CHANGES IN SERUM GLUCOSE AND LIPID PROFILE AS INTRAOCULAR PRESSURE BIOMARKERS IN PLASMODIUM BERGHEI INFECTED MICE TREATED WITH AQUEOUS LEAF EXTRACT OF NAUCLEA LATIFOLIA

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ABSTRACT

*Nauclea latifolia* has been shown to have hypocholesterolaemic and hypoglycaemic effect, and cholesterol and glucose have been implicated to play significant roles in the pathogenesis of glaucoma. This study investigates the serum glucose and lipid profile of *Plasmodium berghei* infected mice treated with aqueous leaf extract of *Nauclea latifolia*. Adult albino male mice, 8 weeks old, weighing 12g-25g and divided into 6 groups of 5 mice per group were used for the experiment. Mice were inoculated intraperitoneally with 0.1ml parasitized blood suspension and parasitaemia assessed by thin blood films stained with Geimsa stain. Aqueous leaf extract of *Nauclea latifolia* was orally administered at different doses (200mg/kg. body weight and 300mg/kg body weight daily) to both normal and *P. berghei* infected mice for a period of 4 days. Serum glucose, triglyceride, high density lipoprotein and cholesterol levels were estimated. Significant (p<0.05) reduction in serum glucose and cholesterol was revealed in the untreated parasitized control when compared with other groups but no significant change (p>0.05) in serum triglyceride and high density lipoprotein were observed in parasitized control when compared with normal control mice. However, oral administration of *Nauclea latifolia* significantly (p<0.05) maintained the cholesterol and glucose levels in the infected mice toward the normal value when compared with the untreated parasitized mice with no significant change in the triglyceride and high density lipoprotein levels. These results suggest that aqueous extract of *Nauclea latifolia* may not cause or lead to the increase in the intraocular pressure (glaucoma) both in normal and malaria infected mice.

**Keyword:** Plasmodium berghei, Glucose, Cholesterol, TAG, HDL and Nauclea latifolia.

INTRODUCTION

Malaria is an endemic parasitic infection in Africa and remains a contributing factor to morbidity and mortality to many populations in the world in general and Africa in particular. About 40% of the world population is at risk of malaria infection (W.H.O, 2007). Almost all the estimated over one million deaths from malaria infection each year worldwide especially in sub-Saharan Africa is attributed to *Plasmodium falciparum* (Wadie, 2002). Severe malaria is almost exclusively caused by *Plasmodium falciparum* in humans, but other forms of *Plasmodium* include *P. vivax, P. ovale, P. malariae and P. Knowlesi*. Any infection, including malaria activates the immune system of the body causing the release of reactive oxygen species (ROS), which can attack the plasma membrane of the erythrocyte compromising its integrity (Kulkarni *et. al.*, 2003). In the early times, plants were a vital source of raw material for medicines. Techniques have been developed to produce synthetic replacements for many of the medicines that had been derived from the forest. But recently, problems with drug resistant microorganisms, side effects of modern drugs, and emerging diseases where no medicines are available, have encouraged an interest in plants once again as a significant source of new medicines. Modern-day researchers are coming to appreciate fully the vast medicinal knowledge of the trado-medical practitioners. *Nauclea latifolia* is used profusely by traditional medicine practitioners. The leaves are used for the treatment of malaria in East Africa (Kokwaro, 1976) and in Nigeria (Akubue and Mittal, 1982). *N. latifolia* is active against
Plasmodium falciparum (Benoit-Vical et al., 1998; Traore et al., 2000; Gidado et al., 2004) and also has hypotensive effect (Gidado et al., 2004). Aqueous extracts of N. latifolia have been shown to contain sugars, saponins, polyphenol and flavonoids (Nworgu et al. 2008) alkaloids, anthraquinones, terpenoids, tannins (Wagner, and Ulrich-Merzenich, 2009). Saponins and flavonoids have been reported to have hypotensive activities in laboratory animals (Segesaka-Mitaane et al., 1996; Peng, 1999). Disease conditions which are accompanied by changes in the composition of blood could give rise to an elevation or depression of the intraocular pressure (Orban et al., 1966). Blood lipids play an important role in the patho--mechanism of glaucoma and cataracts (Gupta et al., 2012).

