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Antiplasmodial, analgesic and anti-inflammatory effects of crude *Guiera senegalensis* leaf extracts in mice infected with *Plasmodium berghei*

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ABSTRACT**:** *Guiera senegalensis* Gmel (combretaceae) is 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*. Phytochemical screening indicated the presence of alkaloids, glycosides, tannins and flavonoids. The extracts had no adverse effects at 600 mg/kg body weight (bw) with LD_{50} of 2800 mg/kg body weight. *G. senegalensis* extract significantly ($p < 0.05$) suppressed the parasites in mice but exhibited no prophylactic activity. It also exhibited analgesic activity with no anti inflammatory effect.

Keywords; 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 global 500 million acute infections and 3 million deaths annually (Fletcher, 2007; WHO, 2008). Vulnerable groups are mostly pregnant women and children under 5 years. 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. This is more so that viable and relevant vaccines are as of now not yet unavailable against malaria (Jigam *et al.*, 2010a). There is the need, therefore, to evaluate scientifically relevant plant species commonly used in the herbal treatment of the disease (Okunji *et al.*, 2000).

Guiera senegalensis G.mel ("Sabara" in Hausa Nigeria), is a shrub of the savannah region of West and Central Africa (Zeljan *et al*., 1998). The leaves which are 3-5 cm long and 1.5 – 3.0 cm broad, are opposite or subopposite, oblong elliptic, rounded or slightly cordate at the base and mucronate at the apex. In addition, the leaves 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. The usual form of preparation for internal use is decoctions or mixture with food preparations. *G. senegalensis* leaves are widely used for managing pulmonary and respiratory complaints, coughs, colic, diarrhoea, syphilis, beriberi, leprosy, impotence, rheumatism, diuresis and expurgation. It is also claimed to be used as a febrifuge (Hutchinson and Dalziel, 1965; Zeljan *et al.*, 1998). In Northern Nigeria, the powdered leaves are mixed with food as a general tonic and blood restorative and as a galacta gogue in women (Koumare *et al.*, 1968). In Ghana and other West African Countries, the leaves are used to treat dysentery and fever (Abbiw, 1990).

Previous study by Etkin (1997) indicated that the *Guiera* leaf extracts markedly oxidized glutathione and generated high levels of methaemoglobin *in vivo*; 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 properties necessitated *in vivo* screening of the plant with parasitized animals. This is with a view to

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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 identified at the Department of Biological Sciences, Federal University of Technology, Minna

Preparation of crude extracts

A known amount (40 g) of air-dried leaves were micronized 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 was 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 sexes of about 6 weeks old weighing between 20-30 g and Wistar rats of about 180-200 g were obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigerian. The animals were conveniently housed under standard environmental conditions: (Temperature 27 ± 2°C; 70% relative humidity; 12 hrs daylight/night cycle) and 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 the 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).

Acute toxicity and determination of LD_{50}

Five groups of four mice were used. The animals were given the extracts intraperitoneally (i.p) at the doses of 200, 400, 600, 800, 1200 mg/kg body weight respectively. Extracts were dissolved in dimethylsulphoxide (DMSO) (Sigma chemicals; St. Louis, M. O. USA). A control group was administered normal saline (0.9% w/v NaCl) at 20 ml/kg body weight. Mice were observed over 72 hr and clinical signs and mortality were recorded. LD_{50} was obtained graphically as the intercept of % mortality against the dosages.

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.*

Suppressive test

The method described by Fidock *et al* (2004) was used for the suppressive test. Briefly, twenty four male and female mice were grouped into four consisting of six animals each. A mouse infected with *P. berghei* (parasitaemia of about 20-30%) was anaesthetized with chloroform and the blood collected by cardiac puncture with a sterile syringe and needle that had been flushed with heparin. The blood was then diluted with normal saline such that 0.2 ml contained about 1 x 10^7 infected cells. Each of the mice was inoculated (i.p.) with 0.2 ml diluted blood. The extract at the dose levels of 3000 and 600 mg/kg body weight respectively were administered subcutaneously once daily for four days (D0, D1, D2 and D3). A parallel test with chloroquine (5 mg/kg body weight) in the third group served as the reference group. The fourth group was administered normal saline and served as the control. Thick and thin films were made from tail blood from D1 - D4, fixed in methanol and stained with 4% Giemsa (pH 7.2) for 45 minutes 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 computed. Percentage suppression of parasitaemia was calculated using values from controls and relating to those of treated animals. Reference drug equivalent was also determined from the ratio of chloroquine (reference) 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 body weight (i.p) of the 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 the control. Tail blood smears were examined from each group on the second, third and twenty one days post inoculation. At the end of this period, blood from the animals was injected into clean mice which were examined for infection over fourteen days period. Percentage suppression of parasites and pyrimethamine (reference) equivalent dose were determined.

