



ANTI-PLASMODIAL ACTIVITIES OF SOME PLANTS EXTRACTS USED IN TRADITIONAL MALARIA THERAPY IN KANO-NIGERIA

*Dabo¹, N.T., ³Ofori², M., Nyarko², A. K., Edo³, D. and Bimi, L. ³

¹Department of Biological Sciences, Bayero University, Kano, Nigeria.

² Noguchi Memorial Institute of Medical Research, (NMIMR), University of Ghana, Legon, Ghana

³Department of Animal Biology and Conservation Science (DABCS), University of Ghana, Legon, Ghana

*Correspondence author: ntdabo@gmail.com

ABSTRACT

This work was set to evaluate the antiplasmodial activities of some plants used in traditional malaria therapy in Kano State, Nigeria. Nine medicinal plants, distributed into eight families, used for treatment of malaria fever by the Hausa people of Kano-Nigeria were selected based on the traditional claims of the folks. These were collected and extracted using aqueous and organic solvents. A total of nineteen extracts and forty four extract-fractions were obtained. Thirty extracts (7 aqueous extracts, 12 ethanol extracts and 11 methanol extracts-fraction) were selected and screened for their in vitro anti-plasmodial activities against laboratory adapted chloroquine susceptible Plasmodium falciparum strain 3D7. Seven (23.33%) of these extracts that exhibited 70% or more inhibition at 50µg/mL concentration were further evaluated to establish their potency levels. The respective IC₅₀ values obtained ranged between 0.361±0.15µg/ml and 37.67±4.20µg/ml. CALF₁ from the leaves of C. aurantifolia (Rutaceae) and CBLF₁ (a combination of the leaves of C. aurantifolia, Carica papaya, Mangifera indica and Psidium guajava) were further screened against a clinical isolate of P. falciparum and recorded IC₅₀ values similar to those obtained earlier against the laboratory strain, 12.42±3.94 µg/ml for CBLF₁, and 29.69±0.65µg/ml for CALF₁, respectively. It could be concluded that majority of the plants have demonstrated appreciable concentration dependent in vitro anti-plasmodial effects against the laboratory adapted and field strains P. falciparum.

Keywords: Plants Extracts, Traditional Malaria Therapy, Hausa people, Kano, Nigeria

INTRODUCTION

Malaria is a disease caused by protozoan parasites of the genus *Plasmodium* transmitted by infected female *Anopheles* mosquitoes. Earlier on, four species, namely *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax* were known to cause infection in humans (WHO, 2009). Of recent *P. knowlesi*, a simian species has been implicated to cause infection in humans (Brown, 2011). *P. falciparum* is the most virulent of the four and is responsible for about 80% of all malaria cases and about 90% of the deaths from malaria (WHO, 2010). Malaria is a serious disease with high mortality and morbidity especially in children and pregnant women. Though there had been a slight decline in the malaria incidence over the last few years from 244 million in 2005 to 225 million in 2009. Mortality was projected to have decreased from 985, 000 in 2000 to 781 000 in 2009. However these large absolute decreases were not observed in Africa (WHO, 2010). The Sub-Saharan Africa bears the highest burden of the disease. A vaccine would have been the ultimate and effective control strategy. However none is available for use. Several vaccines candidate such as RTS, S/AS02A and VAR2CSA are being tested and evaluated. Hence, vector control and chemotherapy remain the major interventions for the control of malaria.

Several drugs particularly chloroquine had been used to treat malaria. However, most of the drugs that we use today are becoming less effective because of the

problem of drug resistance (McMorran, 2009). The spate of drug resistance by the malaria parasite particularly *P. falciparum* has necessitated the scientific evaluation of many traditional medicinal plants for an alternative antimalarial drug that is effective, safe and affordable, (Oyedemi *et al.*, 2005). The value of plants in traditional medicine cannot be overemphasized and is still the first point of healthcare for many people in sub-Saharan Africa (WHO, *TDR news*, 2007; Hostettmann *et al.*, 2000). Plants still provide a source for effective lead compounds against malaria (Midiwo, 2007). The fact that traditional medicinal plants used for the treatment of malaria provided the lead compounds for synthetic antimalarials in use currently gives hope that plants particularly those used in the unorthodox treatment of the disease may provide new lead compounds for development of new and novel antimalarial drugs. It is for this reason that some plants used in the traditional treatment of malaria or fever by the Hausa people of Kano, Nigeria were selected and evaluated for efficacy against the Plasmodium parasite.

