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Full Length Research Paper

Efficacy of different herbal preparations of *Azadarachta indica* Juss. on a 4-day schizontocidal test on *Plasmodium Berghei*

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A four-day 4 suppressive schizontocidal test for anti malarial activity against Swiss albino mice infected with chloroquine sensitive strain (NK-65) of *Plasmodium berghei* was used to monitor *in vivo* response to different modes of preparing herbal remedies using the stem and bark of *Azadarachta indica* Juss. Four recognized modes of preparing anti malarial remedies from A. indica were tested against a positive control (chloroquine) and a negative control (phosphate buffer saline). On day zero (0), all the animals received 8 × 10^6 million *P. berghei* infected red blood cells corresponding to 0.1 ml of the blood sample. The four herbal recipes (infusion, decoction, tincture and juice (macerate)) were prepared in a 1:1 ratio of stem bark and leaves. After 2 h of plasmodium challenge, the experimental animals received oral doses of 125, 250 and 50 mg/kg in triplicates. The recipes had intrinsic anti malarial activities that were dose dependent. The comparison analysis indicated that 250 and 500 mg/kg body weight of the tincture and decoction in addition to the 500 mg/kg body weight yielded 76.34, 85.08, 71.26, 82.43 and 78.94, respectively when compared with the chloroquine with 70.24% suppression. The result were significant ($p < 0.001$) at $p < 0.05$ when compared with a placebo and support the use of a dose not less than 125 mg/kg body weight of the tincture and decoction as effective.

Key words: *Plasmodium berghei*, schizontocidal, mode of preparations, chloroquine, phosphate buffer saline.

INTRODUCTION

Several published works have appeared on the medicinal activities of *Azadarachta indica* Juss., including its anti malarial actions (Koul et al.,1990, Radrianarivelojosia et al., 1992; Crellin et al., 1997; Subapriya and Nagina, 2005). Biswas et al. (2002), Kirara et al. (2006) and Siddiguin (2003) confirmed that almost all parts of the plant are useful as anti malarial. Gill (1992) listed decoction as an effective preparation mode, while Etukudo (2003) listed infusion and tinctures in addition to decoction as effective and preferred modes of preparing anti malarial remedies. Five herbalists from randomly selected communities from the area were interviewed on their modes for treating malaria and the most effective amongst the methods. After the interviews, all of them were unanimous in listing infusion, decoction, tincture and juices (maceration) as modes of preparing anti malarial remedies, but fell short of mentioning any as the most effective. There are very few literatures available on the efficacy due to the modes of herbal remedies in research journals. Mosihuzzaman and Choudhary (2008), reported a paucity of published information and data on the safety and standardization of the various modes employed in the preparation of herbal recipes in Africa just as Norten and Putz (2000) reported haphazard usage of water and alcoholic based herbal remedies in

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the continent. The present study was therefore undertaken to evaluate the different modes of preparing herbal remedies from *A. indica* with a view to determining the most effective mode amongst them.

MATERIALS AND METHODS

Sample collection

The stem bark and leaves of *A. indica* were collected from UNICAL staff quarters, Calabar, Nigeria. The identification and authentication was done at the Herbarium Unit of the Department of Botany, University of Calabar, where a voucher specimen was deposited.

Extraction procedure

The stem bark and leaves of *A. indica* were sun-dried at ambient temperature (28 ± 0.5℃). After drying, 1 kg each of t he stem bark and leaves were pulverized to coarse powder using sterile pestle and mortar to avoid contamination. 160 g each of the pulverized part was measured using Mettler PN-163 electric weighing machine and transferred into a water pot. Infusion and decoction were made, respectively using 1 L of distilled water at 100℃, and were maintained for 10 min, while tincture was made by soaking the powdered plant parts for 10 min in 1 L of 98% ethanol. Similarly, Juice was made by hand squeezing the grounded sample to yield aqueous paste.

