Is a Combine Therapy of Aqueous Extract of Azadirachta Indica Leaf (Neem Leaf) and Chloroquine Sulphate Toxic to the Histology of the Rabbit Cerebellum?

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Abstract

Background: Herbal medication is commonly employed in treatment of diseases. Aqueous extract of Azadirachta indica leaf (A. indica) is commonly used in treatment of malaria by Nigerians. Most often, aqueous extract of A. indica leaf is taken in combination with chloroquine in order to cure malaria infection without knowledge of the side effect especially by the rural dwellers in Nigeria.

Objectives: This study is designed to investigate the effects of aqueous extract of A. indica leaf, and concomitant administration of chloroquine phosphate + aqueous extract of A. indica leaf on the Brain tissue (cerebellum) of rabbit.

Methods: Eight adult male Rabbits with average weight range between 1.29kg – 1.52kg obtained from Department of Zoology University of Ekpoma, Edo state were used for this study. They were weighed at intervals of five days before and after the experiment. They were randomly divided into four groups (A – D) of two rabbits each. The chloroquine and aqueous extract of A. indica leaf was administered to the animals orally via a cannula inserted through the oral cavity. They were treated as follows; group A received (100mg ml⁻¹ dry extract solution of aqueous extract of A. indica), group B received (15mg kg⁻¹ of chloroquine sulphate), group C received (100mg ml⁻¹ dry extract solution of aqueous extract of A. indica + 15mg kg⁻¹ of chloroquine sulphate and the control animals (group D) were given normal saline. Both the treatment and control animals were sacrificed at the end of the experiment. The cerebellum was carefully dissected out and immediately fixed in Bouin’s fluid for histological studies.

Results: Groups A-C animals showed normal Cerebellar histoarchitecture and average weight gain of 2.1% (group A), 1.4% (group B), 0.7% (group C) and 1.4% (group D) respectively. When the average weight gain by the treated animals was compared to the average weight gain by the control animals, it was statistically not significant (P>0.06).

Conclusion: Our findings revealed that aqueous extract of A. indica has no effect on the histology of the Cerebellum and weight of adult male rabbits, even when it is administered concomitantly with Chloroquine Sulphate at a reported and recommended safe dose.

Key words: Aqueous extract; Azadirachta indica leaf; Chloroquine sulphate; histoarchitecture; cerebellum; rabbits.
Introduction
The use of herbal preparations in the treatment of malaria is popular in many parts of Africa and Asia where malarial infestation is endemic. Azadirachta indica A.Juss (Meliaceae), in many countries referred to as the neem tree (Dogonyaro) has been extensively reported as being effective in the treatment of malaria caused by various strains of plasmodium, even those resistant to traditional antimalarial drugs. Iwu et al.,(1987),[1] Okpanyi et al.,(2000),[2] Obaseki and Fadunson (1958),[3] Khalid et al.,(1998)[4] and Nokov et al.,(2001)[5] reported that various phytochemical constituents have been isolated from the neem plant and demonstrated to possess antimalarial properties. The use of chloroquine as a single first line drug treatment is now increasingly limited following the evolution of chloroquine–resistant Plasmodium Falciparum. Nevertheless, the low cost and easy availability makes chloroquine the choice for most patients in Africa.[6]

Malaria is a tropical disease that poses serious problems on human well-being, especially in tropical countries where the environment provides the necessary requirements for the parasite to thrive well. It is a global problem because of migration and the mutant potential of the parasite in living organisms. With legal introduction of herbal medications into National Health Schemes, scientists have embarked on researches to validate the potency and efficacy of these herbs, especially A. indica extracts in the treatment of diseases and infections amongst which is malaria. However, some of these plants extracts have the potentials of destroying several organs in the body, especially the liver, Ucheya and Anibeze (1994)[7] which are responsible for detoxification of drugs. Malanei (1986) [8] reported that any substance ingested into the body is capable of altering the normal biological system of the body hence they should be regarded as drugs. He therefore concluded that Herbal plants extracts are drugs because they consist of biologically active compounds.