MATERIALS AND METHODS

Collection and Handling of Plant Materials

Nine plants used in the treatment of malaria or fever (Table 1) were collected on the basis of personal communication with the folks between 21/11/2008 to 01/01/2009.

Taxonomic identification was confirmed by an Ethnobotanist, Professor B. S. Aliyu of the Department of Plant Science, Bayero University Kano, Nigeria. Voucher specimens were deposited in the herbarium of the Bayero University.

Table 1: Plant names, Voucher number, Family and parts collected

Plant Name	Herbarium Voucher #	Common name	Family	Part used
<i>Acacia nilotica</i>	287/05	Bagaruwa	Fabaceae	Stem bark
<i>Adansonia digitata</i>	77/164	Kuka	Bombacaceae	Seeds & S/bark
<i>Carica papaya</i>	70/12	Gwanda	Caricaceae	Leaves
<i>Cassia occidentalis</i>	287/11	Rai-rai	Fabaceae	Leaves
<i>Citrus aurantifolia</i>	20/07	Lemon-tsami	Rutaceae	Leaves
<i>Mangifera indica</i>	55/10	Mangwaro	Anacardiaceae	Leaves
<i>Moringa oleifera</i>	30/02	Zogale	Moringaceae	Leaves
<i>Psidium guajava</i>	66/01	Goba	Myrtaceae	Leaves
<i>Ziziphus mauritiana</i>	190/01	Magarya	Rhamnaceae	S/bark & Leaves

Six of the plants, (*M. Indica*, *C. Papaya*, *P. guajava*, *C. aurantifolia*, *C.occidentalis* and *M. oleifera*) were prepared as water decoctions. Fresh leaves were collected and washed in clean tap water. They were then drip dried and 50g was weighed using Triple Beam Balance 700 SERIES (OHAUS® USA). The leaves were then placed in 500ml Pyrex® beakers and gently boiled for an hour in clean tap water at 1:5 w/v (plant part: solvent); i.e. 50g/250ml. The four plant leaves used as combination (*M. Indica*, *C. Papaya*, *P. guajava*, *C. aurantifolia*) were prepared by weighing equal proportions of the respective plants (12.5g each) and stirred to squeeze out the maximum extract from the leaves. The extract was allowed to cool to about 55°C before it was filtered using Whatman No. 1 filter paper into a pre-weighed beaker. The filtrate was then concentrated by evaporating the water in shallow containers using Griffin's® Student Water Bath (WHO, 2002). Individual plant yield was then obtained by differential weighing. Seven aqueous extracts were prepared.

The nine plants parts were shade dried to minimize deterioration of active components. Each dried plant material was then ground into powdered form using mortar and pestle. Two hundred and fifty grams (250g) portions was then weighed and extracted as described by Fatope *et al.* (1993). This carried out by percolating in absolute ethanol (Rjedel-de Haan®) at 1:5 w/v (plant part: solvent); for two weeks and filtered using Whatman® No.1 filter paper. The filtrate was then concentrated by evaporating the solvent using rotary evaporator at 40 - 45°C in a pre-weighed round bottom flask. The yield was then determined by differential weighing. A portion of the extract was transferred into pre-weighed specimen bottle and designated F1. This was done for all extracts obtained for each of the plants. The remaining bulk of the F1 extract was further successively extracted in 100 ml (40, 30, and 30) portions of n-Hexane, chloroform, ethyl acetate and finally in methanol for five minutes each. This was allowed to settle for another five minutes before the supernatant was gently decanted into a clean beaker leaving behind the marc. The marc (residue) was allowed to dry before extracting with the next solvent. The decanted portions for each solvent were pooled together and the solvent removed

by rotary evaporation and designated F₂, for n-hexane; F₃, for chloroform; F₄, for ethyl acetate and F₅, for methanol fractions respectively. A total of 55 extracts and fractions were thus obtained in this process. A total of sixty three extracts (7 aqueous and 55 organic) were obtained using the two procedures.

