International Scholars Journals

African Journal of Malaria and Tropical Diseases ISSN 4123-0981 Vol. 2 (12), pp. 092-095, December, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

An evaluation of *Guiera senegalensis* as a common herbal antipyretic and antimalarial among some tribal groups in northern Nigeria

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Accepted 12 November, 2014

***Guiera senegalensis* is acclaimed as a common herbal antipyretic and antimalarial among some tribal groups in northern Nigeria. Leaf extracts of the plant were thus tested for antiplasmodial, analgesic and anti-inflammatory effects *in vivo.* Results indicated the safe dose of extracts as 600 mg/kg body weight of mice with LD_{50} of 1100 mg/kg bw. Only the methanolic fraction had antiplasmodial effect while ethylacetate and hexane fractions were ineffective. Furthermore the methanolic extract produced a significant ($p<0.05$) suppression of up to 67.52% levels. The extracts had no prophylactic effect and high parasitaemia including mortality of sub-inoculated mice were obtained on day 14 post treatment. It gave 44.83% analgesic effect but was devoid of anti-inflammatory activity. Phytochemical screening indicated the presence of alkaloids, glycosides, tannins and flavonoids.**

Key words: Antipyretic, analgesic, antiplasmodial, anti-inflammatory, phytochemical.

INTRODUCTION

Malaria poses a formidable challenge to the realization of improved healthcare and life expectancy among the poor in Sub-Sahara Africa and south-east Asia. Recent statistics on the disease are quite alarming- an estimated 500 million acute infections and 3 million deaths annually (WHO, 2008; Fletcher, 2007). Vulnerable groups are mostly pregnant women and children under 5 years of age. Despite this statistic, the causative parasites, *Plasmodium* species have acquired resistance to most common drugs (Bloland, 2001). New drugs thus have to be sourced to replace the ones already compromised by this phenomenon. This is more so that viable and relevant vaccines are as of yet unavailable against malaria (Jigam et al., 2010a). There is hence the need to evaluate scientifically relevant plant species commonly used in the herbal treatment of the disease (Okunji et al., 2000).

Guiera senegalensis Gmel ("Sabara" in Hausa Nigeria), is a shrub of the savannah region of West and Central Africa (Zeljan et al., 1998). Its leaves 3 to 5 cm long and 1.5 to 3.0 cm broad are opposite or sub opposite, oblong elliptic, rounded or slightly cordate at base and mucronate at the apex. They are softly tomentos on both surfaces, with scattered black glands underneath (Hutchison and Dalziel, 1965).

The leaves are bitter-tasting and have widespread acknowledgement in African medicine as a "cure-all" in herbal concoctions (Hiermann and Bucar, 1994). The usual form of preparation for internal use is in decoctions or mixed with food preparations *G. senegalensis* leaves are widely administered for pulmonary and respiratory complaints, for coughs, as a febrifuge, colic and diarrhea, syphilis, beriberi, leprosy, impotence, rheumatism, diuresis and expurgation (Hutchinson and Dalziel, 1965; Zeljan et al., 1998). In Northern Nigeria powdered leaves are mixed with food as a general tonic and blood restorative and also to women as a galacta gogue (Koumare et al., 1968).

In Ghana and other West African Countries, leaves are used to treat dysentry and fever due to malaria (Abbiw, 1990). Earlier findings by Etkin (1997) indicated *Guiera*

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leaf extracts markedly oxidized glutathione and also generated high levels of methaemoglobin *in vitro*, both conditions being unfavourable for the survival of *Plasmodium* in red cells. These reported effects and the application of the plant in herbal medicine for analgesic and antimalarial properties necessitated *in vivo* screening with parasitized animals. This is with a view to ascertaining the rationale behind using the plant species in malaria and related conditions.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *G. senegalensis* were collected between May and June in Minna, Northern Nigeria and authenticated at the Department of Biological Sciences, Federal University of Technology, Minna.

Preparation of crude extracts

40 g of air dried leaves were micronised and extracted exhaustively (48 h) in the cold with 1.5 L each of hexane, (Sigma-Aldrich Europe), ethylacetate and methanol in that order. The marc were filtered with muslin cloth and solvents removed under reduced pressure in a rotary evaporator. Green coloured pastes were obtained and weighed prior to further analysis.

Animals

Healthy swiss albino mice of either sex of about 6 weeks old weighing between 20 to 30 g each and wister rats of about 180 to 200 g weights obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigerian and were used for the experiments. The rodents were conveniently housed under standard environmental conditions. (Temperature 27 ± 2°C; 70% relative humidity; 12h daylight/night cycle) and had free access to commercial feed pellets and water. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian council on animal care guidelines and protocol review (CCAC, 1997).

Parasites

P. berghei NK65 chloroquine sensitive strain was obtained from NIPRD Abuja, Nigeria and maintained in our laboratory by serial passage in mice.

Phytochemical analysis

Standard screening tests were used to detect secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides and volatile oils e.t.c. in the crude extract (Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

Safe dose and acute toxicity (LD_{50})

Five groups (A, B, C, D and E) of four mice were used. The animals were given extracts intraperitoneally (i.p) at doses of 200, 400, 600, 800, 1200 mg/kg body weight (bw) in A, B, C, D and E respectively. Extracts were dissolved in dimethylsulphoxide (DMSO) (Sigma chemicals; St. Louis, M. O. USA).

