

Effects of Nigerian red propolis in rats infected with *Trypanosoma brucei brucei*

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Abstract Propolis has a long history of use in folk medicine and possesses various biological activities. Effects of the methanol extract of Nigerian red propolis (NRP) in trypanosome-infected rats were studied. Mature albino Wistar rats (30) were randomly grouped into six (A–F). Groups A–E were infected with 1.0×10^6 *Trypanosoma brucei brucei* organisms intraperitoneally. Group F was uninfected. On establishment of infection, groups A–C were treated orally with NRP at 600, 400 and 200 mg/kg body weight for five consecutive days while group D received diminazene aceturate at 7 mg/kg body weight intraperitoneally. Group E received equal volumes of DMSO solution for five consecutive days. Parameters monitored were mean group parasitaemia, packed cell volumes (PCV), haemoglobin concentrations (Hbc) and bodyweight changes. By day 16 post-infection (PI) parasitaemia was significantly higher in the DMSO control group E than in the NRP-treated groups. Mean group PCV, Hbc and weight loss were significantly ($p < 0.05$) lower in DMSO control group when compared with all the other experimental groups. Rats treated with NRP (600 and 400 mg/kg) had significant ($p < 0.05$) reduction in

parasitaemia (day 16 PI), higher PCV, Hbc and weight gain than the DMSO control.

Keywords Bioactivity · Nigerian red propolis · *Trypanosoma brucei brucei* · Diminazene aceturate · Rats

Introduction

Propolis is a resinous substance collected by bees mainly from plants around their hives to reinforce their combs and to seal the unwanted openings in their hives. Composition of propolis varies with source but in general, propolis contains 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% of other substances including organic debris (Sha et al. 2010). Propolis has a long history of use in folk medicine, and it has been reported to possess various biological activities such as anticancer (Burdock 1998), antioxidant (Bankova 2010), anti-inflammatory (Zhang et al. 2014), antifungal (Brito and Chaves 2010) and anti-HIV activities (Ito et al. 2001).

Over 200 compounds have been isolated from propolis samples from around the world (Huang et al. 2014). Studies have shown that compounds in propolis originate from plant exudates, secreted substances from bee metabolism and materials produced during propolis elaboration (Marcucci 1995). Most of the biological activities have been found in the water or polar (ethanol/methanol) extracts, which contain mainly flavonoids, cinnamic acid and polyphenolic compounds (Popova et al. 2007). Ten phenolic compounds were isolated from a similar sample of NRP (from Bonny, Rivers State, Nigeria) by Omar et al. (2016). The compounds were tested in vitro against a wild type of *Trypanosoma brucei* and two pentamidine resistant strains, and they displayed moderate to high activity. Cytotoxicity assay of a methanol

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extract of propolis dissolved in DMSO was done by Altwaijry (2014) using non-differentiated U937 cells (human leukaemia cell line, monocytes). The result showed that the extract was non-toxic at 100 µg/ml.

African trypanosomosis is a tsetse-borne parasitic disease caused by flagellated protozoa of the genus *Trypanosoma*. Large populations of animals are at risk of debilitating infections with trypanosomes in Nigeria. Animal trypanosomosis is a major obstacle encountered in livestock production in Nigeria since it causes reduction in milk, weight gain, reproduction and eventually death of the affected animals (Hoet et al. 2007). Yearly death of about 3 million cattle has been attributed to trypanosomosis (Ogbadoyi et al. 2007). There is an annual loss of about US\$4.75 billion due to AAT (DFID 2001).

Drugs used as veterinary trypanocides are diminazene aceturate, homidium, quinapyramine and isometamidium (Brun et al. 2001). Most of these drugs have been in use for more than half a century (Hoet et al. 2004) and are chemically related (Bizimana et al. 2006) thereby increasing the risks of parasite resistance. New trypanocides that are safe and cost-effective are urgently needed.

This study reports the effects of Nigerian propolis in rats infected with *Trypanosoma brucei brucei*.

