ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Effect of Ethanol Leaf Extract of *Moringa oleifera* on the Lipid Profile of Malaria Infected Mice

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ABSTRACT

The effect of ethanol leaf extract of *Moringa oleifera* on the lipid profile of malaria induced mice was analysed. The experimental animals were divided into six groups with each group consisting of four mice.Groups 1 (positive control) and 6 (negative control) were treated with 5mg/kg body weight of distilled water, group 5 (standard control) was treated with 5mg/kg body weight of artesunate while groups 2 ,3 and 4 were treated with 45,90 and 180 mg/kg body weight of *Moringa oleifera* ethanol leaf extract .The result shows that the mean total cholesterol for mice in groups 2,3,4 and 5 were non-significantly ($p>0.05$) lower than the values for mice in groups 1 (Positive control) and 6 (negative control).There was a non-significant ($p>0.05$) increase in the high density lipoprotein (HDL) concentration of mice in all the test groups administered graded doses of the extract (45,90 and 180 mg/kg body weight) when compared to the HDL concentration of mice in the three control groups (positive ,negative and standard). The low density lipoprotein (LDL) concentration of mice in groups 2, 3, 4 and 5 were non-significantly (p $p>0.05$) lower than that for mice in group 1 (positive control) and 6 (negative control). Triacylglycerol (TAG) concentrations of mice in groups 2, 3, and 5 were non-significantly ($p>0.05$) lower than the value for mice in group 1 (positive control),4 and 6 (negative control).

Keywords: *Moringa oleifera,* Total cholesterol, Triacyglycerol, High density lipoprotein, Low density lipoprotein , Malaria.

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ISSN: 0975-8585

INTRODUCTION

Cardiovascular diseases remain the biggest cause of deaths worldwide, though over the two decades, cardiovascular mortality rates have declined in many high income countries. At the same time cardiovascular deaths have increased at an astonishingly fast rate in low income and middle income countries [8]. Although cardiovascular diseases usually affect adults, the antecedents of cardiovascular disease, notably atherosclerosis begins early in life, making primary preventive efforts necessary from childhood [6].

Malaria is a parasitic disease that is caused by plasmodium which is also a killer disease in the low and middle income countries .Malaria has been and still the major cause of human morbidity and mortality [3]. It is directly responsible for one in five childhood deaths in Africa and indirectly contributes to illnesses and deaths from other diseases [10].

Moringa oleifera has long been used in the Ayurvedic traditional medical practice in India to combat cardiovascular ailments and obesity [9]. Cholesterol is essentially needed for the building and repairing of cells within the body especially in the synthesis of hormones like estrogen and testosterone. There are two basic types of cholesterol, which include the low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs). They play different roles in body. The high-density lipoproteins (HDLs) help in the elimination of fatty deposits from the bloodstream, thereby enhancing cardiovascular health, healthy veins and arteries [7]. The low-density lipoproproteins (LDLs) which are better called the bad cholesterol have nearly the opposite effects on the body, causing lipid deposits in the blood vessels and consequently leading to heart disease, stroke and other cardiovascular diseases [10].

This study was designed to determine the effect of ethanol leaf extract of *Moringa oleifera* on the lipid profile of malaria induced mice.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Moringa oleifera* were obtained from Ovoko, Igbo-Eze South L.G.A of Enugu State, Nigeria. The leaves were identified by Mr. O. Chijioke of the *Hebarium* unit of the Department of Botany, University of Nigeria, Nsukka.

Animals

The experimental animals used for this study were white albino mice of either sex weighing 20-34g. The mice were between 3-4 months old and were obtained from the animal unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

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Chemicals/Reagents

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstand, Germany. Reagents used for the assays were products of Radox commercial kits.

Extraction Procedure

The fresh leaves of *Moringa oleifera* plant were plucked and dried under room temperature at (29±^{o}C -35±^{o}C) for three weeks , after which the leaves were pulverized into coarse form with a crestor high speed milling machine. The coarse form (130g) was then macerated in absolute ethanol. This was left to stand for 48 hours. After that the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature of between 40 and 45±^{o}C to avoid denaturation of the active ingredients. The concentrated extract was stored in the refrigerator.

