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EFFECT OF *AZADIRACHTA INDICA* LEAF EXTRACT ON LIPID PARAMETERS IN WISTAR ALBINO MICE INFECTED WITH *PLASMODIUM BERGHEI*

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ABSTRACT

Malaria, which is caused by a plasmodium parasite, and transmitted by the bite of infected mosquitoes is endemic in developing countries especially in Africa where it pose serious health challenge to the populace. This study evaluates the effect of *Azadirachta Indica* leaf extract on lipid parameters in Wistar Albino mice infected with *Plasmodium berghei.* A total of ninety (90) mature male swiss albino mice (free from infection and weighing between 25-35g) were used for the study. The animals were grouped into six classes (A-F) of fifteen (15) mice per group, per cage. Groups A to C served as the control groups [normal (uninfected plus distilled water), standard (infected plus lonart (4mg/kg) and negative (infected plus distilled water)] respectively while groups D, E and F served as the treatment groups and were orally administered 100 mg/kg, 200 mg/kg and 400 mg/kg doses of leaf extract of *Azadirachta Indica* for five (5) days consecutively. Malaria parasites (*Plasmodium berghei*, Anka strain) were inoculated using standard methods. At the end of the experimental periods, the animals were sacrificed and blood collected through cardiac puncture for bioassay studies. Concentrations of total cholesterol, triglycerides, high density lipoproteins (HDL) as well as low density lipoproteins (LDL) were carried out using standard methods. Results generally showed a significant decrease ($p>0.05$) in serum concentrations of total cholesterol, triglycerides, HDL and LDL in extract treated animals when compared with the untreated control. It could therefore be concluded that administration of *Azadirachta Indica* leaf extract has lipid lowering effects and could therefore ameliorate lipid related disorders.

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Azadirachta Indica, *Plasmodium berghei*, Malaria, Lipids.

INTRODUCTION

Malaria is a mosquito borne disease of humans caused by protozoan parasite of the genus Plasmodium. It is transmitted from one human to another by the bite of an infected female anopheles mosquito and tt is widespread in tropical and sub-tropical regions, including the sub-Sahara Africa, Asia and the Americans (Okpe *et al*., 2019) and a major cause of mortality and morbidity worldwide (Rich *et al*., 2019). These parasites have a complex life cycle in their mosquito appearance of drug-resistant strains of the parasite, the spread of insecticide-resistant strains of the mosquito and the lack of licensed malaria vaccines of proven efficacy (WHO, 2017).

Once in the human bloodstream, *P. falciparum* sporozoites for instance, reach the liver and penetrate the hepatocytes, they undergo asexual replication known as exo-erythrocytic schizogony. Although the mechanism of targeting and invading the hepatocytes is not yet well understood, studies have shown that sporozoite migration through several hepatocytes in the mammalian host is essential for completion of the life cycle. Uncomplicated malaria is diagnosed when symptoms are present, but there are no clinical or laboratory signs to indicate a severe infection or the dysfunction of vital organs. Individuals suffering from this form can eventually develop severe malaria if the disease is left untreated, or if they have poor or no immunity to the disease. Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction. This could be as a result of reactive oxygen species that are generated by the parasitic infection through Fenton and Weiss reaction.

In the absence of an effective vaccine, the fight against malaria depends on chemotherapy as well as the reduction and prevention of breeding and anopheles contacts with humans. Therefore to

combat malaria, new knowledge, products and tools especially new drugs of traditional origin could be promising sources of potential anti-malaria drugs.

Azadirachta indica, commonly known as neem in many countries of the world, is a large evergreen tree that belongs to the family Meliaceae. It is believed to have originated from Assam and Burma in South Asia (National Research Council, 1992), and grow well in tropical and sub-tropical regions around the world (Okpe *et al*., 2019), with ability to withstand many adverse environmental conditions such as drought, infertile soil, shallow or acidic soil (Okpe *et al*., 2019). *Azadirachta indica* have been noted to be of great medicinal value as many biologically active compounds, including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones which have been noted to mitigate malarial infections have been well extracted from it. The leaf extract of *A. indica* has been prescribed orally for the treatment of malaria by Indian ayurvedic practitioners for centuries (National Research Council, 1992). Despite the widespread usage of this plant in combating several diseases, there is a dearth reports on its effect on lipid parameters. Hence, it is important that research focuses on the effects that continuous administration of this plant may have on lipid homeostatsis, thus, evaluating the effect of *Azadirachta indica* leaf extract on lipid parameters in wistar albino mice infected with *plasmodium berghei* remain crucial.

