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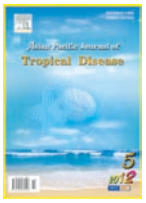

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Impact of *Ganogerma applanatum* extraction on haematological profiles of laboratory rats: a preliminary study in Nigeria

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ABSTRACT

Objective: Extracts of *Ganoderma* species have been widely used as herbal medicines in the treatment of several infections. This study was carried out to ascertain the haematological properties of aqueous *Ganoderma applanatum* (*G. applanatum*). **Methods:** Sixty albino rats grouped into six equal groups (10 each) of A to F consisting of tests and controls. Laboratory albino rats in groups A, B and C were infected with *Trypanosoma brucei brucei (T. brucei brucei)* while groups A and B (test) were treated with aqueous *G. applanatum* extract; other groups served as control. Microscopy and haematological profiles from the albino rats were monitored on daily basis for blood parasites, packed cell volume (PCV), haemoglobin concentration (HC), total red blood cell count (RBC), mean cell haemoglobin (MCH), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), and total white blood cell count (WBC). **Results:** Albino rats in groups A, B and C infected with *T. brucei brucei* and treated with various concentrations of aqueous *G. applanatum* showed a progressive reduction in PCV, HC, RBC, MCH and MCHC compared to the controls ($P<0.05$). All the infected rats died by day 14 of the experiment from parasitaemia. **Conclusions:** *G. applanatum* lacks ability to boost haematological profiles of anaemic laboratory rats and also of no use in the treatment of African trypanosomiasis. Higher doses of the fungal extract may be required to test on laboratory rats with less lethal biological stimulants of anaemia before proving or otherwise its true haematological properties.

1. Introduction

Ganoderma species belong to the genus *Basidiomycete* of the higher fungi. It has a global distribution as taxonomists have traced its pan-continental presence for several centuries in the past[1-3]. Its medicinal value is also not new as it can probably be traced to the Greek, Medieval, Persian as well as the rich Chinese herbal medicine dating as far back as 2500 BC[4-6].

Ganoderma has a unique double walled basidiospore with a shining skin. Some of the active compounds identified in the cell wall of the mushrooms include protein-bound polysaccharides or long chain glucose[7-9]. These compounds along with probably others have been found useful in the treatment of malignancies such as leukaemias as well as immunodeficiency states. Similarly extracts of *Ganoderma lucidum* specifically has been found useful in the treatment of viral, bacterial as well as some parasitic infections and infestations[10-12].

In Nigeria as well as several other parts of Sub-Saharan Africa, the pharmacologic potential of *Ganoderma* species appear to be grossly underutilized even in its crude form as there is little available literature on its activity[13-15]. This study was therefore set up to ascertain the haematological impact of *Ganoderma applanatum (G. applanatum)* on albino rats induced anaemia[16-18]. The findings would be useful as preliminary information when more attention is eventually drawn to exploit the medicinal benefits inherent in the fungus.

2. Materials and methods

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2.1. Study area and setting

The study was carried out in Vom about 25 kilometres south-east of Jos, the Plateau state capital in north-central Nigeria. In Vom, it was sited in the Federal College of Veterinary and Medical Laboratory Technology, and National Veterinary Research Institute where the study was carried out. Experimental rats were obtained from Nigerian Institute for Trypanosomiasis Research (NITR), Vom. The rats were kept in laboratory cages, fed with commercially prepared feeds (vital feed) and allowed to acclamatise for four weeks. Blood samples were then collected from the tail vein on a microscope slide and examined under the microscope to exclude the presence of trypanosomes. Also *Trypanosoma brucei brucei(T. brucei brucei)* infected laboratory rats were obtained from NITR, Vom which supplied *Trypanosoma* species for the study.

2.2. Ganoderma applanatum extraction

One kilogram of the powder of *G. applanatum* was dissolved in three litres of distilled water. The sample was boiled for three hours, stirring every thirty minutes. It was then allowed to stand for 24 hours and then filtered using Whatman number 1 paper. The filtrate was evaporated to dryness in hot air oven set at 45 ℃, the extract obtained was reconstituted using sterile distilled water to obtain concentrations 500 mg/mL and further diluted to obtain 250 mg/mL[19].