Materials and methods

Extraction and isolation of constituents

The propolis sample used in this work was obtained from Bonny, Rivers State, Nigeria in April 2014. Dried, ground propolis (150 g) was extracted successively with analytical grade hexane, ethyl acetate and methanol (600 ml) 72 h each. The extracts were filtered and the solvents evaporated.

Experimental animals

Thirty adult male albino rats (81.8–110.6 g) were used for the study. They were randomly allotted into 6 groups of 5 rats each and were kept in metal cages in the Laboratory Animal House of the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were housed under normal environmental conditions of temperature (25 ± 3 °C). There was an acclimatization period of 7 days before the start of the experiment. They were fed pelleted grower's mash containing 14.5% crude protein (Vital Feeds, Grand Cereals and Oil Mills Ltd. Jos, Plateau State, Nigeria) throughout the course of the study. Rats received feed and water ad libitum and were humanely cared for in accordance with the principles of laboratory animal care (NAS 2011).

Trypanosomes

The trypanosome parasites used in this study were obtained from a naturally infected hunting dog presented at the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The parasites were identified morphologically (Soulsby 1982) and by the blood incubation infectivity test (BIIT) (Rickman and Robson 1970). A donor mouse was first infected with the field isolate and from it experimental rats were infected.

Infection of experimental animals

Blood from the donor mouse was obtained from the retrobulbar plexus via the median canthus of the eye. Infected blood was put into vacutainer tubes containing ethylenediaminetetraacetate (EDTA). Degree of parasitaemia was estimated using the rapid matching technique of Herbert and Lumsden (1976). Infected blood was diluted with phosphate buffered saline (PBS) to give a final concentration of 1×10^6 trypanosomes in 0.4 mL which was used to infect each rat intraperitoneally.

Nigerian red propolis stock solution

The bulk methanol extract of NRP required for treatment at 600, 400 and 200 mg/kg body weights of rats for 5 days was calculated and dissolved separately in 1 mL of DMSO initially and then with 4 mL of distilled water to obtain 1 mL doses which were administered daily for five consecutive days per os. For rats in group E, 1 ml of DMSO was diluted with 4 mL of distilled water and each rat received 1 mL of it for 5 consecutive days per os.

Experimental design

Rats in groups A–E were infected while those in group F were the uninfected control. Treatments were carried out 7 days post infection (PI). Three groups of rats (A, B, C) were treated with NRP at 600, 400 and 200 mg/kg body weights via oral gavage, respectively, for 5 consecutive days. Rats in group D were treated once with diminazene aceturate at 7 mg/kg body weight intraperitoneally (according to manufacturer's instruction for treatment of *T. brucei*) while those in group E received 1 ml of DMSO solution daily for 5 days per os.

Parameters for assessing efficacy of treatment

Efficacy of treatment was assessed by monitoring parasitaemia using the rapid matching technique of Herbert and Lumsden (1976) packed cell volume (Thrall and Weiser 2002) haemoglobin concentration (Higgins et al. 2008) changes in weight gain and survivability.

Statistical analysis

Data obtained were computed into means and analysed using one-way analysis of variance (ANOVA). The means were separated at post hoc using Duncan’s multiple range test at 95% confidence interval.

Results

Extraction

The methanol extract was a chocolate brown sticky solid.

Parasitaemia

The result of parasitaemia in infected rats following treatment with graded doses of propolis is shown in Fig. 1. Average prepatent period was 5 days. Parasitaemia increased steadily until day 7 PI when rats in groups A–D were treated. Three-day post-treatment parasitaemia was cleared in the diminazene aceturate-treated group D, resurfaced day 14 PI before complete clearance by day 16 PI in that group. There were no significant differences in parasitaemia between the 400 and 600 mg/kg NRP-treated and DMSO control groups until day 16 of the experiment when parasitaemia was significantly ($p < 0.05$) higher in the DMSO control group E.