Many workers had also in the past established the relationship between the use of herbal medications, their curative potentials, beneficial importance and adverse effects, Skeptics.[9] The aqueous extract of neem leaf has been found to offer protection against paracetamol induced liver necrosis in rats. [10] Varying degrees of central nervous system (CNS) depressant activity in mice was observed with the aqueous extract of A. indica leaf.[11] Fractions of acetone extract of A. indica leaf also showed significant CNS depressant activity.[12] Aqueous extract of A. indica leaf up to a dose of 200 mg/kg body weight produces significant anxiolytic activity in rats.[13] It has also been reported that anti-malarial extract from A. indica leaves is anti-retroviral and also has antimalarial activities superior to chloroquine.[14] Reports have indicated its safety on the histology of the liver,[15] if administered at the recommended safe dose (≤ 350mg/kg/day). It has been shown that the indiscriminate use of chloroquine induces the derangement of Brain cells.[16] Furthermore, the interaction between Chloroquine sulphate and aqueous extract of A indica caused a significant decrease in the serum concentration, slower absorption and elimination as well as longer half – life of chloroquine sulphate in rabbit.[17] There has been various reports on the antimalarial and hypoglycemic effect of this extract in rats[18-22] and recently, hypoglycemic effect was observed with leaf extract and seed oil, in normal as well as
alloxan-induced diabetic rabbits.\textsuperscript{[23]} The possible mechanisms underlying the hypoglycemic activity of the aqueous leaf extract have also been discussed.\textsuperscript{[24]}

The above studies reports the various biological active compound contained in A. \textit{indica} leaf, CNS activity, and Hypoglycemic activity, but this work was designed to investigate the histological effects of aqueous extract of A. \textit{indica} leaf on the cerebellum of rabbits when administered alone and when given in combination with chloroquine, since a combined therapy instead of a single therapy is now employed in the treatment of malaria due to drug resistance.

\textbf{Materials And Methods}

\textbf{Materials}
Soxhlet extractor (50ml capacity), Mattler weighing balance, dissecting instruments–set, tissue processing materials (Xylene, Formaline. Alcohol, Rotary microtome, Knife, slides, cover slips, paraffin, and embedding mould. Microscope with digital camera, film mountant. Test chemical– aqueous extract of neem, chloroquine sulphate (May and Baker, products UK), Nivaquine Fort®, a brand of chloroquine sulphate (May and Baker Pharmaceutical PLC, Nigeria), fresh leaves A. \textit{indica} (collected from neem trees at the campus of the University of Nigeria, Enugu Campus. Digital PH meter (P107, Consort, Belgium), ultraviolet-visible spectrophotometer (SP 8-100, Pye Unicam, UK), centrifuge (Beckman GS-15, UK), were used.

\textbf{Animals}
A total of eight healthy adult male rabbits weighing between 1.29 to 1.52kg bought from Zoology Department, Ambrose Ali University Ekpoma, Edo State, Nigeria were used for this study. They were randomly divided into four (4) groups of two Rabbits each. Group A animals were administered aqueous extract of A. \textit{indica} leaf only, Group B animals were administered Chloroquine Sulphate only, group C animals were given a combination of Chloroquine sulphate and aqueous extract of A. \textit{indica} leaf, while group D animals were given feed marsh and saline water ad libitum. They were allowed two weeks for acclimatization and ethical standards of the University were adhered to in the course of this study.

\textbf{Extract Preparation}
The method used by Nwafor \textit{et al.},(2002)\textsuperscript{[16]} was employed. The leaves were cut fresh and allowed to air dry under room temperature. The extract was prepared as follows: fresh leaves of A. \textit{indica} (580g) were thoroughly mashed in distilled water (2 L). The decoction was filtered using a clean sieve cloth. Its concentration was determined by evaporating the extract to dryness. Freshly prepared extracts were used. Concentration of the aqueous extract was found to be 15.25mg mL\textsuperscript{-1} dry extract and PH was %.2, indicating a weakly acidic extract. The extractive yield (expressed as dry mass of the extract relative to the mass weight of the leaves) was determined to be 4.2%. The extract was concentrated so as to prepare 100mg mL\textsuperscript{-1} dry extract solution.