***In vitro* Antiplasmodial Activities of Extracts**

Laboratory strains of chloroquine susceptible strain of *P. falciparum*, 3D7 and a clinical isolate maintained in washed human blood group O+ type suspended in complete parasite medium, CPM (RPMI 1640 medium supplemented with gentamicin + l-glutamine + albumax) in long term continuous *in vitro* cultivation in the Immunology Department of the Noguchi Memorial Institute of Medical Research (NMIMR), University of Ghana, Legon (Trager and Jensen, 1976), with slight modifications (Ofori *et al.*, 2002) were used for the assay. Thirty extracts [7 aqueous extracts, 12 ethanol (F₁) and 11 methanol (F₅) extracts] were selected for the preliminary assay against stabilised parasite cultures containing largely ring stages. The extracts were dissolved in microquantities of water, ethanol or methanol depending on the solvent of extraction. The resulting solutions were then diluted with RPMI 1640 such that the final ethanol and methanol concentrations did not exceed 0.01%. For the preliminary assay each extract was tested at three duplicate concentrations of 5, 50 and 100µg/ml using 24-well microtitre plates. Nine hundred microlitres of 1% parasite culture was placed in each well. One hundred microlitres of the solution of the test extract in RPMI 1640 containing the requisite concentration were added to give a final volume of 1000µl in each well. Based on the criterion of Prozesky *et al.*, (2001) (the most active extract giving more than 70% inhibition at 50µg/ml in the preliminary assay) was used as basis of selecting extracts for re-evaluation.

Seven extracts were selected for further evaluation, using two-fold serial dilutions ranging from 0.78125µg/ml to 200µg/ml 24-well microtitre plates in duplicate for each extract. Artesunate was also tested alongside these extracts as standard control. CALF₁ and CBLF₁ extracts were also evaluated against field strain *P. falciparum*.

The assay plates were placed in Billups-Rothenberg Modular Incubating Chamber (patent no. 5352414, USA) and flushed with a gentle flow of a mixture of 2% O₂, 5.5% CO₂ and 92.5% N₂ gases and finally incubated in Galaxy 170S brand incubator (RS Biotech UK) set at 37°C for 48h.

Parasitaemia Estimation and IC₅₀ Determination

Parasitaemia was estimated by microscopic observation of completely dried thin slide films prepared from each assay well under the x100 objective in oil immersion using the Olympus binocular microscope, (Model CH30 Japan) (WHO 1991; NMIMR/SOP). A minimum of 500 RBCs were counted against the parasitized/infected cells in the preliminary assay, while 1000 cells were counted in the extended

assay. Estimate of % parasitaemia was determined by the relation: %parasitaemia = [(no. of infected cells ÷ total cell count) × 100%]

Once the % parasitaemia was determined for a particular extract concentration, this was transformed into percentage inhibition of growth by comparison with control values (Addae-Kyereme, *et. al.*, 2001). This is given by the relation: [(a – b)/a] × 100%; Where a=%parasitaemia in control and b=%parasitaemia in treatment.

The half maximal inhibitory concentration, IC₅₀ for an extract was determined by plotting the % inhibition against the logarithms of the concentrations in Microsoft Office Excel Worksheet. Regression equation was used to compute the IC₅₀ values based on the equation of the slope given as: [y = ax + c].

RESULTS

Aqueous extraction of the plants gave extract yields ranging from 4.5g (9%) for *C. papaya* to 8.04g

(16.09%) for *C. occidentalis* (Table 2). The extracts were generally green in colour and pasty or gummy in texture.

Table 2: Yields from Aqueous Extraction

PLANT NAME	FAMILY	PART	CODE	FRESHWT. (g)	EXTRACT WT. (g)	%YIELD
<i>C. aurantifolia</i>	Rutaceae	Leaves	CALW	50	5.05	10.10.
<i>C. occidentalis</i>	Fabaceae	Leaves	COLW	50	8.04	16.09
<i>C. papaya</i>	Caricaceae	Leaves	CPLW	50	4.50	9.00
<i>M. indica</i>	Anacardiaceae	Leaves	MILW	50	7.30	14.60
<i>M. oleifera</i>	Moringaceae	Leaves	MOLW	50	7.80	15.60
<i>P. guajava</i>	Myrtaceae	Leaves	PGLW	50	6.50	13.00
Combination*	-----	Leaves	CBLW	50	6.50	13.00

*A combination of *M. indica*, *C. papaya*, *P. guajava* & *C. aurantifolia* in equal proportions;

The yields for the F₁ extracts ranged from 6.9g (4.6%) for the stem bark of *A. digitata* to 58.3g (23.32%) for the leaves of *P. guajava*. The leafy parts mostly yielded products which were green or grey in colour, and gummy or oily in texture. Extract of the leaves of *P. guajava* (PGLF₃) was crystalline. The stem-bark materials mostly yielded materials which were brown, yellow or red with their texture varied from pasty to powdery substances.