This study attempts to investigate the effect of N. latifolia on serum glucose and lipid profiles as intraocular pressure biomarkers in Plasmodium berghei infected mice.

MATERIALS AND METHODS
Collection of Nauclea latifolia

Nauclea latifolia was harvested behind Sudoz Nigeria limited (Fuel Filling Station) in neighbourhood of Abraka, in Ethiope East Local Government Area of Delta State, Nigeria. The leaf was identified to the species level at the Forest Research Institute of Nigeria, Ibadan, Nigeria, where a copy of the leaf is deposited.

Preparation of extract

Nauclea latifolia leaves (50g) was weighed, sliced into pieces and then boiled in 1000 ml of distilled for 30 minutes, and then allowed to cool and filtered. Preparation was done in accordance to the local consumption within the Ethiope East Local Government Area of Delta State, Nigeria.

Experimental animals and care

Thirty albino mice of mixed sexes weighing between 12-26 grams, 8 -12 weeks old purchased from the Nigeria Institute of Medical Research (NIMR), Yaba-Lagos, Nigeria. The animals were fed on growers mash obtained from Top-Feeds, Sapele, Delta State, and were given water ad libitum. The animals were housed in transparent polypropylene cages lined with wood chip bedding under controlled conditions of 12 hours light/12 hours dark cycle. The animals used in this study were maintained in accordance with the guidelines approved by the Animal Ethical Committee, Delta State University, Abraka.

Inoculation of animals

The mice were infected with parasites (P. berghei) by obtaining parasitized blood from the cut-tip of the tail of an infected blood (3 to 4 drops) and diluted in 0.9 ml phosphate buffer, pH 7.4. The mice were inoculated intraperitoneally with One hundred microlitre (100µl or 0.1ml) parasitized suspension. Parasitaemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Geimsa stain. Inoculation was carried out in the Biochemistry Laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

Experimental design

After the confirmation of parasitaemia, the infected (parasitized) and non-infected (normal) mice were divided into six groups of 5 mice per group and treated as follows: Group A: Normal control; Group B: Parasitized control; Group C: Parasitized mice + N. latifolia (200 mg/kg b. wt); Group D: Parasitized mice + N. latifolia (300 mg/kg b. wt); Group E: Normal mice + N. latifolia (200 mg/kg b. wt); Group F: Normal mice + N. latifolia (300 mg/kg b. wt). The administration of the extract was carried out using an automated micropipette (as an improved oral cannula) for a period of four days. On the fifth day mice were made to starve overnight, sacrificed by partial decapitation and the blood and eyes were collected for various biochemical estimations.

Preparation of eye-tissue homogenate

The wet eye was homogenized in 0.5 ml of freshly prepared normal saline and then centrifuged at 3000rpm for 10 minutes. The
supernatant obtained was used for this experiment. This was done on ice to avoid denaturation of biological content.

**Biochemical investigations**

The biochemical investigations in ocular humour homogenate samples were carried out with the following using commercially available kits as supplied by TECO Diagnostic, Anaheim, USA: Cholesterol and HDL levels was determined by Cholesterol Oxidase method and Randox Reagent kit for triglyceride via triglyceride Oxidase method.

**Statistical analysis**

The results were expressed as Means ± Standard Deviation. Level of significance was assessed by One-way ANOVA (Analysis of variance) and those with a significant difference were further analysed using Student’s t-test to know the groups that had the significant differences. A P-value of 0.05 was considered as statistically significant.