Materials and Methods

Preparation of Plant Material

Azadirachta indica leaf were collected from the environment of Nnamdi Azikiwe University, Awka, Anambra State, and were identified at the Herbarium unit, Botany Department, Nnamdi Azikiwe University, Awka, by Taxonomist, Mr. Iloka Finian, with herbarium number 'NAUH-

14B'. The plant material was washed with clean water, shredded with a knife and air-dried under shade for 15 days.

Extraction of Plant Materials

The dried plant (leaves) was pulverized using a laboratory grinder and the fine powder obtained was stored in an airtight container at room temperature until further use. Two hundred gram (200 g) of the powdered sample was weighed and steeped in 1000 ml of 70% ethanol (by maceration) for 48 hours. The solution was then filtered and the filtrate gotten was concentrated under vacuum in a rotary evaporator which yielded a gummy residue, as extracts of the leaves. The extracts were kept in a tightly closed bottle in a refrigerator until further used.

Procurement of Experimental Animals

Mature male Swiss albino mice (90) free from infection and weighing between (25-35g) were obtained from Chris Farm Ltd Mgbakwu, Awka, Anambra State. They were sorted, housed in standard cages with housing conditions of 12:12 light: dark cycles. They were fed with standard grower's mash pellets and water *ad libitum.* All the experimental procedures and protocols used for this study were in accordance with the guidelines and principles of Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka.

Inoculation and Treatment of Albino Mice

Malaria parasites (*Plasmodium berghei*, Anka strain) were obtained from Mr. Ike Chibueze, research Centre, University of Nigeria, Nsukka. The strain was maintained in the laboratory by serial blood passage from mouse to mouse. The success of the infection was done using the Giemsa and Leishman stain method for malaria parasite detection. Treatments commenced 72 hours after successful induction.

Dose Preparation and Treatment

The hydro-ethanolic leaf extract of *Azadirachta indica* was prepared with distilled water in three divided dose (100, 200, and 400) mg/kg, Lonart (4 mg/kg) was used as a reference drug, distilled water as untreated group. The animals were grouped into six different groups of fifteen (15) mice per group per cage and administered the extract and drug for five consecutive days with water per os and feed *ad libitum* as shown in table 1.

Table 1: Grouping and Dose Administration of Experimental Animals

Group	Treatment
A (Normal)	Uninfected plus distilled water
B (Malaria untreated)	Infected plus distilled water
C (Standard control)	Infected plus 4 mg/kg standard drug (Lonart)
D (Treatment)	Infected plus 100 mg/kg *A. indica* leaf extract
E (Treatment)	Infected plus 200 mg/kg *A. indica* leaf extract
F (Treatment)	Infected plus 400 mg/kg *A. indica* leaf extract

Collection of Blood Sample for Bioassay

At the end fifth day, the experimental animals were anaesthetized with chloroform vapor, and sacrificed. A 5 ml sterile syringe with needle was used for collection of blood via cardiac puncture and the sera obtained were used for bioassay studies.

Biochemical assays

Lipid profile; High density lipoprotein (HDL), Low density lipoprotein (LDL), Triglycerides (TAG) as well as total cholesterol (TC) assay were carried out, using standard assay kits sourced

from Randox Laboratories Ltd., BT29 4QY, United Kingdom with strict adherence to manufacturer's instructions.

Data Analysis

The results obtained in this research were expressed as Mean ± S.D of triplicate determinations. One way analysis of variance (ANOVA) was carried out on the results and significance was accepted at $p<0.05$. GraphPad Prism5 Program (GraphPad Software, San Diego, CA, USA) was used for the graphical analyses of the results obtained.