2.3. Source of T. brucei brucei

Albino rat as parasite donor was obtained from NITR, Vom. About 0.5 mL blood was collected from the parasite donor rat and diluted (50:50) with normal saline. A drop of the diluted blood was examined under the microscope to ensure that there was presence of the parasites. The parasitaemia examined was on the average of 5/field. About 0.1 mL of the diluted was used for injecting the infected group of albino rats intraperitoneally[20].

2.4. Rat groupings

Sixty rats were used in the study and were grouped into six with 10 rats in each group. Group A: rats infected and treated with 250 mg of aqueous *G. applanatum* extract/body weight of the rats. Group B: rats infected and treated with 500 mg of aqueous *G. applanatum* extract/body weight of the rats. Group C: rats infected and not treated with *G. applanatum* extract. Group D: rats uninfected but treated with 250 mg of aqueous *G. applanatum* extract/body weight. Group E: rats uninfected but treated with 500 mg aqueous *G. applanatum* extract/body weight. Group F: rats uninfected and untreated.

T. brucei brucei was used to induce anaemia in albino rats infected with the parasites. Group C served as positive control while group F served as negative control.

2.5. Blood sample collection

Rats used in the study were bled through the ocular vein into Ethylene diamine tetra-acetic acid (EDTA) bottles. The samples were analysed immediately in Haematology and Microbiology Laboratories of Federal College of Veterinary and Medical Laboratory Technology, Vom.

Estimation of haematocrit packed cell volume (PCV)-blood was collected using capillary tubes (length of 75 mm and diameter of 1 mm) by capillary action, leaving 15 mm unfilled. The tubes were sealed by flaming and spun in a microhaematotocrit centrifuge at 1 200 g for 5 minutes. PCV was then measured using haematocrit reader[21].

2.6. Haemoglobin estimation

A 1: 250 dilution of blood was made by adding 0.02 mL of blood to 5 mL of Drabkins solution in a test tube. This was mixed and allowed to stand for 5 minutes, for complete conversion. The test was read colorimetrically at a wavelength of 540 nm.

2.7. White blood cell count (WBC)

A 1:20 dilution of blood was made by adding 0.02 mL of blood to 0.38 mL of Turks solution in a 75 mm×10 mm plastic tube. After tightly corking the tube the suspension was well mixed by rotation. The improved Neubauer counting chamber was loaded with the diluted blood by means of pasteur pipette. The loaded counting chamber was allowed for two minutes for cells to settle, after which the preparation was viewed under the microscope ×10 mm objective. The cells were counted in the 4 large corner squares of the counting chamber. The calculation of total white blood cells was made using the first principle[22].

2.8. Red blood cell count (RBC)

A 1:200 dilution of blood was made in formol citrate solution by diluting 200 mL of blood into 4 mL of diluents in a plastic tube. A clean dry improved Neubauer counting chamber with cover slip already in position was loaded with diluted blood using pasteur pipette. The chamber was left undisturbed for 2 minutes for the cells to settle. The cells were counted under the microscope using ×10 mm objective. Cells were counted in 80 small squares in the central ruled area of the counting chamber[22].

2.9. Data analysis

Data obtained was analysed using simple descriptive methods of arithmetic mean, mode and standard deviation (SD) as well as Epi Info statistical software 2006 version.

