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Assessment of Anti-Malaria Efficacy of *Azadirachta indica* Leaf Extracts in Albino Wistar Rats

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ABSTRACT

Azadirachta indica (Neem) is variously referred to as "wonder tree", sacred "tree", "Heal All", "Nature's Drugstore", "Village pharmacy" and "Panacea for all diseases". Its anti-malaria efficacy was investigated using hot (100°C) and cold water extracts. Twenty-four albino rats of both sexes (average age: 3months, weight 200-220gram) were divided into three groups of eight rats. Group 1 (the control group) received only feed, group 2 received feed, hot and cold water extracts 6 days before being infected with malaria parasite while group 3 rats were infected with malaria parasite 6 days before receiving hot and cold water extract. Blood samples were collected from the rats through eye puncture with capillary tubes 24hrs and 72hs later and examined for malaria parasite load with giemsa and leishman stained films using tally method of counting. There was no malaria infection in the control and group 2 rats. In group 3, the administered parasite load (+++) was completely eradicated within 72hs. The observed anti-malaria effect of neem leaf suggests a dual role in preventing and eradicating plasmodia activity.

Keywords: Neem leaf, extracts, malaria, treatment, prevention.

INTRODUCTION

Malaria is a mosquito borne disease caused by protozoan parasite of the genus plasmodium and transmitted to human and animal hosts by the bite of blood sucking mosquitoes. It is widespread in tropical and subtropical regions including part of America, Asia and Africa. Each year there are millions of cases of malaria with death rate between one and three million people, mostly young children in sub-Sahara Africa (Mirathy *et al.,* 2011, Keiser et al, 2009). Five species of malaria parasite (plasmodium) exist and each specie can infect man depending on locality or country of residence. The most severe form of malaria is caused by plasmodium falciparum and is the specie common in Nigeria. Malaria caused by plasmodium vivax,

plasmodium ovale or plasmodium malariae is a milder disease that is not generally fatal. Plasmodium knowlesi is a zoonosis that causes malaria in apes but can also infect human (Bledsoe, 2011). Malaria is commonly associated with poverty but it is also a cause of poverty and a major hindrance to economic development (Bremen, 2012).

Neeem is botanically called *Azadirachta indica* and gujarati or dongoyaro in Nigeria. It is a tree in the mahogany family *meliaceae* and native to South Asia. It is one of the two species in the genus *Azadirachta* which grows in hot valley area, semi tropical and tropical regions. Neem is a fast growing tree that can reach a height of 15-20 meters (Mujumda et al 2012). It is evergreen but in severe drought it may shed most or nearly all of its leaves. The branches are wide spread. The fairly dense crown is roundish or oval. The bark is grey or dark reddish brown with numerous scattered tubercles. The bark exudes a gum known as East Indian gum. The leaves are alternate and 20-30cm long. The flower is white or pale yellow, little scented and numerous on long auxiliary panicles with a honey-like scent that attracts many bees. The fruit is an ovoid bluntly pointed smooth drupe, green when young and turns yellow with a thin epicarp (Natarajan et al., 2011).

Rational of Study

Malaria causes about 250 million cases of fever and approximately one million deaths annually (Roestenberg, 2012). It is becoming increasing difficult in many endemic areas to control malaria because of resistance to commonly used anti malaria drugs. Malaria in humans develops via two phases, exo-erythrocytic and erythrocytic phases. The exo-erythrocytic phase involves infection of the hepatic system (liver) whereas the erythrocytic phase involves infection of red blood cells (erythrocytes).

Malaria is at present endemic around the equator in areas of the Americas, many parts of Asia and much of Africa. It is in sub-Sahara Africa where 85-90% of malaria fatalities occur.

The geographical distribution of malaria within large regions is complex and malaria affected and malaria free areas are often found close to each other. In Africa malaria is present in both rural and urban areas. The development of resistance to commonly used drugs poses the greatest threats to malaria control. Thus there is a great need for alternatives that can eradicate drug resistant malaria parasites. This study was aimed to ascertain the efficacy of Neem leaf for the treatment and control of malaria.

METHODOLOGY

Collection of Neem sample

The neem leaves were collected from Agbani in Nkanu West Local Government Area of Enugu State.

Animals

All the experimental animals used for the study were albino wistar rats of both sexes obtained from the Animal house of the University of Nigeria, Nsukka. They weighed between 200-220grams and were housed in a deep lit housing system with adequate ventilation. The rats were fed with growers feed and water was provided. They were allowed to adjust to the environment for one month before the commencement of the experiment. During the period of acclimatization, thorough physical assessments of the rats were carried out and all the animals were handled according to the guidelines for research and evaluation of traditional medicine and the international guiding principles for biomedical research involving animals.

Experimental Design

The rats were separated into 3 groups of 8 rats.

Group 1 (control rats) received only feed.

Group 2 rats were given feed, hot and cold water extracts before they were infected with blood containing malaria parasite.

Group 3 rats were infected with malaria parasite before receiving hot and cold water extracts.

PREPARATION OF EXTRACTS

Hot water Extract

Ten grams of neem leaves were weighed with mettler weighing balance. The leaves were sliced and placed in 100ml of hot (100°C) distilled water in a conical flask and mixed thoroughly. The flask was covered and kept for 5 days at room temperature. The solution was filtered and 94.3ml of the leaf extract collected.