RESULTS

Figure 1 presents the effect of *Azadirachta Indica* leaf extract on total cholesterol levels in mice infected with *Plasmodium berghei.* There was no significant difference in the total cholesterol levels of the treatment group when compared to the untreated control. However, a significance decrease ($p>0.05$) was observed in the treatment groups compared with the normal control.

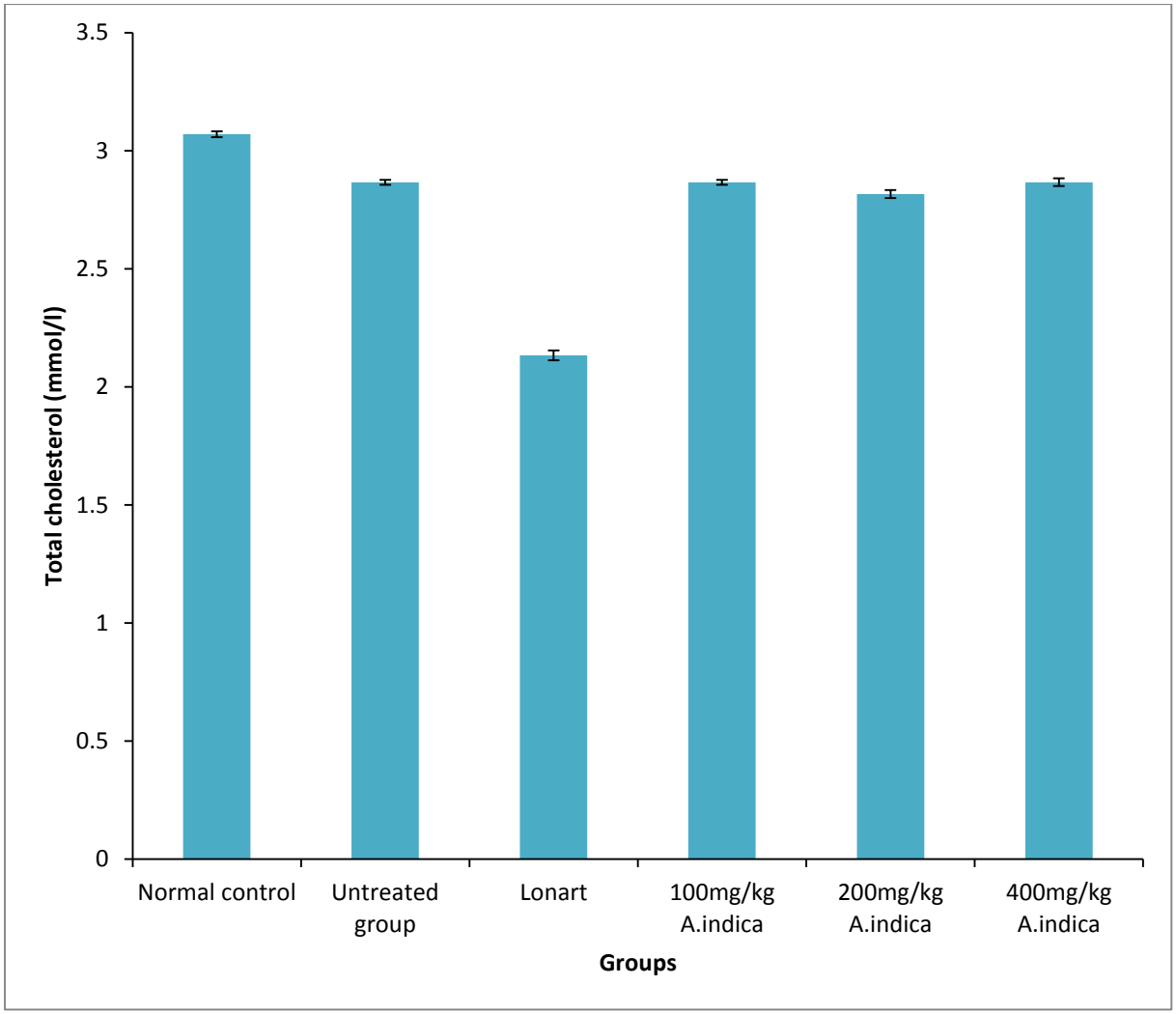

Figure 1: Effect of *Azadirachta Indica* leaf extract on total cholesterol levels in mice infected with *Plasmodium berghei.*

Figure 2 presents the effect of *Azadirachta Indica* leaf extract on triglyceride levels in mice infected with *Plasmodium berghei.* There was no significant difference in the triglyceride levels of the treatment group when compared to the untreated control. However, a significance decrease ($p>0.05$) was observed in the treatment groups compared with the normal control.

