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**Original Research Article**

# Medicinal Potency of Lime *(Citrus aurantifolia)* Juice and Ogogoro (Local gin) Mixture on Ascariasis and its Assessment on Hepato-Renal and Inflammatory Biomarkers in Ikotun Egbe Lagos State Nigeria

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## Abstract

Ascariasis is an infection of the small intestine caused by *Ascaris lumbricoides*. The aim of this study was to evaluate the medicinal potency of lime (*Citrus aurantifolia*) juice and ogogoro (Local gin) mixture on ascariasis as well as assessing its effects on hepato-renal and inflammatory biomarkers. Thirty primary school children whose stool samples upon microscopical examination at x40 were found to have 2+ ova of *Ascaris lumbricoides* (experimental group) and another thirty whose stool samples had no ova of *Ascaris lumbricoides* (control group) were recruited for this study. Each of the subjects in the experimental group was administered orally with ten milliliters of the mixture twice daily for five consecutive days. Two weeks after the completion of this oral administration, their stool samples were re-examined microscopically using x40 objective and it revealed 70% significant therapeutic response to *Ascaris lumbricoides* i.e. zero ova. Thereafter, five milliliters of blood specimens were collected into lithium heparin anti-coagulated bottles from each subject in the control and experimental groups respectively. These specimens were spun and the obtained plasma used for the quantitative measurement of hepato-renal and inflammatory biomarkers which revealed no statistically significant differences ($p>0.05$) when compared. In conclusion, this study has given an insight on the medicinal potency of this mixture in the treatment of ascariasis and its use is therefore recommended.

**Keywords:** Medicinal potency, lime (*Citrus aurantifolia*) juice, ogogoro (Local gin), Ascariasis, primary school children, Lagos State.



## INTRODUCTION

Ascariasis is an infection of the small intestine caused by the accidental ingestion of *Ascaris lumbricoide* eggs [1]. This infection is associated with symptoms such as severe abdominal pain, fatigue, vomiting, weight loss, malnutrition, worm in stool or vomit etc. Approximately 807 million-1.2 billion people are infected with this roundworm globally [2] which is a significant clinical problem particularly in developing countries where it is responsible for morbidity and mortality in adults and children [3].

Lime which is scientifically known as *Citrus aurantifolia* is a word derived from French. It is also known as lima in Arabic and lemu in Persian. It is a hybrid citrus fruit which is round and green in colour, measuring 3-6 centimeters in diameter and containing acidic juice vesicles [4, 5]. This fruit which is sour and rich in vitamin C is used as food flavor as well as beverages [6]. It was first planted in Indonesia from where it was transported to the Mediterranean region and North Africa around 1000C.E. [5] with a world production rate of 19.4 million tones in 2018 [7]. Its juice is squeezed from either fresh limes or purchased in bottles as unsweetened and sweetened varieties [5]. In the 19$^{th}$ century the British sailors were given a daily allowance of lime as a preventive measure of scurvy, thus were nicknamed "Limey" [8, 9].

Ogogoro is an alcoholic local gin that is common in West Africa and nicknamed in Nigeria as kparaga, akpeteshie, push me I push you, wuru, kaikai, sapele water, ufofobi, baba erin, oyinbogo, robirobi etc. This local gin is distilled from the juice of raphia palm tree via local fermentation. This distillation involved incision of the tree trunk with a gourd placed by it for

collection of the juice after 1-2 days followed by its extraction and boiling of the sap which forms steam that condenses and finally collected for consumption [10]. This local gin contains 30-60% ethanol as its active ingredient and is presumably dangerous to human health when consumed in large quantity leading to intoxication and neurotoxicity [11].

Helminthes infections occur mostly in the tropical regions worldwide [12] and is one of the commonest causes of infant and childhood mortality in developing countries [13] thus de-worming is strongly advocated for in both children and adults [17-21]. It is on this basis that this study which is aimed at the medicinal potency of lime (*Citrus aurantifolia*) juice and ogogoro (Local gin) mixture on ascariasis and its assessment on hepato-renal and inflammatory biomarkers was embarked on with a view that any updated information will go a step further in the scientific exploration of the mixture to the advantages of man taking into consideration the gradual use of herbs in treating diseases in many countries worldwide [22].

## MATERIALS AND METHODS

Thirty primary school children within the age range of 7-12 years whose stool samples were found to have 2+ ova of *Ascaris lumbricoides* infection upon microscopical examination using x40 objective (experimental group) were recruited for this study while another thirty primary school children within the same age range who had no evidence of *Ascaris lumbricoides* infection upon microscopical examination of their stool sample using x40 objective served as control group. This study which was carried out in strict compliance with the principle of Helsinki declaration of 1975 as revised in 2008 got the approved parental verbal informed consent from all the recruited primary school children as well as the Authorities of Foundation Nursery, Primary School Ikotun Egbe which the school children attend.