After 10 min, the herbal extracts were filtered using clean white handkerchief and the filtrates were concentrated in a rotary evaporator to yield various gram ages of the recipes. Prior to use, each recipe was made to125, 250 and 500 mg/kg. These preparations were done daily and were administered once per day for four consecutive days.

Experimental animals

Healthy Swiss albino mice, 4 weeks old and weighing 16 to 21 kg was obtained, from the animal house of Department of Genetics and Biotechnology, University of Calabar, Calabar. They were kept in plastic cage measuring 29 × 11 × 12 cm. The animals were fed on standard diets (Pfizer Livestock Feeds Ltd, Nigeria) and had free access to water. Before the experiment, the animals were bred for one week at the Botany Research Laboratory of the University of Calabar, where the research took place, for proper acclimatization.

Development of parasitaemia

Chloroquine-sensitive *P. berghei* (NK-65) used for this research was obtained from the Malaria Research Laboratory of the Nigeria Institute of Medical Research (NIMR), Yaba, Nigeria. Parasites were maintained through weekly passage in mice by inoculation of known amount of parasite into healthy mice every week.

Inoculation and treatment

Peter's 4 day test (Peter and Anatoli, 1988; David et al., 2004; Ogbonna et al., 2008) was followed to evaluate the blood schizontocidal action against *P. berghei.* Donor albino mice previously infected with chloroquine-sensitive *P. berghei* and with rising parasitaemia of 20% as determined using thin blood film were sacrificed and the blood was collected using ethylenediaminetetraacetic acid (EDTA) bottle. The blood sample was diluted using phosphate buffered saline (concentration of 137 mM NaCl, 10 mM phosphate and 2.7 mM KCl, pH 7.4) such that 0.1 ml contains eight million parasites. To avoid variability in parasitaemia, all the animals were infected from the same source. Eighteen mice were used to assess the effects of herbal recipes. On day 0, the experimental groups as well as the control groups of animals were inoculated with 8 million *P. berghei* infected red blood cells. The mice were then randomly divided into groups of three per cage, and groups of experimental animals were given oral doses of 125, 250 and 500 mg/kg of each of the recipe consecutively from day 0 to 3. The initial treatment started 2 h after parasitaemia challenge. The other two groups received either 5 mg/kg chloroquine per day (positive control) or 0.2 ml of phosphate buffered saline (concentration of 137 mM NaCl, 10 mM phosphate and 2.7 mM KCl, pH 7.4) (negative control). All experiments were carried out in triplicate. The animals were adequately fed and no death was recorded throughout the duration of the experiment.

On day 4 of the test, thin blood smear were prepared using well-labeled and properly cleaned slides (Ogbonna et al., 2008). Blood was collected from the tail vein of each animal using heparinized capillary tube. The dry blood films were fixed with methanol and subsequently stained with geimsa for 25 min. They were then washed with phosphate buffer (pH 7.2) (Dikaso et al., 2006) and were allowed to dry. To obtain optimal film quality, Thirty six slides were made, each animal in duplicate. The slides were then microscopically examined using x100 magnification in oil immersion. The percentage suppression of parasitaemia by each recipe level was calculated by comparing the percentage in infected controls with that of treated mice. Average percentage suppression of parasitaemia was calculated using the following formula:

$$A = [(B-C)]/B \times 100$$

where A is average percentage parasitaemia, B is average percentage parasitaemia in the placebo group; C is average percentage parasitaemia in the test groups (Abosi and Raseroke, 2003).

Student t-test was used to compare the differences in the results between the groups.

