (Chauhan and Dixit, 2008).According to the classification by Noarkes *et al.*, (2004), sperm abnormality in the present study was aberrations of spermatogenesis, and that the plant extract interfered with the maturation stage of spermatogenesis in the seminiferous tubules or that the abnormal cells matured from damaged seminiferous tubules (Hafez, 1987).

The blood is a vital fluid, which contains the Red Blood Cells (RBC's), White Blood Cells (WBC's) and platelets suspended in the serum in homeostatic concentrations. The circulatory blood volume makes up about 8% of the weight of an average animal. The blood cells take up about 45% of the blood, while plasma constitutes about 55 % (Guyton and Hall, 2000).

The blood is important for pulmonary and tissue respiration, as a medium of endocrine and neuro-humoral transmissions, biotransformation and metabolic excretion, (Adebayo *et al.*, 2005) nutritional and immunological processes, as well as homeostatic responses (Oze, 1992). Reactive oxygen species have been implicated in the mechanism of damage of red blood cells in diabetic patients (Rice-Evans *et al.*, 1986; Corrons *et al.*, 1995; Rao *et al.*, 2003). As a result, haematological complications develop which consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (Comazzi *et al.*, 2004).

Packed Cell Volume measures the percentage by volume of packed RBC's in a whole blood sample after centrifugation. The significant decrease in the level of packed cell volume (PCV) in plant extract treated rats (Table 5) may be as a result of the cellular damage on the erythrocyte membrane as a result of oxidative stress. The rats treated with varying doses of *Chromolena odorata* leaves extract also showed significant decrease ($p<0.05$) in values of Hb, WBC, RBC and platelet when compared to normal rats. Haemoglobin test measures the amount of HB in grams in 1 dl of whole blood and provides an estimate of oxygen carrying capacity of the RBC's. Red blood cell counts can be a factor in erythropoietin process.

Moreover, white blood cell count can indicate that there is a disease or condition affecting white blood cells, but it cannot determine the underlying cause. It is also related to the immune system and bone marrow. Platelet count is used to diagnose and/or monitor bleeding and clotting disorders. Since, a marked reduction was observed in these aforementioned parameters this may be an indication of anaemia in *Chromolena odorata* leaves extract treated rats. White blood cell differentials are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes. Neutrophils attack and destroy bacteria in the blood (Dacie and Lewis, 1995).

The finding suggests that the acetone extracts of *Chromolena odorata* leaves may cause haematopoietic dysfunction and safety or toxicological implications on fertility and reproduction when used on a prolonged time.

CONCLUSION

In conclusion, the decrease in red blood cell, packed cell volume (PCV or haematocrit), total white blood cell, platelet and lymphocyte counts following administration of the acetone extracts of *Chromolena odorata* leaves may signify the negative effects of extract on the haemopoietic system of experimental rats and might be capable of causing the hematological abnormalities associated with pathophysiology of various blood disease and toxic effect of *Chromolaena odorata* leaves on the sperm cells was as a result of degeneration of seminiferous tubules. Which is an indication that the prolonged administration of this extract will induce infertility in the male.

Recommendation:

It is established facts that prolonged administrations of acetone extracts of *Chromolena odorata* leaves on rats affect the blood and sperm cells adversely, therefore the use of the herbal preparation of the plant extract should cautiously use for any its important role earlier reported and in particular to be cautiously used in animal meant for breeding.

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