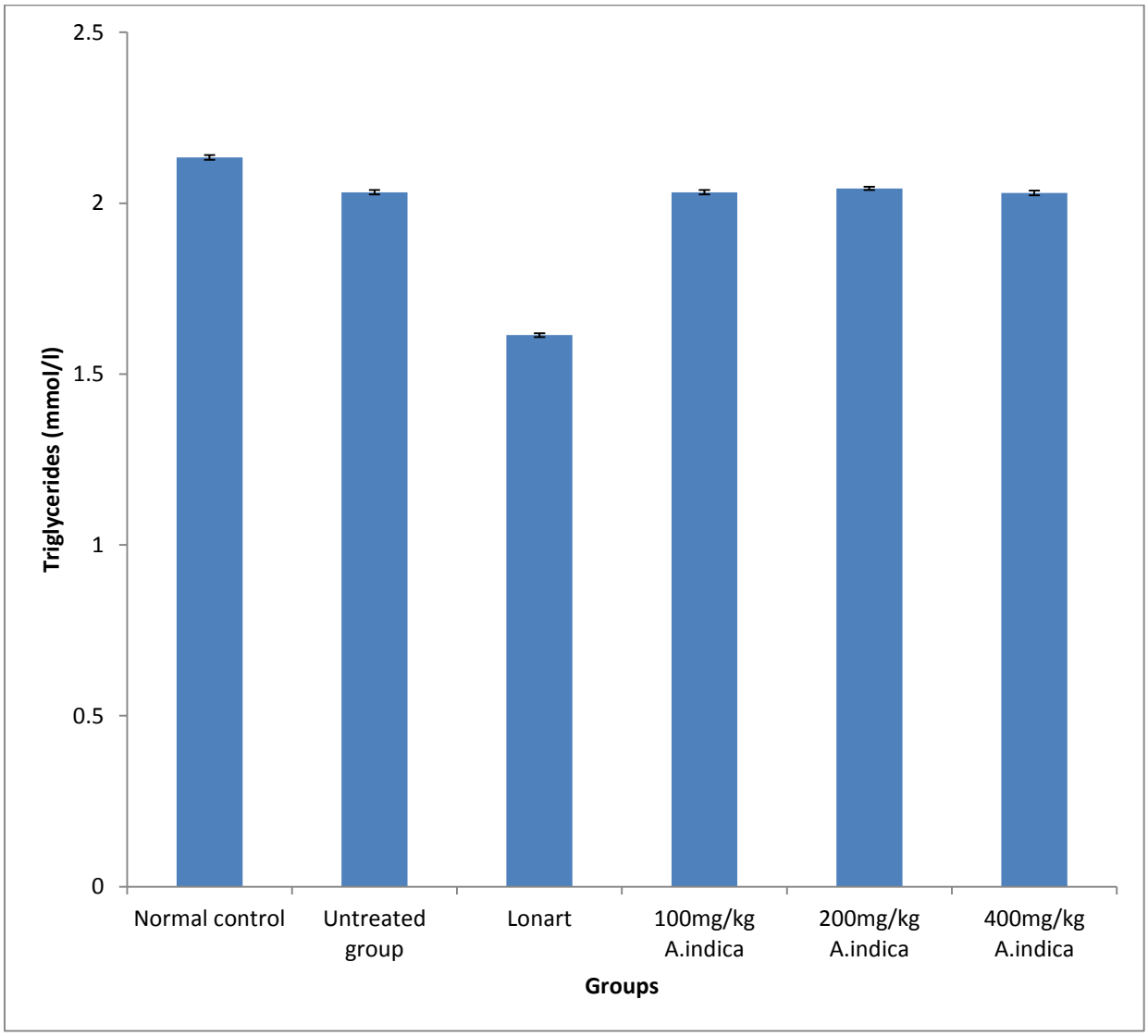

Figure 2: Effect of *Azadirachta Indica* leaf extract on triglyceride levels in mice infected with *Plasmodium berghei.*