At 5µg/ml concentration, ADSF₁ (with 54% inhibition) and ZMLF₁ (with 54.82% inhibition) exhibited the best inhibition. No extract had up to 70% inhibition at 5µg/ml concentration (Table 4). Seven (or 23.33%)

exhibited up to 70 or more % inhibition at 50µg/ml concentration. These extracts were CBLF₅ (88.49%), MILF₁ (82.51%), MILF₅ (80.41%), ANSF₁ (78.33%), CALF₁ (75.97%), ANSF₅ (74.45%) and CBLF₁ (70.07%). However, at 100µg/ml concentration, 15 or (50%) of the extracts had 70% or more inhibition.

The extracts evaluated in the detailed analysis, exhibited concentration dependant inhibition activities on the parasite as indicated by the mean percentage inhibitions in the two experiments. A graphical presentation of this interactions developed using the Sigma Plot Program (Version 9) is presented in Figure 1.

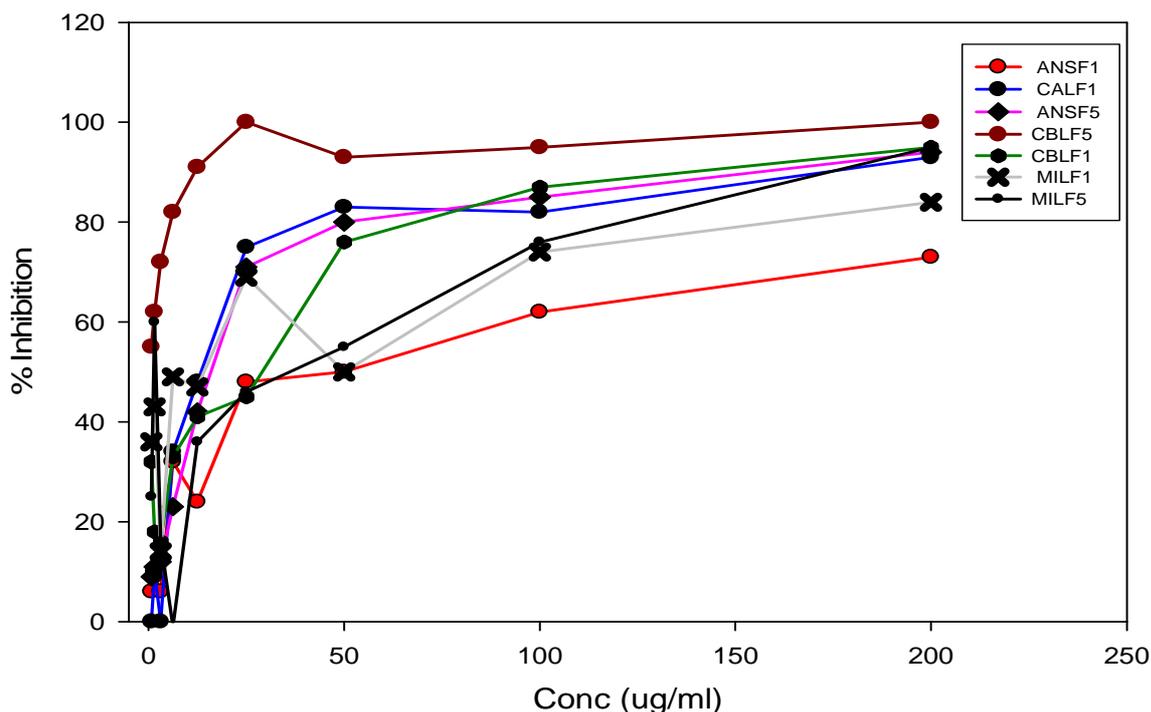


Figure 1: Inhibition Characteristics of Seven Selected Extracts in the Detailed Evaluation Assay against 3D7 Pf