Cold water Extract

Ten grams of the neem leaf sample were weighed, sliced and soaked in 100ml of distilled water in a conical flask at room temperature and kept for 5 days. The solution was filtered and 89.8ml of the leaf extract collected.

Administration of the extracts

The first group of rats (control rats) received only feed and water. In group 2 with 8 rats, 4 of the rats were given the hot extract while the other 4 rats received cold extract. This was carried out with the help of a syringe connected to a thin pipe passed through the mouth down to the stomach. After 6 days, these rats were infected with malaria parasite (Plasmodium falciparum). In group 3, the 8 rats were first infected with malaria parasite. After six days, 4 of the rats received the hot extract while the other 4 rats were given the cold extract either to treat the rats or to reduce the parasite load in the system.

Bleeding

The rats were bled through the eye vein with capillary tubes. Two milliliters (2ml) of blood was collect into ethylenediamine tetra acetic acid (EDTA) tubes.

Initial parasite count with human blood sample

Thin and thick blood films were made on microscope slides and allowed to air dry. The thick film was stained with giemsa stain for 10 minutes while the thin film was stained with leishman stain for 30 minutes. The slides were washed with normal saline, the back cleaned

with cotton wool, air dried and examined with oil immersion objective lens (x 100) of Nikon microscope to count the parasite load per 100 red blood cells using the tally counting method. All the blood samples collected from the rats (control, group 2 and 3) were examined using the same procedure.

RESULTS

The control rats were not infected with malaria parasite. In table 3, the rats given hot and cold water extracts six days before been infected with blood containing malaria parasite did not habour the parasite in their system. In table 4, the rats infected with malaria parasite before receiving the extracts showed reduced quantity (+) of malaria parasite after 24hours from the initial load (+++) of malaria parasite administered and no malaria parasite at all after 72hrs.

Table 1: Malaria parasite load of blood used for infection

Sample	Malaria Parasite Load
Human blood	+++

Table 2: Malaria parasite infection of control rats

Sample	Number of Rats	Malaria Parasite Load
Control rats (No extract)	8	Nil (No infection)

Table 3: Result of group 2 rats given extract before been infected with human blood

Sample	Rats given extract prepared in hot water 6 days before being infected	Rats given cold water exact 6 days before been infected
No of rats	4	4
Initial parasite load administered for infection	+++	+++
Parasite load 24hrs after infection	No infection	No infection

Table 4: Result of group 3 rats infected with malaria parasite before receiving extract

Sample	Rats infected with malaria parasite 6 days before receiving extract papered in hot water	Rats infected with malaria parasite 6 days before receiving cold water extract
No of rats	4	4
Initial parasite load for infection	+++	+++
Parasite load 24hrs after extract administration	+	+
Parasite load 72hrs after extract administration	Nil	Nil

DISCUSSION

Malaria is caused by a eukaryotic protist of the genus plasmodium. The human species plasmodium falciparum is dangerous since it changes the adhesive properties of the cell it inhabits and causes the cell to stick to the walls of blood vessels. It becomes highly dangerous when the infected blood cells stick to the capillaries in the brain, obstructing blood flow and resulting in cerebral malaria. In the pathogenesis, anopheles mosquito infects a person by taking a blood meal; sporozoites enter the blood stream and migrate to the liver.

They infect liver cells (hepatocytes) where they mature into merozoites, rupture the live cells and escape back into the blood stream. The merozoites infect the red blood cells and develop into ring forms, then trophozoites (feeding stage), schizonts (reproductive stage) and back to merozoites. Sexual forms called gametocytes are also produced which if taken up by a mosquito, infects the insect and the life cycle continues (Alkawa, 2013).

The development of resistance to drug poses one of the greatest challenges to malaria eradication. Many drugs are currently in use but resistance still persists. Therefore new approaches or effective alternatives need to be sought. Neem leaf (or dongoyaro) contains many active compounds which include nimbin, nimbidin, nimbidol, gedumin, quercetin and azadirachta which is the main chemical compound (chattopadhyay and Bandypadhay 2011). The finding of this work shows that the cold and hot water extracts of neem leaf posses anti-malaria properties due to the presence of secondary plant metabolites. Wister rats that were given neem leaf extract before being infected with malaria parasite did not develop malaria parasite infection (table 3). This suggests that neem leaf extract could be used as prophylaxis. In table 4, the wistar rats infected with malaria parasite before the administration of neem extract showed significant reduction (+) in malaria parasite from the initial malaria parasite load (+++) after 24hrs and no infection at all after 72hrs. Neem leaf extract greatly increases the state of oxidation in red blood cells which prevents normal development of malaria parasite. Drug resistance has been confirmed in plasmodium falciparum and plasmodium vivax infections (Roestenberg, 2012). Although resistance to malaria drugs tends to vary geographically in some areas of the world, the impact of multi-drug resistant malaria can be extensive.

Neem extracts block the development of gametes in an infected person. Stimulation of the immune system is a major factor in Neem's effectiveness against malaria. The plant lowers the fever (increase in temperature) and increases appetite, enabling a strong body to fight the parasite and recover quickly.

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