After this each of the primary school children in the experimental group who was free from anti-helminthic therapy before the commencement of the study and had 2+ ova of Ascaris *lumbricoides* after the examination of their stool samples microscopically using x40 objectiive orally administered with 10 milliliters of the mixture (limejuice and local gin) daily for a period of 5 consecutive days, followed by the collection of their stool samples two weeks after the completion of the oral administration of the mixture and re-examined for the presence of ova of *Ascaris lumbricoides* infection microscopically using x40 objective. Subsequently five milliliters blood specimens was withdrawn from each of them (experimental group) as well as each of the primary school children that were monitored as controls (control group) into lithium heparin anti-coagulated bottles respectively. The specimens were mixed gently to prevent clotting and spun at 2500 revolution/minute for 10 minutes using a macro centrifuge. The plasma obtained was used for the quantitative measurement of liver enzyme biomarkers: alanine aminotransferase according to the colorimetric method as described in the manual of 11th February, 2009 revised edition of Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom [14], aspartate aminotransferase according to the colorimetric method as described in the manual of 5th January, 2007 revised edition of Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom [14], and alkaline phosphatase according to the colorimetric endpoint method as described in the manual of September, 2001 revised edition of Teco Diagnostics, 1268 N, Lakeview Avenue, Anaheim, CA 92807 1-800-222-9880 [14], renal biomarkers: urea according to the urease Berthelot's method as described in the manual of 7th January, 2011 revised edition of Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom [15] and creatinine according to Jaffe reaction method previously described by Jaffe in 1886 and revised on the 15th September, 2010 by Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom [15], inflammatory biomarker: C-reactive protein according to Latex turbidimetry method using reagents as described by Spin-react Diagnostic, Spain [16]. The demographic data collected from all the *Ascaris lumbricoides* infected primary school children (experimental group) and the uninfected primary school children (control group) included age, gender, weight and preferred therapy. The adverse reactions to the mixture were also assessed in the primary school children infected with ova of *Ascaris lumbricoides* (experimental group).

### Preparation of Mixture

Two hundred lime (*Citrus aurantifolia*) oranges purchased in a local market in Mushin, Lagos state, Nigeria were thoroughly washed with distilled water and wiped with a clean towel to dryness. The oranges were peeled with the aid of a sterile knife, thereafter each of them was cut into two parts horizontally and the juice squeezed manually into a clean sterile container and filtered using a clean white handkerchief. 4.0 liters of the filtered juice was added to 1.0 liter of the ogogoro (local gin) and the content shaken thoroughly in order to ensure homogenous mixture.

### Statistical analysis

The demographic data obtained from the recruited infected primary school children (experimental group) and un-infected primary school children (control group) via questionnaire were analyzed using descriptive statistic of frequency and percentage while the results obtained from the quantitative measurement of their plasma biochemical parameters were expressed as mean and standard

deviation with the differences between the groups compared using the student's 't' tests. A p-value of $p<0.05$ was considered statistically significant

## RESULTS AND DISCUSSION

Organisms that live inside humans or other organisms who act as hosts are referred to as parasites. These organisms which are unable to produce food and energy for themselves are solely dependent on their hosts for survival, thus making them harmful to their hosts with the subsequent resultant morbidity as seen all over the world.

**Table-1: Demographic data of the *Ascaris lumbricoides* infected primary school children (experimental group) and the uninfected (control group).**

| Variables | Frequency | | Percentage | |
|---|---|---|---|---|
| | Experimental (n=30) | Control (n=30) | Experimental (n=30) | Control (n=30) |
| **Study population** | | | | |
| Male | 15 | 15 | 50 | 50 |
| Female | 15 | 15 | 50 | 50 |
| **Age** | | | | |
| 7-12years | 30 | 30 | 100 | 100 |
| **Weight of subjects** | | | | |
| 30-45kg | 30 | 30 | 100 | 100 |
| **Are you currently on any anti-helminthes** | | | | |
| Yes | 0 | 0 | 0 | 0 |
| No | 30 | 30 | 100 | 100 |
| **How long have you de-wormed** | | | | |
| ≤11months | 0 | 30 | 0 | 100 |
| ≥1year | 30 | 0 | 100 | 0 |
| **Preferred anti-helminthes therapy** | | | | |
| Herbal | 0 | 0 | 0 | 0 |
| Modern | 30 | 30 | 100 | 100 |
| **Ova of *Ascaris lumbricoide* seen in stool before the oral administration of the mixture** | | | | |
| 1+ | 0 | 0 | 0 | 0 |
| 2+ | 30 | 0 | 0 | 100 |
| **Ova of *Ascaris lumbricoide* seen in stool after the oral administration of the mixture** | | | | |
| Nil | 21 | 0 | 70 | 0 |
| 1+ | 9 | 0 | 30 | 0 |
| 2+ | 0 | 0 | 0 | 0 |

Key: n=Number of subject

As shown in Table 1 the demographic data revealed gross infection of *Ascaris lumbricodes* among the primary school children in Ikotun Egbe Lagos State Nigeria, this is as established in this study. The data further revealed that all the primary school children recruited for this study have not been on anti-helminthes for a period of ≥1 year. This however, is presumed to be responsible for the gross infection of *Ascaris lumbricoides* among the primary school children as established. The data which also revealed 100% usage of modern anti-helminthes therapy among the primary school children as compared with the herbal therapy as shown in Table 1 may be suggestive that the usage of herbal anti-helminthes therapy for *Ascaris lumbricoides* is unkown and this may be associated with its unavailability in the study area as established. Thus findings from this study may influence the scientific exploration of this mixture to the advantages of man as well as influence its gradual use in the treatment of *Ascaris lumbricoide*s

The results as seen in Table 1 further revealed that there was a significant reduction in the infection with ova of *Ascaris lumbricoides* from 2+ to nil in 70% of the primary school children in the experimental group after the oral examination of mixture. This may be suggestive of the medicinal potency of this mixture against *Ascaris lumbricoides* as established in this study.