Experimental Design

Twenty-four white albino mice of either sex weighing 20 – 34kg were housed in separate cages, acclimatized for one week and then divided into six groups of four mice each. The route of administration (treatment) was via oral route with the aid of an oral intubation tube.

Group 1 was the (positive control) inoculated with malaria parasite (Mp^{+}) and treated with 5mg/kg body weight of distilled water.
Group II was inoculated with malaria parasite and treated with 45mg/kg body weight of *Moringa oleifera* ethanol leaf extract.
Group III was also inoculated with malaria parasite and treated with 90mg/kg body weight of *Moringa oleifera* ethanol leaf extract.
Group IV was inoculated with malaria parasite and treated with 180mg/kg body weight of *Moringa oleifera* ethanol leaf extract.
Group V which was also inoculated with malaria parasite (standard control) and was treated with 5mg/kg body weight of artesunate (standard drug).
Group VI was the negative control which was not inoculated with malaria parasite and was finally treated with 5mg/kg body weight of distilled water.

Determination of yield of extract

The percentage yield of the extract was determined by weighing the coarse *Moringa oleifera* leaf before extraction and the *Moringa oleifera* ethanol leaf extract after concentration and then calculated using the formula.

ISSN: 0975-8585

$$\text{Percentage (\%) yield} = \frac{\text{Weight (g) of concentrated extract}}{\text{Weight (g) of ground Moringa leaves}} X100$$

Determination of Total Cholesterol Concentration

Total cholesterol concentration was determined by the method of Allain *et al* .(1976) using Randox kit.

Determination of High-Density Lipoproteins (HDL) - Cholesterol Concentration

The concentration of high-density lipoprotein (HDL) was determined by the method of Albers *et al*. [1] using Randox kit.

Determination of Low-Density Lipoprotein (LDL) – Cholesterol Concentration

The concentration of low density lipoprotein (LDL) was determined by the method of Assmann *et al.* (1984) using Randox kit.

Determination of Triacylglycerol Concentration

The concentration of triacylglycerol (TAG) was determined by the method of Allain *et al* [2] using Randox kit.

RESULTS

Percentage yield of the extract

Table 1: The percentage yield of the ethanol leaf extract of *Moringa oleifera*

Initial weight of ground extract (g)	Final weight of extract (g)	Percentage (%) yield of extract
130	23.20	17.85

From the result in Table 1 the (%) yield of the ethanol leaf extract of *Moringa oleifera* was found to be 17.85%.

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Total Cholesterol Concentration in Mice

Fig.1 Shows that on day 28 of post treatment mean total cholesterol concentration for mice in groups 2 , 3 , 4 and 5 were non-significantly ($p>0.05$) lower than the values for mice in groups 1 (positive control) and 6 (negative control).

ISSN: 0975-8585

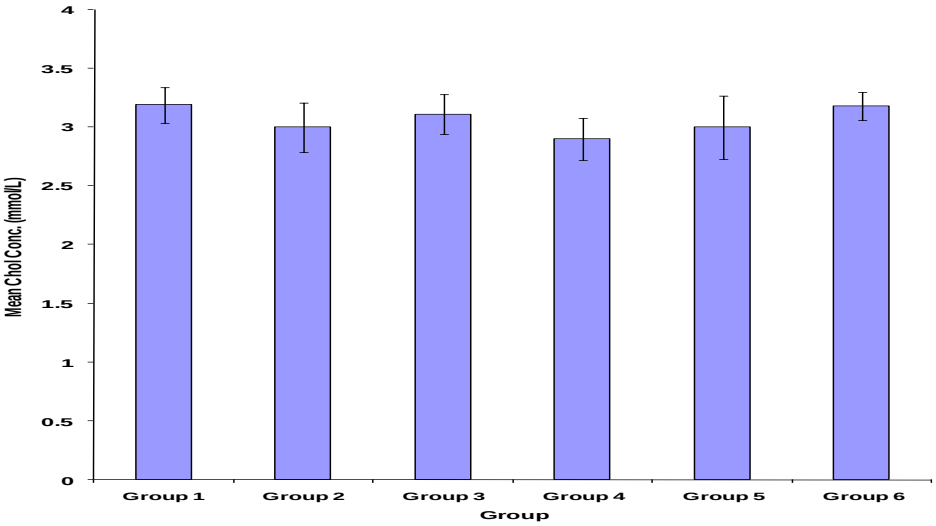

Fig. 1: Effect of ethanol leaf extract of *Moringa oleifera* on total cholesterol concentration in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Total High Density Lipoprotein Concentration in Mice