Packed cell volume

Packed cell volumes of rats in the different experimental groups are shown in Fig. 2. By day 9 PI, mean group PCV of DMSO control rats was significantly ($p < 0.05$) lower than that of the uninfected rats but when compared with the treated groups, there was no significant difference. By day 13 PI, mean group PCV was significantly ($p < 0.05$) lower in the DMSO control rats than in those of the 600 and 400 mg/kg body weight NRP-treated and 7 mg/kg diminazene aceturate-treated groups. By day 16 PI, there was no significant

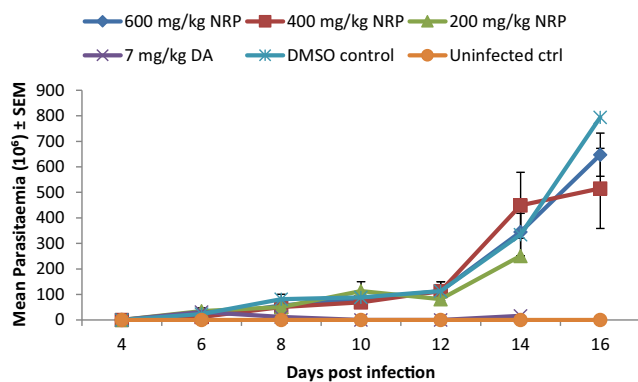


Fig. 1 The effect of treatment with methanolic extract of NRP on parasitaemia of *T. b. brucei*-infected rats

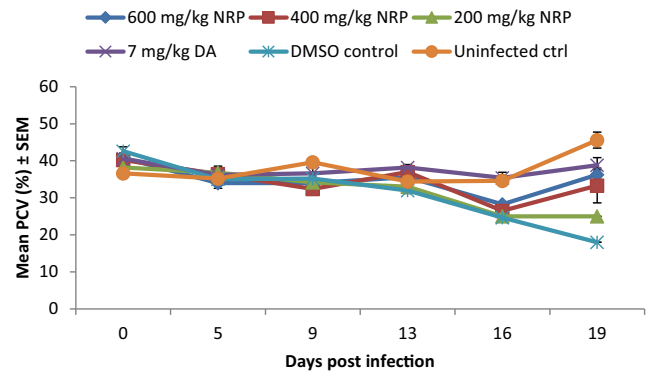


Fig. 2 The effect of treatment with methanol extract of NRP on packed cell volumes of *T. b. brucei*-infected rat

difference in mean group PCV between the uninfected and diminazene aceturate-treated groups. Mean group PCV was significantly ($p < 0.05$) higher in the 600 mg/kg NRP-treated group than in the other extract treated and DMSO control groups. By day 19 PI, there was no significant difference between the 600 and 400 mg/kg NRP-treated and diminazene aceturate-treated groups. There was no significant difference in mean group PCV of the 200 mg/kg NRP-treated group and that of the DMSO control group. Mean group PCV was significantly ($p < 0.05$) higher in the uninfected group than in all the other experimental groups except the diminazene aceturate-treated group.

Haemoglobin concentration

Haemoglobin concentrations of rats in the different experimental groups are shown in Fig. 3. By day 9 PI, mean group Hbc of the uninfected rats was significantly ($p < 0.05$) higher than those of the other experimental groups except the diminazene aceturate-treated group. By day 13 PI, there was no significant difference between the uninfected control and other experimental groups. By day 16 PI, mean group Hbc of the uninfected was significantly ($p < 0.05$) higher than that of the diminazene aceturate-treated group which in turn was significantly ($p < 0.05$) higher than those of the other