Figure 3 presents the effect of *Azadirachta Indica* leaf extract on High density lipoprotein (HDL) levels in mice infected with *Plasmodium berghei.* Results showed as significant decrease ($p>0.05$) in the HDL levels of the treatment group when compared to the untreated control.

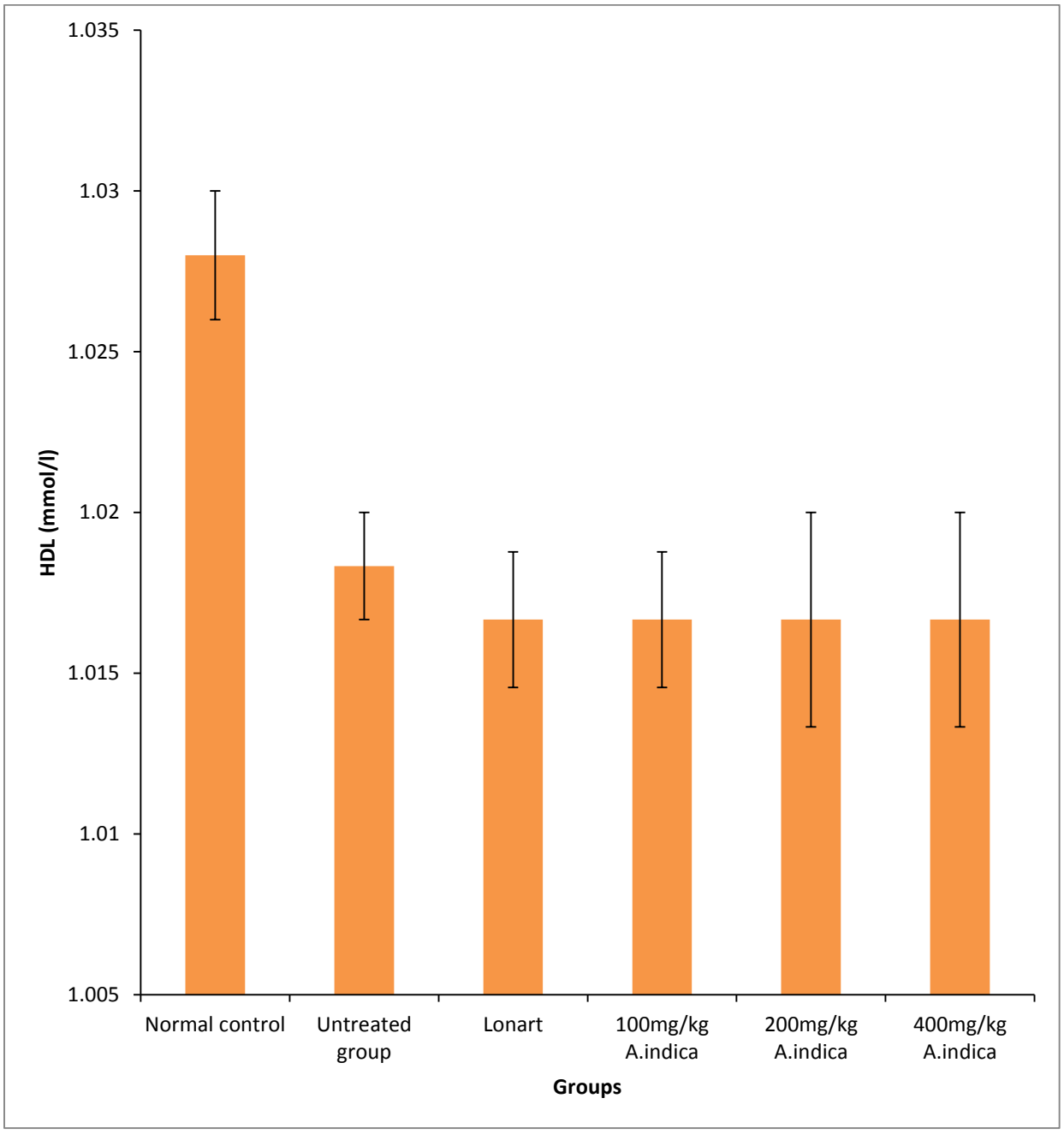

Figure 3: Effect of *Azadirachta Indica* leaf extract on High density lipoprotein (HDL) levels in mice infected with *Plasmodium berghei.*

Figure 4 presents the effect of *Azadirachta Indica* leaf extract on Low density lipoprotein (LDL) levels in mice infected with *Plasmodium berghei*. Results showed as significant decrease ($p>0.05$) in the LDL levels of the treatment group (200 mg/kg) when compared to the untreated control.