Fig. 2 Shows that on day 28 of post-treatment ,there was a non-significant ($p>0.05$) increase in the high density lipoprotein (HDL) concentration of mice in all the test groups administered graded doses of the extract (45,90 and 180 mg/kg body weight) when compared to the HDL concentration of mice in the three control groups (positive ,negative and standard).In the same vein, the HDL concentration of mice in groups 5 (standard control) and 6 (negative control) decreased compared to the HDL concentration of mice in group 1 (positive contro). However, such decrease was found to be non-significant ($p>0.05$).

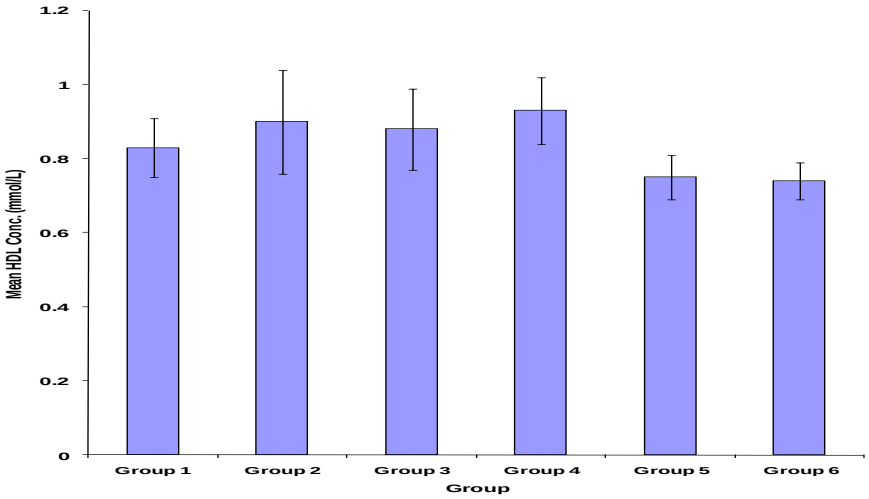

Fig. 2: Effect of ethanol leaf extract of *Moringa oleifera* on total high density lipoprotein concentration in mice

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Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Effect of Ethanol Leaf Extract of *Moringa oleifera* Low Density Lipoprotein Concentration in Mice

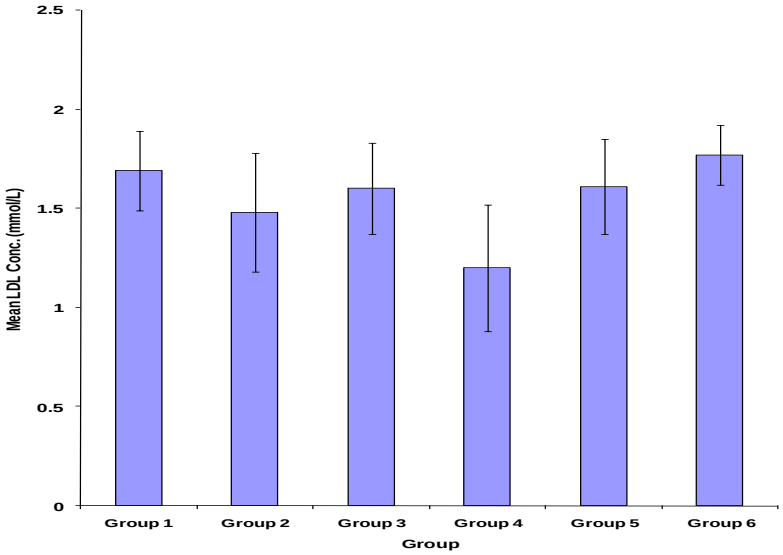

Fig. 3: Effect of ethanol leaf extract of *Moringa oleifera* on low density lipoprotein concentration in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Fig.3 Shows that on day 28 of post-treatment mean values of LDL concentration for mice in groups 2,3,4 and 5 were non-significantly ($p>0.05$) lower than that for mice in groups 1 (positive control) and 6 (negative control) .But the mean value obtained for LDL of mice in group 6 was similar to that for group 1 (positive control) when compared.