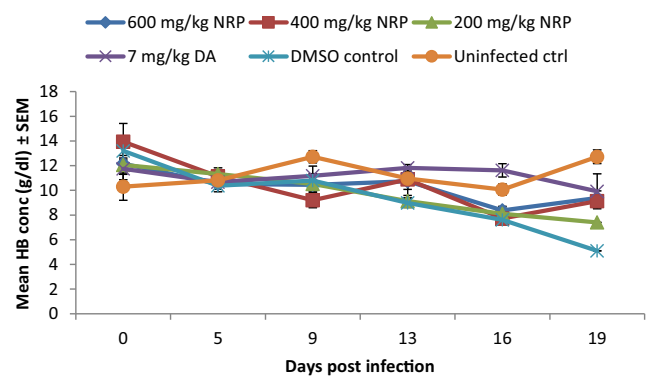


Fig. 3 The effect of treatment with methanol extract of NRP on haemoglobin concentration of *T. b. brucei*-infected rat

experimental groups. By day 19 PI, there was no significant difference between the NRP and diminazene aceturate-treated groups. Mean group Hbc was significantly ($p < 0.05$) higher in the 600 and 400 mg/kg NRP treated than in the DMSO control group. Between the 200 mg/kg NRP-treated and DMSO control groups however, there was no significant difference. Mean group Hbc was significantly ($p < 0.05$) higher in the uninfected when compared with all the other experimental groups.

Body weight

Body weights of rats in all the experimental groups are shown in Fig. 4. Increase in body weights was steady in all the experimental groups until day 14 PI when there was a significant decline in both the DMSO control and 400 mg NRP-treated groups when compared with the other experimental groups.

Other clinical signs observed in all infected rats were anorexia, huddling, weakness and depression.

Survivability

Survivability of infected rats following treatment is shown in Table 1. For rats treated with 600 and 400 mg/kg of NRP survivability was 11 and 13 days post-treatment, respectively. While for the 200 mg/kg NRP treated and DMSO control groups survivability was 6 and 7 days, respectively. In the diminazene aceturate-treated group, survivability was 100% by the end of the experiment.

Discussion and conclusion

Trypanosomes were observed in the blood of infected rats from the 4th day PI with an average prepatent period of 5 days. This finding agrees with those observed by other workers (Nweze et al. 2009). Diminazene aceturate completely cleared parasites from the blood 3-days post-treatment though relapse infection occurred 4 days after clearance. This highlights one

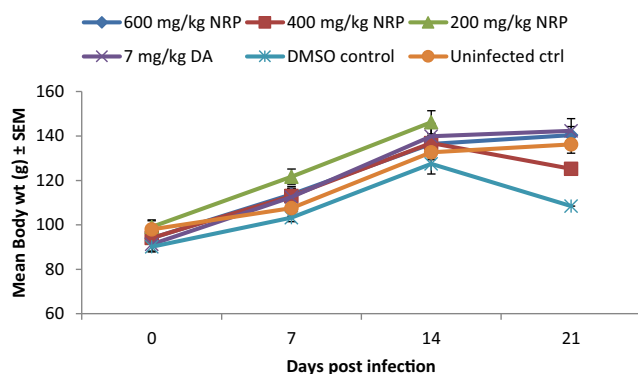


Fig. 4 The effect of treatment with methanol extract of NRP on body weights of *T. b. brucei*-infected rat

Table 1 Survivability of rats infected with *T. b. brucei* and treated with methanol extract of NRP

Days post-infection	Experimental groups				
	A	B	C	D	E
0	5/5	5/5	5/5	5/5	5/5
5	5/5	5/5	5/5	5/5	5/5
7 ^a	5/5	5/5	5/5	5/5	5/5
9	5/5	5/5	5/5	5/5	5/5
11 ^b	5/5	5/5	5/5	5/5	5/5
13	5/5	5/5	5/5	5/5	5/5
15	4/5	4/5	1/5	5/5	3/5
18	4/5	2/5	0/5	5/5	1/5
21	2/5	1/5	0/5	5/5	0/5
23	0/5	1/5	0/5	5/5	0/5
25	0/5	0/5	0/5	5/5	0/5

Numerator number of rats alive, *denominator* number of experimental animals in group

^a Onset of treatment

^b End of treatment

Group A, infected and treated with 600 mg/kg NRP; Group B, infected and treated with 400 mg/kg NRP; Group C, infected and treated with 200 mg/kg NRP; Group D, infected and treated with 7 mg/kg diminazene aceturate; Group E, DMSO control

of the limitations of treatment with diminazene aceturate encountered in the field which often necessitates repeat treatment of affected livestock. A number of factors are responsible for relapse infection including extravasation of trypanosomes to brain tissues where they are inaccessible to drugs circulating in the plasma due to the blood brain barrier. Development of drug resistance in trypanosomes also leads to relapse parasitaemia and treatment failures.