Analgesic activity

Analgesic activity of the extract was assessed by the method of Koster *et al* (1959). Twenty five mice were assigned into five groups. The extracts (300 and 600 mg/kg body weight) were administered to the mice in groups A, B and C, one hour before they were challenged with acetic acid (0.75% v/v). Animals in group D were however pre-treated with 150 mg/kg body weight of acetyl salicylic acid (ASA) as reference drug, while animals in group E which were administered normal saline (20 ml/kg body weight) served as the controls. At the end of five minutes post-treatment, the number of abdominal constrictions induced by the acetic acid was counted. Observations were made over ten minutes and mean value for each group calculated. Percentage inhibition of abdominal constriction by the extracts at the two doses and ASA were determined in relation to the control. ASA equivalent was also computed.

Anti-inflammatory activity

The anti-inflammatory activity of the extract was evaluated using egg albumin induced paw oedema in rats (Winter *et al*., 1962). Adult Wistar rats of either sex were assigned into six each per treatment group. Inflammation was induced following the injection of 0.01 ml of egg albumin into the sub-planter surface on the right hind paw 30 min after administering the extracts (300 and 600 mg/kg body weight, 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 hours. Control rats received an equivalent amount of normal saline while ASA (150 mg/kg body weight) served as reference. The percentage inhibition of oedema was calculated for each dose.

RESULTS AND DISCUSSION

G. senegalensis crude leaf extract yields were 1.38 g (hexane), 1.74 g (ethylacetate) and 1.85 g (methanol) corresponding to 3.45%, 4.35% and 4.63% w/w respectively of the original dry leaves. Alkaloids, glycosides, tannins and flavonoids were detected. The doses below 800 mg/kg body weight of the extract were safe in the mice and devoid of adverse clinical symptoms. LD_{50} was determined to be 2800 mg/kg body weight.

Only crude methanolic extracts were effective against *P. berghei* in mice (Table 1). Other results of the parasitological tests as indicated in Table 2 are the high dose dependent. The plant however produced no prophylactic activity (Table 3). Residual effects declined considerably with increased number of days, hence, the high drug equivalent compared to pyrime thannine and the death of the mice under test.

G senegalensis exhibited moderate analgesic activity in mice irrespective of the dose used. Acetylsalicylic acid equivalent of the extract was low hence, favourable (Table 4). *G. senegalensisi* did not possess antinflammatory potential in mice (Table 5).

The phytochemicals detected were among some secondary plant metabolites reported to be contained in *G. senegalensis* in the literature. These include mucilage, tannins, flavonoids, alkaloids and amino acids (Kaumare *et al.,* 1968; Combier *et al.,* 1977). Flavonol aglycones, flavonol glycosides and their acetylated derivatives have also been reported to be present (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 are analgesics e.g. morphine; anti-malarials e.g. equinine and tranaquilizers (reserpine).

The relative high safety level of the leaf extracts of *G. senegalensis* explains in part its widespread use in herbal medicine. The extract can be standardized and packaged to be used as phytomedicine. The long term consumption

Table 1: Preliminary antiplasmodial screening of *G. senegalensis* extracts

Treatments	**Dose (mg/kg body weight)**	**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: Suppression of *P. berghei* in mice by methanolic extract of *G. senegalensis*

Treatments	**Dose (mg/kg body weight 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]	20ml	89.48±2.25	-

[a]Normal saline [b]mean ± SEM; n = 6

Table 3: Results of Prophylactic Tests of *G. senegalensis* against *P. berghei* in mice

Treatments	Dose (mg/kg body weight)	Activity (%)	Pyrimethamine 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

Treatments	**Dose (mg/kg body weight)**	**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]	20ml/kg	40.6±3.21	-

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

Table 5: Effects of *G. senegalensis* on mice paw oedema

Treatments	**Dose (mg/kg body weight)**	**Paw oedema (mm^3)**	**Inhibition (%)**
G senegalensis	300	0.68	0.00
G senegalensis	600	0.67	0.00
Acetylsalicylic acid	150	0.28	58.82
Normal saline	20 ml/kg	0.69	-

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 effect of G. senegalensis in mice is 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 and 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). In addition, 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 use in herbal medicine for the treatment of fever (Zeljan *et al.,* 1998). This effect is also an added advantage to the antiplasmodial effect (Fletcher, 2007).

G. senegalensis can be better utilized as an herbal component in the management of malaria especially in endemic zones when used in combination with plasmodicidal agents. It is also suggested that other organs of the plant other than leaves be similarly analysed.

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