3. Results

Albino rats in groups A, B and C infected with *T. brucei brucei* and treated with various concentrations of aqueous *Ganoderma applanatum* showed a progressive reduction in PCV, HC, RBC, MCH and MCHC compared to the controls, (P<0.05) (Table 1, 2, 3, 6 and7), [Table 1: Group A X^2(Mantel-Haenszel)=7.73, OR=1.28-4.63, RR= 1.23-3.18, P= 0.0034, Group B X^2(Mantel-Haenszel)=6.32, OR=1.17-4.11, RR=1.15-2.87, P=0.011, Group C X^2(Mantel-Haenszel)=5.47, OR=1.11-3.77, RR=1.10-2.65, P=0.01; Table 2: Group A X^2(Mantel-Haenszel)=4.77, OR=1.02-4.09, RR=1.03-2.25, P= 0.02, Group B X^2(Mantel-Haenszel)=5.50, OR=1.08-4.47, RR=1.06-1.78, P=0.01, Group C X^2(Mantel-Haenszel)=7.15, OR=1.20-5.11, RR=1.13-2.67, P=0.007; Table 3: Group A X^2(Mantel-Haenszel)=112.11, OR=2.32-3.43, RR=1.68-2.17, P=0.0000, Group B X^2(Mantel-Haenszel)=23.65, OR=1.98-5.63, RR=1.30-1.82, P=0.0000, Group C X^2(Mantel-Haenszel)=23.65, OR=1.98-5.63, RR= 1.30-1.82, P=0.0000; Table 6: Group A X^2(Mantel-Haenszel)= 7.46, OR=1.16-2.86, RR=1.09-1.82, P=0.006, Group B X^2(Mantel-Haenszel)=5.61, OR=1.07-2.60, RR=1.05-1.70, P= 0.01, Group C X^2(Mantel-Haenszel)=12.37, OR=1.39-3.57, RR=1.16-1.65, P=0.0004 and Table 7: Group A X^2(Mantel-Haenszel)=23.36, OR=1.43-2.39, RR=1.23-1.65, P=0.0000, Group B X^2(Mantel-Haenszel)=28.36, OR=1.53-2.61, RR= 1.28-1.75, P=0.0000, Group C X^2(Mantel-Haenszel)=22.75, OR=1.40-2.37, RR=1.21-1.62, P=0.0000].

Table 1
Packed cell volume of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	47.40±0.28	46.00±0.22	46.60±0.35	36.30±1.36	31.90±1.24	36.00±0.35
7	23.40±0.18	21.70±0.23	19.00±1.6	29.60±1.92	16.10±0.52	14.80±0.91
14	Died	Died	Died	34.60±1.04	46.10±0.40	44.00±0.07

Table 2
Haemoglobin concentration (g/dL) with mean±SD of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	13.30±0.57	13.60±0.10	12.80±0.68	10.10±0.35	9.40±0.27	10.60±0.16
7	7.30±0.61	5.80±0.60	5.10±0.42	8.50±0.56	4.30±0.26	4.10±0.28
14	Died	Died	Died	11.30±0.42	14.10±0.64	12.60±0.14

Table 5
Mean cell volume of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	67.10±3.84	65.50±1.95	69.00±1.57	69.80±1.30	66.30±2.75	69.20±0.42
7	66.9±6.3	63.70±0.64	66.40±4.85	72.20±8.66	77.10±4.96	75.00±1.41
14	Died	Died	Died	75.90±5.05	76.90±3.28	71.90±6.57

There was no significant change in MCV and WBC among the treated infected rats compared to the controls (P>0.05) (Table 4 and Table 5), [Table 4: Group A X^2(Mantel-Haenszel)=0.04, OR=0.20-6.95, RR=0.49-2.38, P=0.84, Group B X^2(Mantel-Haenszel)=0.11, OR=0.29-5.46, RR=0.57-2.21, P=0.73, Group C X^2(Mantel-Haenszel)=0.13, OR=0.27-6.20, RR=0.55-2.35, P=0.719, Group D X^2(Mantel-Haenszel)=4.66, OR=0.48-0.98, RR=0.61-0.98, P=0.03, Group E X^2(Mantel-Haenszel)=4.66, OR=0.48-0.98, RR=0.61-0.98, P=0.03; Table 5: Group A X^2(Mantel-Haenszel)=0.00, OR=0.61-1.69, RR=0.79-1.20, P=0.951, Group B X^2(Mantel-Haenszel)=0.02, OR=0.62-1.72, RR=0.80-1.30, P=0.901, Group C X^2(Mantel-Haenszel)=0.03, OR=0.63-1.73, RR=0.80-1.30, P=0.854]. Albino rats in group A, B and C all died from day 12 to day 14 (Table 1-7).