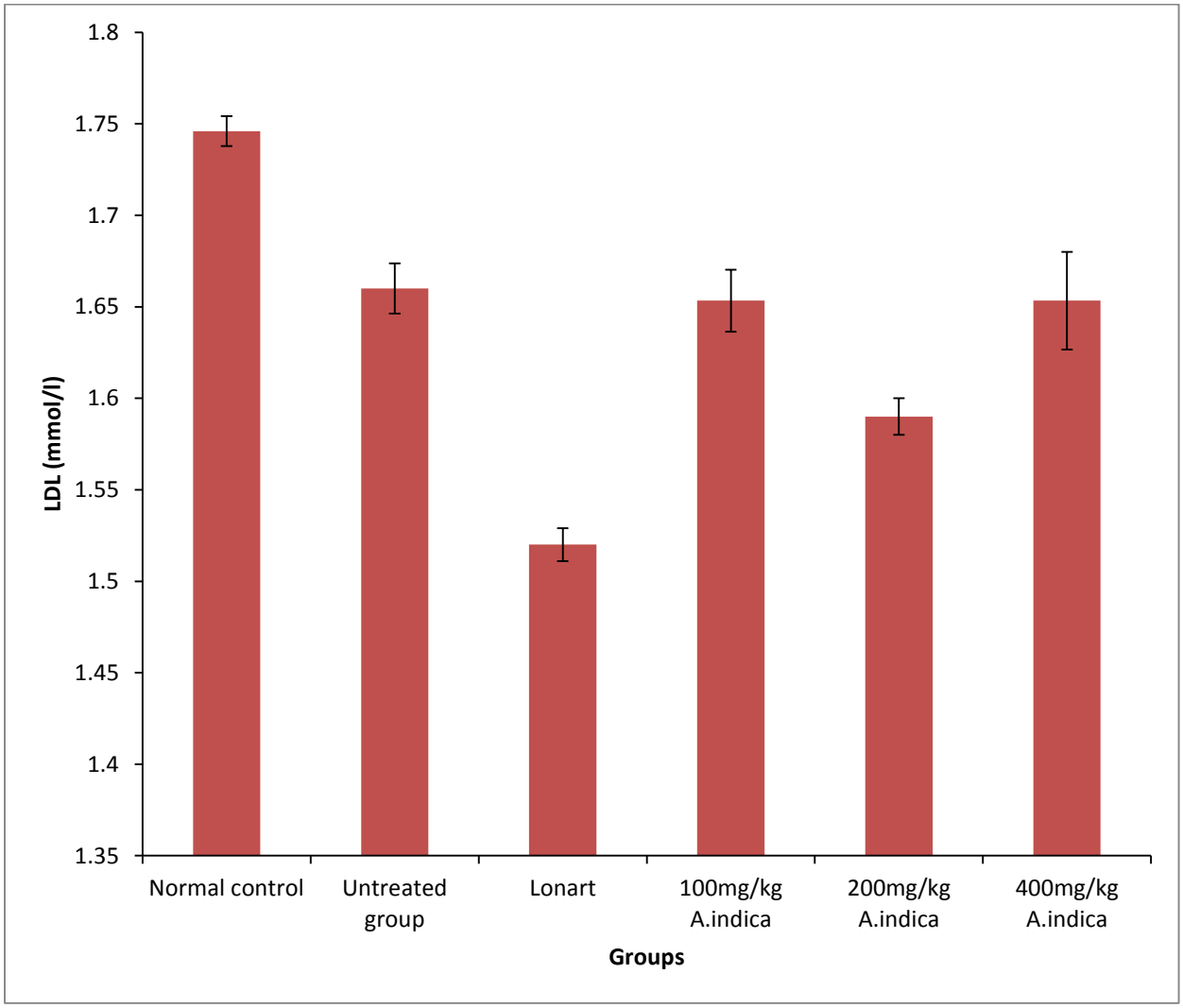

Figure 4: Effect of *Azadirachta Indica* leaf extract on Low density lipoprotein (LDL) levels in mice infected with *Plasmodium berghei.*

DISCUSSION

Lipid profiling is a common blood test that is carried out to monitor and screen for risk of cardiovascular diseases. These include cholesterol, triglycerides, high and low density lipoproteins.

In this study, as depicted in figure 1, total cholesterol levels in the extract groups, standard drug, and untreated groups were lower than those in the control group, which had an average higher total cholesterol level (3.07±0.01mmol/l). The elevation of total cholesterol concentration in the control group could be attributed to indirect stimulation of HMG CoA (3-hydroxy-3-methylglutaryl-Coenzyme A) reductase following induction of malaria. Hence the possible total cholesterol lowering effects of *neem* extract could be attributed to decreased activity of hepatic HMG CoA reductase and/or stimulation of cholesterol-7-alpha-hydroxylase, which converts cholesterol into bile acids. It could also be due to the presence of saponins, a phytochemical which forms insoluble complexes with cholesterol or their bile salt precursor, thus making them unavailable for absorption (Banti and Bajo, 2020). The extract groups and untreated groups had almost equal average total cholesterol levels. This shows that the onset of malaria with subsequent treatment could lead to the lowering of cholesterol levels. These findings were in line with the findings of Venugopal *et al*. (2020), who concluded that malaria infection leads to hypocholesterolemia. The reduction in cholesterol level may be associated with the modulation of lipid metabolism and inflammation by the extract. Since malaria infection has been reported to trigger an inflammatory response in the body, inflammatory mediators, such as cytokines and chemokines, can stimulate the production of acute-phase proteins, including C-reactive protein (CRP). CRP has been shown to inhibit the activity of enzymes involved in cholesterol synthesis, such as HMG-CoA reductase; this inhibition can lead to a decrease in cholesterol production,