A control group was given normal saline (0.9% w/v NaCl) at 20 ml/kg bw. Mice were observed over 72 h clinical signs and mortality were recorded. LD_{50} was obtained graphically as the intercept of % mortality (yaxis) and dosages (x-axis).

Antiplasmodial screening

Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was necessary to exclude the possibility of test animals harboring rodent *Plasmodium* species. Preliminary antiplasmodial tests with ethylacetate, hexane and methanolic fractions of the plant extracts were conducted in parasitized animals.

Suppressive test

The method by Fidock et al. (2004) was used. It involved treatment with the extract immediately after mice had been inoculated (early infection). Twenty four male and female mice were divided into four groups of six each. A mouse infected with *Plasmodium berghei* (parasitaemia of about 20 to 30%) was anaesthetized with chloroform and its blood collected by cardiac puncture with a sterile syringe and needle earlier flushed with heparin. The blood was diluted with normal saline such that 0.2 ml contained about 1×10^7 infected cells. Each of the twenty four clean mice were inoculated (i.p.) with 0.2 ml diluted blood. The extract at dose levels of 300 and 600 mg/kg bw respectively were administered subcutaneously once daily for four days (D^0, D^1, D^2 and D^3). A parallel test with chloroquine (5 mg/kg bw) in the third group served as reference. The fourth group was given normal saline and served as control. Thick and thin films were made from tail blood from D^1 - D^4, fixed with methanol and stained with 4% Giemsa (pH 7.2) for 45 min before being examined under a microscope. Five fields were examined on each slide and the number of infected and uninfected red blood cells (RBC) counted and means taken. Percentage suppression of parasitaemia was calculated using values from controls related to those of treated animals. Standard drug equivalent was also determined from the ratio of chloroquine (standard) dose to dose of test drug giving identical average percentage suppression.

Prophylaxis

Twelve mice were kept in three groups of four animals each and administered 600 mg/kg bw (i.p) plant extracts for three days. A group was inoculated with *P. berghei* on D4, another on D7 and the third on D14. Equal number of untreated mice was also infected to serve as controls. Tail blood smears were examined from each group on the second and third days post inoculation and for twenty one days subsequently. At the end of this period, bloods from the animals were injected into clean mice which were examined for infection over fourteen days. Percentage suppression of parasites and pyrimethamine (standard) equivalent dose were determined.

Analgesic activity

Analgesic was assessed by the method of Koster et al. (1959). Twenty five mice were divided in five groups. The extract (300/600 mg/kg bw) were administered mice in groups A, B and C an hour before they were challenged with acetic acid (0.75% v/v). Animals in group D were however pretreated with acetyl salicylic acid (150

Table 1. Results of the preliminary antiplasmodial screening of *G. senegalensis* fractions in mice.

Treatment	Dose (mg/kg bw)	Parasitaemia	
		A	B
G senegalensis (e)	600	+++	+++
G senegalensis (h)	600	+++	+++
G senegalensis (m)	600	++	++
Chloroquine	5	+	+
Normal saline	20	+++	+++

m – methanolic extract, e – ethylacetate extract h – hexane extract , a, b – mice of either sex ±slightly present, ++ - moderately present +++ - high present - - absent.

Table 2. *P. berghei* suppression in mice by MeOH Extracts of *G. senegalensis.*

Treatments	Dose (mg/kg bw per day)	Mean parasitaemia	%
G senegalensis	300	50.67±1.23	43.07
G senegalensis	600	29.06±2.11	67.52
CQ	5	15.33±1.12	83.01
N.S[a]	20 ml	89.48±2.25	-

[a]Normal saline, [b]mean ± SEM, n = 6.

mg/kg bw) as reference drug, while group E which were given normal saline (20 ml/kg bw) served as controls. Five minutes elapsed before the numbers of abdominal constrictions induced by acetic acid were counted. Observations were made over ten minutes and mean value for each group calculated. Percentage inhibition of abdominal constriction by the plant extracts at the two doses and ASA were determined in relation to the control. ASA equivalent was also calculated.

Anti-inflammatory activity

The anti-inflammatory activity of the extract was tested using egg albumin induced paw oedema in rats (Winter et al., 1962). Adult rats were divided six per each treatment group and used for the analysis.

Inflammation was induced by the injection of 0.01 ml egg albumin into the sub-planter surface on the right hind paw 30 min after administering the extracts (300/600 mg/kg bw i.p). The increase in volume (cm^3) of the hind paw was measured with a LETICA digital Plethysmometer (LE 7500) before and at 20 min interval after the injection of egg albumin for a period of 2 h. Control rats received an equivalent amount of normal saline while ASA (150 mg/kg bw) served as reference. The percentage inhibition of oedema was calculated for each dose.