RESULTS AND DISCUSSION

The results of the study indicated that *in vivo* herbal recipes of the stem bark and leaves of *A. indica* showed a very good activity against chloroquine-sensitive *P. berghei* (N-65 strain) malaria parasite when given at graded doses of 125, 250 and 500 mg/kg, respectively. Comparative evaluation of the results indicated that decoction and tincture at 250 mg/kg in addition to infusion at 500 mg/kg displayed a statistically significant difference ($p < 0.001$) at 0.05 confidence interval in a 4-day schizontocidal test when compared with the phosphate buffer sulphate-negative control. The highest percentage of parasitaemia inhibition (85.08%) was recorded when tincture was administered at 500 mg/kg (Table 1). The research findings also implicated the activity of the recipes as dose-dependent. For instance, all the recipes showed a non significant statistical difference at 125 mg/kg, while 50 and 75% of the recipes proved statistically significant ($p < 0.001$) at 250 and 500 mg/kg, respectively. Similarly, juice as a form of herbal recipe showed varied parasitaemia inhibition amongst the dosages, but no one proved statistically significant ($p <$

Table 1. *In vivo* anti malarial effect of herbal recipes.

Herbal recipe	Control		Herbal dosage in mg/kg body weight (mg/kg)		
	+ve control (CQ)	-ve control (PBS)	125	250	500
Infusion; n = 3			5.47 ± 3.93 (41.56)	3.93 ± 2.01 (58.12)	2.93 ± 0.84 (68.70)*
Decoction; n = 3			4.69 ± 3.36 (49.90)	3.01 ± 2.14 (67.85)*	2.10 ± 0.64 (77.57)*
Tincture, n = 3			4.60 ± 3.31 (50.86)	2.88 ± 0.91 (69.24)*	2.06 ± 0.77 (78.00)*
Juice; n = 3			6.70 ± 4.06 (28.42)	4.99 ± 3.37 (46.69)	4.37 ± 3.28 (53.32)
	0.64 ± 2.17 (70.24)	9.36 ± 5.64			

N, No. of animals in each group; *, significance at $p < 0.05$; values indicate mean percentage parasitaemia; values in parenthesis indicate percentage reduction in parasitaemia as compared to control. CQ, Chloroquine; PBS, phosphate buffer saline.

0.05) when compared with the phosphate buffer saline used as negative control. Also, infusion was proved statistically significant only at 500 mg/kg at the same confidence limit. It should be stated also that the positive control induced a high level of chemo-suppression at 500 mg/kg.

Mode of herbal preparation was reported by Mosihuzzaman and Choudhary (2008), as an integral arm in the safety and standardization of herbal remedies. Aibinu and Adelowotan (2005) noted that the therapeutic activity of a medicinal plant is closely related not only to the plant chemicals in it, but also the mode of preparation which facilitates the extraction of these active ingredients. For instance, water is almost universally the solvent of choice in extracting delicate herbs (leaves and fresh tender parts)-infusion and tougher fibrous parts, such as stem barks and roots which have water soluble metabolites-decoction. Alcohol on the other hand is used to extract chemicals (tinctures) that are readily soluble in it and also, when bulk volume and long-term preservation are of essence. Macerate, also called juice is a preferred mode of preparing much tendered parts or those plant parts with delicate chemicals that might be harmed by heating or which might be degraded by alcohol. Three chemicals in the neem plant (gedunin, nimbidol and quercentin) were implicated by Okpanyi and Ezeukwu (1981) and Ross (2001) as being active against the plasmodium parasite. The high percentage inhibition observed in decoction and infusion at 250 and 500 mg/kg could be due to the water soluble properties of gedunin and quercentin (since they are soluble in water) just as the use of alcohol in preparing the tincture may have released nimbidol in the sample that brought significant reduction in average percentage parasitaemia. This position is enhanced by Ross (2001), who posited that hot water extract of fresh leaf of *A. indica* by gastric intubation in mice releases gedunin that produced a weak to moderate activity on *P. berghei* at a dose of 500 mg/kg on days 1 to 4.

Conclusion

The modes of preparing and administering plant extracts to ensure maximum efficacy and maintaining consistency have been central to the practice of pharmacy. Extraction procedures for instance, whether water or alcohol is used and how much heat is employed have been known to produce variations in quality. This is as shown in this study where equal dose of same plant extract produces different activity in different recipes. To ensure standardization and safety of herbal remedies, attention should be paid to the mode of preparation that produces best results.

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