CBLF₅ [prepared from four combined plants namely *M. indica*, (Anacardiaceae); *C. papaya*, (Caricaceae); *P. guajava*, (Myrtaceae); and *C. aurantifolia*, (Rutaceae)] exhibited the highest % inhibition (55% Inhibition) at the lowest concentration (0.78125µg/mL) and IC₅₀ of 0.361µg/mL. ANSF₁ on the other hand had the least effect (IC₅₀ of 37.67µg/ml). However, as the concentrations of the extracts increases, the percent

inhibition also increased. In addition the ethanol extracts [CALF₁ (Rutaceae) and CBLF₁ (combination)] selected and evaluated against the field strain of *P. falciparum* also exhibited concentration dependant inhibition characteristics with IC₅₀s of 12.42±3.94 µg/ml for CBLF₁ and 29.69±0.65µg/ml for CALF₁ against the field strain.

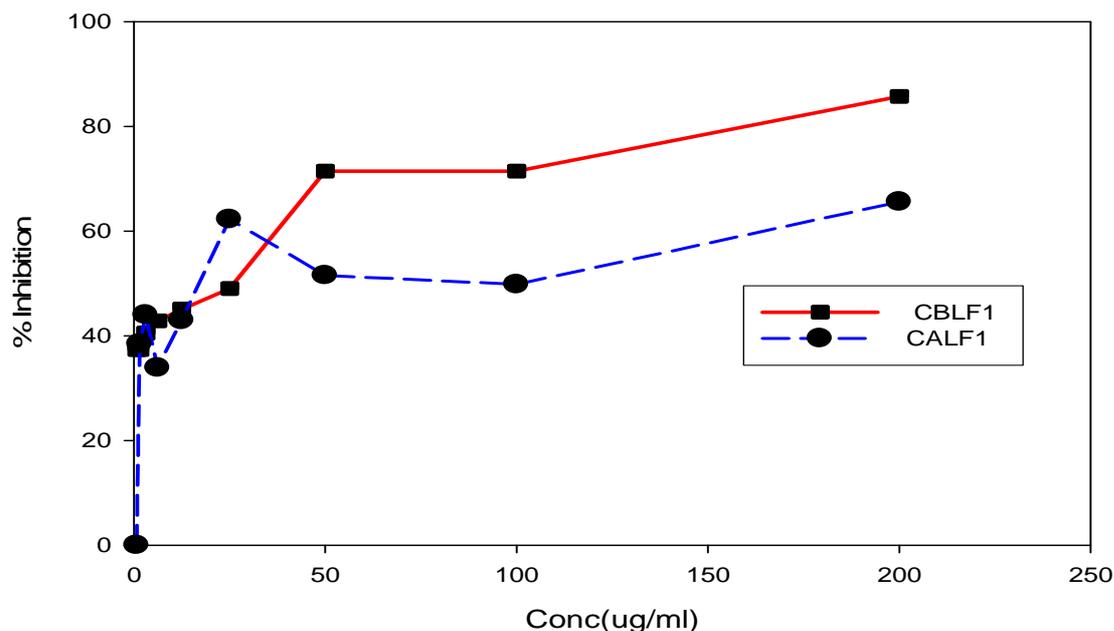


Figure 2: Inhibition Characteristics of Two Selected Extracts in the Detailed Evaluation Assay against a field isolate Pf

Artesunate (a standard drug) evaluated against 3D7 *P. falciparum* also exhibited concentration dependant inhibition characteristic with extremely low IC₅₀ of 0.00012±0.000041µg/ml. This result was not to compare with the plant extracts.