Anaemia is a cardinal sign of trypanosomosis. Clearance of trypanosomes from the blood of treated animals reversed clinical signs like anaemia and weight loss. This was evident in increased PCV and Hbc recorded in the diminazene aceturate-treated group. However, in the NRP-treated groups (especially 600 and 400 mg/kg body weight) significant increases in both PCV and Hbc were recorded despite the fact that parasitaemia was not cleared. It is either that treatment with NRP reduced the rate of parasite multiplication or helped the animals to contain the infection perhaps through immunomodulation. Orsatti et al. (2010) and Sforzin (2007) reported that propolis has an effect on both innate and adaptive immunity. In the former report, increased proliferation of Toll-like receptors (TLR) 2 and better stimulation of TLR-4 in peritoneal macrophages indicated higher response towards gramme negative bacteria. Propolis also stimulates the formation of oxygen products which have a microbicide effect (Sforzin 2007).

Emaciation is one of the clinical signs of trypanosomosis (Hoet et al. 2004) especially, the chronic type. Weight loss

observed in infected rats was perhaps due to anorexia, huddling, weakness and depression associated with infection. Rats treated with diminazene aceturate and NRP at 600 mg/kg body weight had better weight gains post-treatment than others treated. This may be an indication of greater efficacy of treatment in those groups.

Treatment with NRP at 400 and 600 mg/kg appeared to increase survivability more than the 200 mg/kg NRP dose regimen. This could be due to the immunomodulatory effects of propolis. When treatment with NRP was stopped, parasitaemia increased steadily until the animals all died. This is because trypanosomiasis is highly debilitating and invariably fatal if left untreated (Molyneux et al. 1996).

Treatment with methanolic extract of Nigerian red propolis had some degree of antitrypanosomal effect with improved PCV, Hbc, weight gain and survivability on oral administration especially at 600 and 400 mg/kg body weight. Further optimisation of the method of administration and fractionation to produce increased activity could yield an improved outcome.

Compliance with ethical standards

Funding No funds were received from any external bodies for the conduct of this research.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical considerations All procedures carried out were in accordance with institutional and national/international guidelines for animal experiments.