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Triacylglycerol Concentration in mice

Fig.4 Shows that the mean TAG concentrations of mice in groups 2, 3 and 5 were non-significantly ($p>0.05$) lower than the values for mice in groups 1 (positive control), 4 and 6 (negative control). But the mean TAG value for mice in group 1 (positive control) was similar to that in group 6 (negative control) when compared.

ISSN: 0975-8585

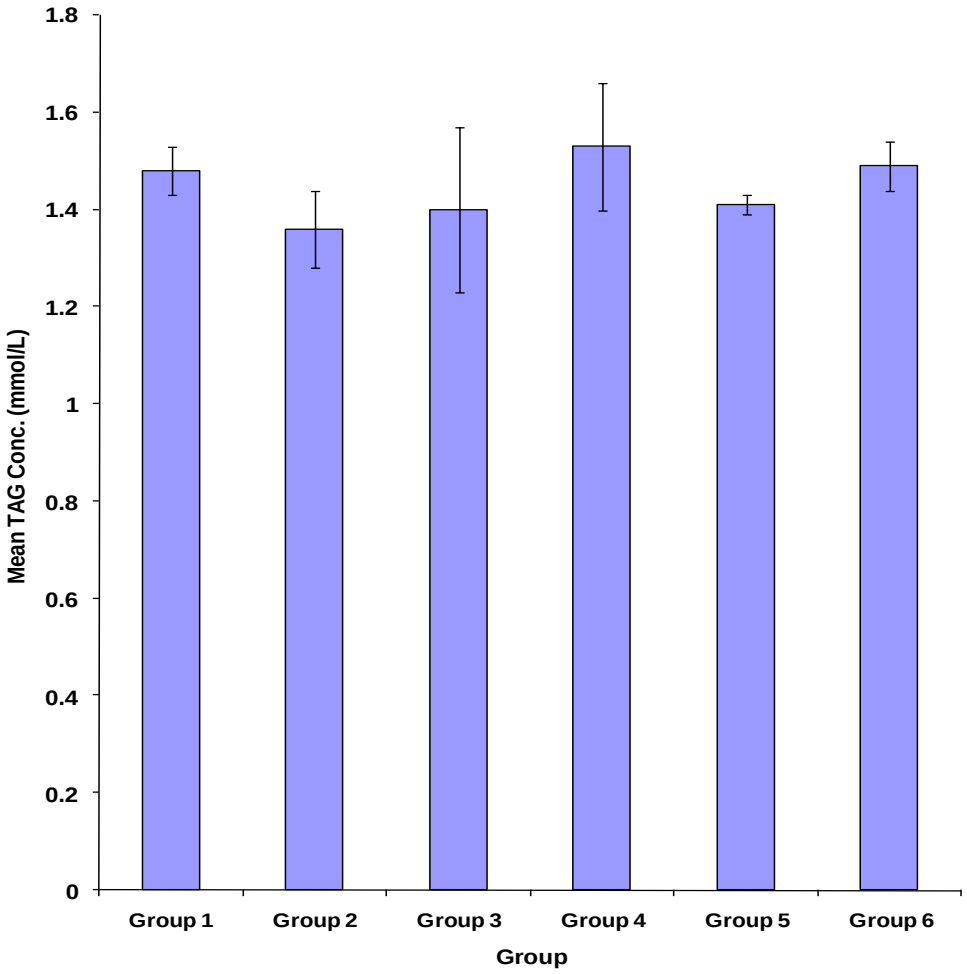

Fig. 4: Effect of ethanol leaf extract of *Moringa oleifera* on triacylglycerol concentration in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

DISCUSION

Lipids are group of naturally occurring molecules of fats, waxes, sterols, fat soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. They play many important roles in the body but can also lead to cardiovascular disease when their concentration is abnormal in an organism.