DISCUSSION

Hot aqueous extractions (decoctions) were employed in this work in a bid to evaluate the rationale of the traditional method of boiling fresh herbs / leaves in clean potable water. In addition, a combination of four of the plants (*M. Indica*, *C. Papaya*, *P. guajava*, and *C. aurantifolia*) also was to depict the typical traditional concoction mode of preparation of these plants. The yields obtained for both protocols shows how maximally the phyto-contents of the drug plants can be exploited in the traditional method thereby exposing the parasite to reasonably high dosages of the active phytochemicals in the patient. The moderate *in vitro* activities exhibited by the aqueous extracts despite the hot mode of extraction, in the preliminary assay confirms the stability of the active principles in these plants which gives credence to the traditional mode of preparation in malaria therapy. The activity exhibited by some of the aqueous extracts confirms the scientific rationale of the use of the plants in traditional malaria therapy. The lower efficacy exhibited by aqueous extracts in the assay compared to the organic solvents extracts was not unusual as earlier observed by Kirira *et al.*, (2006), and Mostafa *et al.*, (2007). Also in an *in vivo* study, the aqueous extract of *C. occidentalis* was reported to be less active than the corresponding ethanolic extract (Tona *et al.* 2001). The organic extracts F₁ and F₅ demonstrated relatively higher activities in the preliminary *in vitro* assay compared to the aqueous extracts. This was consistent with the report of Mbatchi *et al.*, (2006). Perhaps this sharp difference in bioactivity over the aqueous extracts points to the minimal destruction of other active compounds and/or a refining effect of synergistic properties of compounds in the organic fractions.

The activity of the F₁ extract of ZMLF₁ (Rhamnaceae) on the 3D7 strain of *Pf* corroborates the report by Panseeta *et al.* (2011). The activity exhibited by ADSF₁ (Bombacaceae) is in consonance with the report of Kohler *et al.*, (2002), who reported the activity of the lipophilic extract of *A. digitata* (Bombacaceae) on the laboratory adapted *P. falciparum* species PoW and Dd2 with IC₅₀ > 50µg/ml. The activity demonstrated by the concoctions' extracts only confirms the scientific worth of this traditional therapy. It also suggests that the constituent plants possibly inhibit the *P. falciparum* malaria parasite in a synergistic mode. Fiot *et al.*, (2006) had reported a similar phenomenon with *G. senegalensis*, *Mitragyna*

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inermis and *Pavetta crassipes* plants commonly used in combination to treat malaria.

The preliminary *in vitro* assay provided baseline data on the antiplasmodial properties of the plants and warrants a closer evaluation to generate a more reliable data for further considerations; hence the subsequent phases of the assays on selected plants extracts. Result obtained strongly indicated that the plants possess significant antiplasmodial action against the 3D7 *Pf. in vitro*. Six out of these extracts yielded IC₅₀s below 20µg/ml. The activity demonstrated by CBLF₁ (15.07±1.74µg/ml) and CBLF₅ (0.361±0.15µg/ml) only further affirms the value of combination of plants (*C. papaya*, *C. aurantifolia*, *P. guajava* and *M. indica*) normally used in traditional malaria treatment by the Hausas of Kano.

The IC₅₀ for ANSF₁ (Fabaceae), 37.67±4.20µg/ml and ANSF₅, 12.51±1.89µg/ml in this work is by far lower than what was earlier reported for *A. nilotica* (73.59±2.87µg/ml and 70.33±1.89µg/ml) on laboratory *P. falciparum* cultures of ENT30CQ resistant isolate and NF54CQ sensitive strain respectively by Kirira *et al.*, (2006). However, El-Tahir *et al.* (1999) earlier reported that the ethyl acetate extract of *A. nilotica* produced an IC₅₀ < 5µg/ml. The efficacy observed against clinical strains of *P. falciparum* (IC₅₀s for CBLF₁ (12.42±3.94 µg/ml and CALF₁ (29.69±0.65µg/ml) indicated that these extract indeed hold promise as antimalarial agents. The activity observed for CALF₁ (Rutaceae) further corroborates earlier report that a number of members of the Rutaceae have indeed antimalarial activity (Schwikkard van and Heerden, 2002; Rukunga *et al.* 2009). Artesunate exhibited a concentration dependant characteristic against the laboratory strain *Pf* and IC₅₀ of 0.00012µg/ml. This observation further confirmed the effectiveness of this drug on the parasite.

CONCLUSION

It can be concluded that some of the plants evaluated in this study indeed possess antiplasmodial effects. Also the extracts prepared from the combination of four plants (*C. papaya*, *C. aurantifolia*, *P. guajava* and *M. indica*) CBLF₁ and CBLF₅ demonstrated the best antiplasmodial activities with IC₅₀ values of 15.07±1.74µg/ml and 0.361±0.15µg/ml respectively. Thus, suggesting that the plants have synergistic effects.

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