**Table-2: Adverse reactions associated with the administration of this mixture**

| Adverse reactions | Number of reactants (n=30) | Percentage of reactants |
|---|---|---|
| Gastrointestinal tract anorexia | 0 | 0 |
| Abdominal cramps | 0 | 0 |
| Nausea | 0 | 0 |
| Vomiting | 0 | 0 |
| Diarrhea | 0 | 0 |
| Dizziness | 0 | 0 |
| Rash | 0 | 0 |

Key: n=Number of study subjects

As shown in Table 2, the administration of the mixture revealed that it was well tolerated in the primary school children as none of them showed adverse reactions such as gastrointestinal tract anorexia, abdominal cramps, nausea, vomiting, diarrhea, dizziness and rash. This finding is as established in this study

**Table-3: Hepatic (enzymes) biomarkers of control and experimental groups**

| Parameters | Control (n=30) | Experimental (n=30) | p-value | Remark |
|---|---|---|---|---|
| ALT (U/I) | 9.50 ± 1.04 | 10.00 ±1.08 | p>0.05 | NS |
| AST (U/I) | 8.97 ± 0.95 | 9.02 ± 0.97 | p>0.05 | NS |
| ALP (IU/L) | 12.10 ± 1.78 | 12.20 ± 1.82 | p>0.05 | NS |

Values are expressed as mean ± SD
KEYS:
ALT= alanine aminotransferase
AST=aspartate aminotransferase
ALP=alkaline phosphatase
n= number of subjects
NS= not statistically significant

The liver is an organ responsible for the metabolism of drugs and toxins thus the quantitative measurement of its enzyme biomarkers was considered vital in this study. As shown in Table 3, comparison was made between the mean values of liver enzyme biomarkers: alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in the experimental group and that of the control group. The results revealed no statistical significant differences (p>0.05) in the experimental group as compared with that of the control group. This finding as established in this study may be suggestive that the administration of this mixture has no adverse effects on the liver.

**Table-4: Renal biomarkers of control and experimental groups**

| Parameters | Control (n=30) | Experimental (n=30 | p-value | Remark |
|---|---|---|---|---|
| Urea (mmol/L) | 7.12 ± 1.78 | 7.16 ±1.80 | p>0.05 | NS |
| Creatinine (µmol/L) | 60.00 ± 1.27 | 60.15 ± 1.29 | p>0.05 | NS |

Values are expressed as mean ± SD
KEYS
n= number of subjects
NS= not statistically significant

The kidneys are organs responsible for the removal of waste products of metabolism thus the quantitative measurement of renal biomarkers such as urea and creatinine was considered vital in this study. As shown in Table 4, comparison was made between the mean values of renal biomarkers: urea and creatinine in the experimental group and that of the control group. The results revealed no statistical significant differences (p>0.05) in the experimental group as compared with that of the control group. This finding as established in this study may be suggestive that the administration of this mixture has no adverse effects on the kidneys.

**Table-5: Inflammatory biomarker of control and experimental groups**

| Parameters | Control (n=30) | Experimental (n=30) | p-value | Remark |
|---|---|---|---|---|
| CRP (mg/L) | 9.50 ± 1.04 | 10.00 ±1.08 | p>0.05 | NS |

Values are expressed as mean ± SD
KEYS
CRP= C-reactive protein
n= number of subjects
NS= not statistically significant

C-reactive protein was also considered vital to measure in this study with a view to assess any inflammatory disorder that may manifest in the primary school children during the administration of this mixture. As shown in Table 5, comparison was made between the mean values of inflammatory biomarker: C-reactive protein in the experimental group and that of the control group. The results revealed no statistical significant differences (p>0.05) in the experimental group as compared with control group. This established finding may be suggestive that the administration of this mixture has no adverse inflammatory effects on human organs.

## CONCLUSION

In conclusion, this study has revealed the medicinal potency of lime (*Citrus aurantifolia*) juice and ogogoro (local gin) mixture on Ascariasis with no resultant health implications.

## RECOMMENDATIONS

(i) This mixture is recommended for the treatment of *Ascaris lumbricoides* infection particularly in children between the age of 7-12 years in the study environment.

(ii) Further study should be carried out to ascertain the medicinal potency of this mixture in the treatment of *Ascaris lumbricoides* as related to dose and age outside 7-12 years.

### COMPLIANCE WITH ETHICAL STANDARDS

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**Disclosure of conflict of interest**

We the authors declare no conflict of interest.

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