References

- Altwayjry NA (2014) The identification of chemical components from UK bee propolis and preliminary screening for potential immunomodulatory effects. MSc thesis, University of Strathclyde, UK
- Bankova V (2010) New biologically active compounds from Kenyan propolis. *Fitoterapia* 81:509–514
- Bizimana N, Tietjen U, Zessin K-H, Diallo D, Melzig MF, Clausen P-H (2006) Evaluation of medicinal plants from Mali for their in vitro and in vivo trypanocidal activity. *J Ethnopharmacol* 103:350–356
- Brito GAB, Chaves MH (2010) β -amyryn, a natural triterpenoid ameliorates L-arginine-induced acute pancreatitis in rats. *World J Gastroenterol* 16:4272–4280
- Brun R, Schumacher R, Schmid C, Kunz C, Burri C (2001) The phenomenon of treatment failures in human African trypanosomiasis. *Tropical med Int Health* 6:906–914
- Burdock G (1998) Review of the biological properties and toxicity of bee propolis. *Food Chem Toxicol* 36:347–352
- DFID (2001) Trypanosomiasis, Tse-tse and Africa: The year 2001 report p. 15
- Herbert WJ, Lumsden WHR (1976) *Trypanosoma brucei*: a rapid matching method for estimating the host's parasitemia. *Exp Parasitol* 40:427–431
- Higgins T, Beutler E, Dumas BT, 2008. Measurement of haemoglobin in blood. In Tietz fundamentals of clinical chemistry (6th edn) Burtis CA, Ashwood ER, Bruns DE (eds). Saunders Elsevier: Missouri; 514–515.
- Hoet S, Opperdoes F, Brun R, Adjakidje V, Quentin-Leclercq J (2004) In vitro antitrypanosomal activity of ethnopharmacologically selected Beninese plants. *J Ethnopharmacol* 91:37–42
- Hoet S, Pieters L, Muccioli GG, Habib-Jiwan J, Opperdoes FR, Quentin-Leclercq J (2007) Antitrypanosomal activity of triterpenoids and sterols from the leaves of *Strychnos spinosa* and related compounds. *J Nat Prod* 70:1360–1363
- Huang S, Zhang CP, Wang K, Li GQ, Hu FL (2014) Recent advances in the chemical composition of Propolis. *Molecules* 19:19610–19632
- Ito J, Chang FR, Wang HK, Park YK, Ikegaki M, Kilgore N, Lee KH (2001) Anti-AIDS agents. 48. 1 anti-HIV activity of moronic acid derivatives and the newmelliferone-related triterpenoid isolated from Brazilian propolis. *J Nat Prod* 64:1278–1281
- Marcucci MC (1995) Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie* 26:88–90
- Molyneux DH, Pentreath V, Doua F (1996) African trypanosomiasis in man. In: Cook GC (ed) Manson's tropical diseases. WB Saunders, London, pp 1171–1196
- NAS (2011) Guide for the care and use of laboratory animals, 8th edn. National Academy Press: National Academy of Sciences (NAS), Washington D. C
- Nweze NE, Fakae LB, Asuzu IU (2009) Trypanocidal activity of the ethanolic extract of *Buchholzia coriacea* seed. *Nig vet J* 29:1–6
- Ogbadoyi EO, Abdulganiy AO, Adama TZ, Okogun JI (2007) In vivo trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei*. *J Ethnopharmacol* 112:85–89
- Omar RMK, Igoli JO, Gray AI, Ebiloma GU, Clements C, Fearnley J, Ebel RE, Zhang T, De Koning HP, Watson DG (2016) Chemical characterization of Nigerian red propolis and its biological activity against *Trypanosoma brucei*. *Phytochem analysis* 27:107–115
- Orsatti CL, Missima F, Pagliarone AC, Bachiaga TF, Bufalo MC, Araujo JP, Sforcin JM (2010) Propolis immunomodulatory action in vivo on toll-like receptors 2 and 4 expression and on pro-inflammatory cytokines production in mice. *Phytother res* 24:1141–1146
- Popova MP, Bankova VS, Bogdanov S, Tsvetkova I, Naydenski C, Marazzan GL, Sabatini AG (2007) Chemical characteristics of poplar type propolis of different geographic origins. *Apidologie* 38:306–311
- Rickman LR, Robson J (1970) The blood incubation infectivity test: a simple test which may distinguish *Trypanosoma brucei* from *T. rhodesiense*. *Bull World Health org* 42:650–651
- Sforcin JM (2007) Propolis and the immune system: a review. *J Ethnopharmacol* 113:1–14
- Sha N, Guan SH, Lu ZQ, Chen GT, Huang HL, Xie FB, Yue QX, Liu X, Lotti C, Campo-Fernandez M, Piccinelli AL, Cuesta-Rubio O, Hernández IM, Rastrelli L (2010) Chemical constituents of red Mexican propolis. *J Agric Food Chem* 58:2209–2213
- Soulsby EJJ (1982) Helminths, arthropods and protozoa of domesticated animals, 7th edn. London, Bailliere Tindall
- Thrall MA, Weiser MG (2002) Haematology. In: Hendrix CM (ed) Laboratory procedures for veterinary technicians, 4th edn. Mosby Inc, Missouri, pp 29–74
- Zhang C, Huang S, Wei W, Ping S, Shen, X, Li, Y, Hu, F (2014) Development of high-performance liquid chromatographic for quality and authenticity control of Chinese propolis. *J Food Sci* 79: C1315–C1322