The effect of ethanol leaf extract of *Moringa oleifera* on total cholesterol concentration in mice showed a non significant difference ($p>0.05$) in all the groups compared to group 6 (negative control). This indicated that malaria had no effect on total cholesterol concentration of the mice. But, there was non-significant decrease ($p>0.05$) in total cholesterol of group 2 (45

mg/kg body weight of the extract), group 3 (90 mg/kg body weight of the extract), group 4 (180 mg/kg body weight of the extract) and group 5 (5 mg/kg body weight of the artesunate) animals compared to group 6 (negative control) animals. This result agrees with the works of Ghasi *et al*., [5] and Mehta *et al*.,[9]. Cholesterol is essential for all animals' life, high levels in blood circulation, depending on how it is transported within lipoprotein, are strongly associated with progression of artherosclerosis and other cardiovascular diseases.

The effect of ethanol leaf extract of *Moringa oleifera* on high density lipoprotein concentration in mice showed non-significant difference ($p>0.05$) in high density lipoprotein concentration of all the groups mice compared to the high density lipoprotein concentration of mice in group 6 (negative control). But, there was a non-significant increase ($p>0.05$) in high density lipoprotein concentrations of group 2 (45mg/kg body weight of the extract), group 3 (90mg/kg body weight of the extract) and group 4 (180mg/kg body weight of the extract) mice. These are consistent with the work of Ghasi *et al*., [5] and Mehta *et al*.,[9]. High density lipoprotein particles transport cholesterol back to the liver for excretion or to other tissues that use cholesterol to synthesize hormones. So, having high concentrations of high density lipoprotein correlate with better health outcomes.

The effect of ethanol leaf extract of *Moringa oleifera* on low density lipoprotein concentration in mice showed a non-significant difference ($p>0.05$) in low density lipoprotein concentration of mice in all the groups compared to the low density lipoprotein concentration of mice in group 6 (negative control).However, there was a non-significant decrease ($p>0.05$) in low density lipoprotein (LDL) concentration of groups 2 (45 mg/kg body weight of the extract), group 3 (90mg/kg body weight of the extract), group 4 (180mg/kg body weight of the extract) and group 5 (5mg/kg body weight of the artesunate) mice compared to group 6 (negative control). These findings are in line with the work of Ghasi *et al*., [5] and Mehta *et al*.,[9].). High concentration of low density lipoprotein (hypercholesterolemia) and lower concentration of functional high density lipoprotein are strongly associated with cardiovascular diseases because these promote antheroma development in arteries (antherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke and peripheral vascular diseases. Since higher low density lipoprotein particle concentrations and smaller low density lipoprotein particle size contribute to this process more than the cholesterol content of the low density lipoprotein particles, low density lipoprotein particles are called bad cholesterol because they have been linked to antheroma formation. On the other hand, high concentrations of functional high density lipoprotein, which can remove cholesterol from cells and antheroma, offer protection and are referred as good cholesterol.

The effect of ethanol leaf extract of *Moringa oleifera* on triacylglycerol concentration in mice showed a non significant difference ($p>0.05$) in triacylglycerol in all the groups compared to the triacylglycerol concentration of mice in group 6 (negative control). This showed that malaria had no effects on the triacylglycerol concentration in the mice.But there was a non significant decrease ($p>0.05$) in triacylglycerol concentration in group 2(45mg/kg body weight of the extract), group 3 (90mg/kg body weight of the extract) and group 5 (5mg/kg body weight of the artesunate) when compared to group 1 (positive control) and these are consistent with

the work of Ghasi *et al*., (2000) and Mehta *et al.* ,(2003). Triacylglycerol (TAG) is a major component of very low density lipoprotein and chylomicrons which play important role in metabolism as energy sources and transporters of dietary fats (Mehta *et al.*, 2003).

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