Statistical analysis

Results are expressed as mean ± standard error of the mean and the data compared using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

G. senegalensis crude leaf extract yields were 1.38 g (hexane), 1.74 g (ethylacetate) and 1.85 g (methanol) corresponding with 3.45, 4.35 and 4.63% w/w respectively of the original dry leaves. alkaloids, glycosides, tannins and flavonoids were detected.

Safe dose and LD_{50} of crude extract

Doses below 600 mg/kg bw of mice were safe and devoid of adverse clinical symptoms. LD_{50} was determined to be 1100 mg/kg bw.

Antiplasmodial activity

Only crude methanolic extracts were effective against *P. berghei* in mice (Table 1). Other results of the parasitological tests as indicated in Table 2 is the high dose dependent. Suppressive effects of methanolic extracts of *G. senegalensis* leaf extracts against *plasmodium* in mice. The extract had no prophylactic activity (Table 3). Prophylaxis of the extracts against *plasmodium* in mice declined considerably with increased

Table 3. Results of prophylactic tests of *G. senegalensis* against *P. berghei* in mice.

Treatment	Dose (mg/kg bw)	Activity (%)	Pyr. Equivalent	Parasitaemia on sub-inoculation
G senegalensis (D4)	300	20.15	548.25	+++
G senegalensis (D7)	300	12.22	988.66	+++
G.senegalensis (D14)	300	2.45	1654.11	*
Pyrimethamine	150	85.66	-	+
N.S[a]	20ml	0.00	-	+++

[a]Normal saline, [b]mean ± SEM, n = 6,+ - slightly present ,++ - moderately present ,+++ - highly present, * - mortality , - - absent.

Table 4. Inihibition of acetic acid induced abdominal constriction by *G. senegalensis* in mice.

Treatment	Dose (mg/kg bw)	A.C./10min[b]	Inhibition (%)
G senegalensis	300	23.0±1.81	43.35
G senegalensis	600	22.4±1.01	44.83
ASA	150	10.2±1.00	74.88
N.S[a]	20 ml/kg	40.6±3.21	-

[a]Normal saline, [b]mean ± SEM, n = 6, a.c. – abdominal constriction.

Table 5. Effects of *G. senegalensis* in mice paw oedema.

Treatment	Dose (mg/kg bw)	Paw oedema mm[3]	Inhibition (%)
G senegalensis	300	0.68	0.00
G senegalensis	600	0.67	0.00
ASA[a]	150	0.28	58.82
N.S.[b]	20 ml/kg	0.69	-

n=6,[a]ASA – acetylsalicylic acid, [b]NS – normal saline.

number of days hence the high drug equivalent compared to pyrimethamine and the death of the mice under test.

Analgesic assay (Table 4) results of *G senegalensis* exhibited moderate activity in mice irrespective of the dose used. Acetylsalicylic acid equivalent of the extract was low hence favourable. In (Table 5), *G. senegalensis* is shown to possess no antiinflammatory potential in mice. The phytochemicals detected are among some secondary plant metabolites reported to be contained in *G. senegalensis* in the literature. These include mucilagines, tannins, flavonoids, alkaloids and amino acids (Combier et al., 1977; Kaumare et al., 1968). Flavonol aglycones, flavonol glycosides and their acetylated derivatives were also isolated (Makkar and Becker, 1994).

Phytochemicals generally have medicinal potentials and serve in some cases as blueprints for the synthesis of potent drugs (Jigam and Atunde, 2001; Jigam et al., 2010b). Some alkaloids analgesics e.g. morphine; antimalarials e.g. equinine; tranaquilizers e.g. reserpine, etc. Tannins and flavonoids are polyphenols with reported antimicrobial properties. Some glycosides e.g digoxin and digitoxigenin, are useful in cardiac disease (Haidet, 2003; Jigam et al., 2009).

The relative high antiparasitic and analgesia levels of the leaf extracts of *G. senegalensis* explains in part its widespread use in herbal medicine. The extract can hence be standardized and packaged to be used as phytomedicine. Its long term consumption should however be weighed viz-a-viz the likelihood of adverse effects on organs as is the case with some reported plant species (Gamaniel, 2000).

The significant *plasmodial* suppressive effects of *G. senegalensis* in mice are noteworthy. Earlier reports were based mostly on *in vitro* studies and did not specify whether the plant acted directly on the parasite (Etkin, 1997). The extract exhibited poor prophylactic potentials. This conforms to the suggestion that crude plant extracts tended to have better plasmodistatic than plasmodicidal and prophylactic effects (Jigam et al., 2010a). The assertion

could be rationalized on the basis that unpurified bioactive principles require initial conversions which time lag allows for parasite proliferation (Noedl et al., 2003). Moreover, active components might not be present in high enough concentrations in the crude extracts as to effect rapid clearance of target organisms (Fidock et al., 2004).

The analgesic potential detected with *G. senegalensis* explains its reported application in herbal medicine for the treatment of fevers (Zeljan et al., 1998). This effect is also an added advantage to the antiplasmodial effect in the management of malaria infection (Fletcher, 2007). *G. senegalensis* can be better utilized as a herbal component in the management of malaria especially in endemic zones when used in combination with plasmodicidal agents and herbs that boosts packed cell volume. It is also suggested that other organs of the plant other than leaves be similarly analysed.

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