\textbf{Determination of Chloroquine Sulphate}
The method used by Nwafor \textit{et al.},(2002)\textsuperscript{[16]} was employed in this study. Nivaquine Fort® (a brand of chloroquine sulphate) was suspended in 3% Tween85 to give 100mg mL\textsuperscript{-1} suspension. The
animals were given between 0.16 and 0.21 mL of the chloroquine sulphate suspension, depending on their body mass. Both chloroquine sulphate and the extract were administered to the animals via a cannula inserted through the oral cavity. The experiment commenced by administration of 15 mg kg\(^{-1}\) of chloroquine sulphate to each rabbit orally. This dosing was based on the 15mg kg\(^{-1}\) loading dose of chloroquine sulphate in humans Emdex Desk.\(^{[25]}\) The second part of the experiment commenced following the end of the 1\(^{st}\) part by administration of 15 mg kg\(^{-1}\) of chloroquine sulphate orally to each rabbit and 100 mg kg\(^{-1}\) of aqueous extract of A. \textit{indica} was immediately administered orally to each rabbit. This was based on the report that aqueous extract of A. \textit{indica} is safe at a concentration ≤ 350mg/kg/body weight of mice. The animals received between 1.1 and 1.4 ml of the aqueous extract relative to their body mass. The third group (c) was administered 15mg kg\(^{-1}\) of chloroquine sulphate concomitantly with 100mg kg\(^{-1}\) of aqueous extract of A. \textit{indica}. The morphology of the cerebellar structure was compared for the drug administered alone with that administered concomitantly with aqueous extract of A. \textit{indica}. The animals were then weighed and the mean weight for each group was noted. This was done for fifteen (15) days at interval of five (5) days from the time of acclimatization of animals’ up to fifteen (15) days after drug administration. At the end of the fifteenth day after drug administration, the animals were sacrificed by inhalation of chloroform. The cerebellum from each experimental and control animals were immediately extracted for histological processing and production of Photomicrograph. The microscopic features of the cerebellum in the Photomicrographs were assessed by comparing the cell architecture of the treated animals in groups A-C with the cell architecture of control animals in group D.

Significance of the difference between the control and test values was evaluated using Student’s \(t\)-test. This was done using the computer programme ‘Statistical Package for Social Sciences (SPSS)’, version 15.0. P<0.05 was taken as the significant level.

Results

**The effect of aqueous extract of A. \textit{indica} leaf on the histology of rabbit cerebellum**

The control animals showed normal hist-architecture as revealed in the photomicrographs of the animal in group D. This is evidenced in the normal cerebellar cell features which Shows normal cerebellar architecture, with no injury or inflammatory reaction of the grey and white matter (Figure 4).

Histology of the cerebellum of Rabbit treated orally with aqueous extract of A. \textit{indica} only, shows normal cerebellar cell architecture, with no injury or inflammatory reaction of the dendrite, nucleus, perikaryon and axon (Figure 1). Histology of the cerebellar tissue of rabbit treated concomitantly with Chloroquine and Aqueous extract of A. \textit{indica} orally (group C) shows a normal histoarchitecture of the cerebellar tissue and this was demonstrated by the normal appearance of the cerebellar cells (Figure 3).
The Effect of Aqueous Extract of A. Indica Leaf on the Body Weight of the Adult Male Rabbit.

Groups A-C animals showed average percentage body weight gain of 2.1% (group A), 1.4% (group B), 0.7% (group C) and 1.4% (group D) respectively. When the average percentage body weight gain by the treated animals in groups A, B and C was compared to the average percentage body weight gain by the control animals (group D), it was statistically not significant (P>0.05) as shown in Table 1.
Fig. 1: Histology of the cerebellum of rabbit treated orally with aqueous extract of A. indica only. Shows normal cerebellar architecture, with no injury or inflammatory reaction. (Mag.) X400. Stains: hematoxylin & Eosin.
Fig. 2: Histology of the cerebellar tissue of Rabbit (control animals) treated orally with chloroquine. Shows normal cerebellar architecture. (Mag.) X400. Stains; hematoxylin & Eosin.
Fig. 3: Histology of the cerebellar tissue of Rabbit treated concomitantly with Chloroquine and Aqueous extract of A. indica orally. Shows a normal histoarchitecture. (Mag) X400 Stains: hematoxylin & Eosin.
Fig. 4: Histology of the cerebellum of Rabbit of the control Animals. Shows normal cerebellar architecture, with no injury or Inflammatory reaction. (Mag) X400. Stains: hematoxylin & Eosin.
**Table 1:** Showing effects of aqueous extract of Azadirachta indica alone, Chloroquine alone and a combined administration of aqueous extract of A. indica leaf and Chloroquine Sulphate on the weight of Rabbits.