Table 3
Total red cell counts with mean±SD of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	5.91±1.65	6.78±0.55	6.62±0.51	5.01±1.57	4.80±1.37	5.21±0.49
7	2.10±3.39	3.42±3.66	2.87±2.41	4.23±3.10	2.15±1.37	1.97±0.25
14	Died	Died	Died	4.99±1.18	5.99±0.94	6.36±0.83

Table 4
Total white cell count of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	6.93±2.24	9.70±3.33	8.80±1.03	6.83±0.45	6.83±0.45	6.85±0.49
7	6.55±2.84	8.10±2.40	7.40±3.36	4.83±2.80	6.20±1.05	3.55±2.33
14	Died	Died	Died	10.33±0.24	10.96±2.46	6.90±0.35

There was no significant change in haematological profiles tested among albino rats uninfected with *T. brucei brucei* but treated with *G. applanatum* in group E similar to those in group F who were neither infected nor treated with the *G. applanatum* extract during the study period (P>0.05) (Table 1-7).

The total white cell count of rats in groups D and E which were uninfected but treated with *G. applanatum* increased significantly at day 14 when the experiment was terminated, (P< 0.05) (Table 4).

The experiment was terminated at day 14 when all the test rats and those ones in positive control died.

Table 6
Mean cell haemoglobin of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	20.00±1.15	19.30±0.64	20.10±1.66	19.90±1.20	18.80±1.80	25.90±2.02
7	11.40±0.57	10.80±2.69	9.00±1.31	20.10±2.88	20.40±1.01	20.70±0.85
14	Died	Died	Died	22.10±3.61	23.70±2.78	20.55±2.93

Table 7
Mean cell haemoglobin concentration of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	299.00±15.58	292.80±13.15	312.50±47.78	286.30±18.01	284.00±33.87	303.50±31.82
7	162.00±15.56	146.50±41.72	173.00±6.24	279.00±19.08	266.30±11.93	278.50±3.54
14	Died	Died	Died	291.00±42.32	308.00±14.53	286.00±1.42

4. Discussion

T. brucei brucei (federe) is a tissue parasite which induces anaemia in infected rats and other susceptible animals such as cattle, dogs and mice[23-25]. This manifested in the fall in PCV, MCH, MCHC and total red cell counts among the test animals and the positive controls in the present study ($P< 0.05$). Although the bleeding intervals of the rats may also have affected the haematological parameters, the non-significance of this effect as seen in the negative controls stresses the negligible effect on the overall result.

WBC showed no significant decrease in rats infected with *T. brucei brucei* and treated with *G.*applanatum ($P>0.05$) but significant decrease in infected but untreated rats ($P<0.05$). This is in line with immunopotentiation and immunomodulatory properties severally attributed to *Ganoderma* species which have found wide clinical applications in the management of malignancies and immunodeficiency states[26-28].

All the test and control rats infected with *T. brucei brucei* died between day 12 to day 14 primarily due to overwhelming parasitaemia and probably secondary anaemia. This points to the fact that the *Ganoderma* extracts had no therapeutic effect on *T. brucei brucei* contrary to its established antibacterial, antiviral, antimycotic and other anti-infectious applications[29,30]. Higher doses may still need to be tried to ascertain the true usefulness or otherwise of this fungus in the management of Trypanosomiasis.

The full impact of aqueous *Ganoderma* species extract on the haematological profiles of rats in the present study which was originally designed to last for a minimum of 28 days was terminated on day 14 when all the test animals died about midway into the test period. The healthy appearance and agility of all uninfected rats equally treated with aqueous *Ganoderma* species at day 14 and beyond implies all the test rats did not die from *Ganoderma* toxicity[31,32].

It is indeed our candid view that the effect of extract of this fungus on haematological parameters would probably have been more pronounced and conclusive had the rats survived the infection beyond day 14 up to 28th day. Further studies using less lethal biological agents to induce anaemia in rats is therefore required to fully study the haematological properties of *Ganoderma* species. The fact that the haematological parameters of uninfected rats picked up by day 14 further strengthens this view[33-35].

In conclusion, *G. applanatum* extracts failed to correct anaemia induced by *T. brucei brucei* in rats and also failed to kill the parasites, although all the test animals died midway into the period of experiment. Higher concentrations of aqueous *Ganoderma* species extract may therefore be tried to fully establish the activity of the fungus or otherwise in this regard[36,37]. Similarly, its level of bioavailability in rats should be assessed to ascertain its suitability as a potential candidate drug for the treatment of haemoparasites such as African trypanosomiasis as well as its ability to boost blood parameters.

Conflict of interest statement

We declare that we have no conflict of interest.

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