**RESULTS**

The result in the table 1 above, showed that there is significant difference (P<0.05) decrease in the level of serum cholesterol and glucose of untreated parasitized mice treated when compared with other groups. Administration of the extract with the parasitized mice in a dose dependent manner significantly (p<0.05) increased the serum cholesterol and glucose to near normal. No significant (p>0.05) change was observed triglyceride and High Density Lipoprotein (HDL) levels.

**Table 1:** Changes in Serum Triglyceride (TAG), Cholesterol (CHOL) and High density Lipoprotein (HDL) levels in both parasitized and non-parasitized mice treated with *Nuclea latifolia*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum TAG (mg/dL)</th>
<th>Serum HDL (mg/dL)</th>
<th>Serum CHOL (mg/dL)</th>
<th>Serum Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>130.00±21.22</td>
<td>88.89±11.68</td>
<td>198.63±23.71</td>
<td>9.46±1.32</td>
</tr>
<tr>
<td>Group B</td>
<td>117.27±20.43</td>
<td>65.00±16.40</td>
<td>140.51±25.26*</td>
<td>5.95±0.61*</td>
</tr>
<tr>
<td>Group C</td>
<td>107.18±28.02</td>
<td>68.00±12.81</td>
<td>166.90±28.83</td>
<td>8.01±1.72</td>
</tr>
<tr>
<td>Group D</td>
<td>114.73±30.76</td>
<td>72.78±24.45</td>
<td>169.92±21.93</td>
<td>8.79±1.18</td>
</tr>
<tr>
<td>Group E</td>
<td>131.82±24.55</td>
<td>70.19±24.24</td>
<td>176.84±23.85</td>
<td>9.29±2.27</td>
</tr>
<tr>
<td>Group F</td>
<td>128.18±21.56</td>
<td>80.84±18.38</td>
<td>186.84±25.26</td>
<td>9.53±2.01</td>
</tr>
</tbody>
</table>

*Values are expressed as means ± SD with n = 5. The column with * have a significant difference (P<0.05) from each other.*
DISCUSSION

In the origin of acute glaucoma attack, the neurovascular circulation plays an important role with a rise in blood lipid concentration (Hanisch et al., 1966). An elevation of blood lipid concentration is generally known in other vascular crises. Researchers have pointed out an elevated lipid concentration in the blood and that the clinical improvement in the condition of the patient is closely accompanied by a reduction in different lipid fractions in the blood (Goth and Blumenfeld, 1964). However, blood cholesterol content of glaucoma patients corresponded to that of healthy individuals (Omale and Haruna, 2011). From this study as shown in Table 1, there was no significant difference (p>0.05) in serum triglyceride (TAG) and HDL cholesterol of the parasitized mice treated with the extract from the normal control. The possible reason might be due to the presence of bioactive agent such as saponins and flavonoids which have been shown to have antihypercholesterolemia and hypotensive effects (Segesaka-Mitaane et al., 1996; Peng, 1999). As regards the other lipid fractions like serum triglycerides and serum cholesterol, similar observations have been made. The correlation between intraocular pressure and blood sugar has been shown by various authors (Armstrong et al., 1960; Lieb et al., 1967; Becker, 1971). It was observed from this research (Table 1) that serum glucose and cholesterol were significantly different (p<0.05) in the groups treated the extract of *N. latifolia* when compared with the *Plasmodium berghei* treated group alone but no significant different (p>0.05) was seen in extract treated groups from the control (non-infected). No specific association between diabetes and glaucoma has been found because only slightly higher levels of blood sugar were registered after producing glaucoma (Bouzas et al., 1971). On the basis of these various biochemical changes occurring in blood in glaucoma, it was concluded that the rise in various lipid fractions especially serum FFA may be associated with acute attack of glaucoma since the rise and fall of the biochemical fractions closely corresponded with the rise and fall in intraocular pressure (Gupta et al., 2012). This study reveals that effect of *N. latifolia* on serum glucose and lipid profiles may not be a threat or contribute to intraocular pressure in *Plasmodium berghei* infection, as it tends to maintain these investigate biomarkers to near normal values.

REFERENCES