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Extract Administered</th>
<th>Mean Weight Before Drug Administration (Kg)</th>
<th>Mean Weight at 5th day of Drug Administration (Kg)</th>
<th>Mean Weight at 10th day of Drug Administration (Kg)</th>
<th>Mean Weight at 15th day of Drug Administration (Kg)</th>
<th>Mean Weight Difference (kg)</th>
<th>Percentage Weight Gain (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aqueous Extract of A. indica</td>
<td>1.41 ± 1.3</td>
<td>1.42 ± 1.3</td>
<td>1.44 ± 1.1</td>
<td>1.44 ± 0.3</td>
<td>0.03 ± 1.0</td>
<td>*2.1%</td>
</tr>
<tr>
<td>B</td>
<td>Chloroquine sulphate</td>
<td>1.43 ± 1.4</td>
<td>1.43 ± 1.4</td>
<td>1.44 ± 3.1</td>
<td>1.45 ± 2.1</td>
<td>0.02 ± 0.7</td>
<td>*1.4%</td>
</tr>
<tr>
<td>C</td>
<td>Chloroquine sulphate + A. indica</td>
<td>1.48 ± 0.4</td>
<td>1.48 ± 0.4</td>
<td>1.48 ± 0.4</td>
<td>1.49 ± 0.3</td>
<td>0.01 ± 0.1</td>
<td>*0.7%</td>
</tr>
<tr>
<td>D</td>
<td>Control (Saline water)</td>
<td>1.43 ± 1.4</td>
<td>1.44 ± 2.2</td>
<td>1.44 ± 3.1</td>
<td>1.45 ± 2.4</td>
<td>0.02 ± 3.4</td>
<td>1.40%</td>
</tr>
</tbody>
</table>

*Statistically insignificant compared to the value of control Mean ± S.D (*P>0.05*)

The weight gained was statistically insignificant when compared to the control at P>0.05

**Discussion**

The results obtained from this study showed that the treated group (A) animals that received aqueous extract of A. indica orally, presented with a normal histoarchitecture of the cerebellum (fig. 1) and weight gained of 2.1% was also recorded for this group of animals (Table 1). This finding is in consonant with a study carried out by the author,[15] which reported that aqueous extract of A. indica is safe on the histology of the liver at a conc. ≤350mg/kg/body weight. The treated group (B) animals that were administered chloroquine sulphate also showed normal cerebellar architecture (Fig. 1) and a percentage increase in body weight of 1.4% (Table 1). This however supports the findings that chloroquine is safe on the organ when used at a normal or safe recommended dose but when administered indiscriminately without prescription, it is capable of inducing derangement of the Histology of the brain tissues.[16] The treated group (C) animals that were
given chloroquine sulphate + aqueous extract of A. indica also showed a normal cerebellar architecture (Fig. 3) and an increase in body weight were also recorded (Table 1). This however contradicts another report [16], which shows that a concomitant administration of chloroquine and aqueous extract of A. indica caused a significant decrease in the serum concentration, slower absorption and elimination as well as longer half – life of chloroquine sulphate.

This present investigations shows that even though a concomitant administration of chloroquine and aqueous extract of A. indica is capable of altering the pharmacokinetic properties of serum level and chloroquine in blood plasma, it has no effect on the histology of the cerebellum when administered at a recommended safe dose.

From our present findings, we suggest that before a concomitant therapy of aqueous extract of A. indica and chloroquine sulphate can be employed in the treatment of malaria, further researches should be carried out in order to confirm its safety on the other organs in the body, and how its effects on the Pharmacokinetics properties in the serum can be prevented.

Conclusively, even though A. indica leaf has been reported consist of biologically active substances,[5-8,23,24] these substances apparently have no effect on the histology of the rabbit brain when administered at a reported/recommended safe dose. It is recommended that further studies be carried out to corroborate these findings.

Acknowledgement
I am grateful to my colleagues, Dr. Iyare E., Dr. Eweka E., and Mr. Nwosu E. O. for their assistance during the preliminary stage of this work and in the preparation of the micrographs.

References