resulting in reduced total cholesterol levels (Kalia *et al*., 2021). On the other hand, neem leaf extract contains bioactive compounds, including flavonoids and limonoids, which possess anti-inflammatory properties (Sandhir *et al*., 2021). Hence, the administration of higher doses of the extract may help modulate the inflammatory response induced by malaria infection. By reducing the production of inflammatory mediators and acute-phase proteins, neem leaves extract may have indirectly contribute to the lowering of total cholesterol levels. Furthermore, neem leaf extract has been reported to inhibit the absorption of dietary cholesterol in the intestines and enhance the excretion of cholesterol through bile acid formation (Yarmohammadi *et al*., 2021).

The result of triglyceride levels in this study is similar to that of total cholesterol. This is however in contrast with the findings of Warjri *et al*. (2016) in their reports. Triglycerides are the main constituent of vegetable and animal fats in diets, and they also serve as the main constituent of the body's fat stores. Accumulation of excess triglycerides in the liver has been implicated in the pathogenesis of hepatic lipidosis. The reduction in triglyceride levels in the extract groups could be attributed to several factors such as its anti-inflammatory activities (Kalia *et al*., 2021; Hashim *et al*., 2021), antioxidant potentials (Sharma *et al*., 2012, Alzohairy, 2016;) as well as immune-modulatory properties (Sarkar *et al*., 2021; Kalia *et al*., 2021).

As depicted in figures 3 and 4, there was significant decrease in High Density Lipoprotein (1.02±0.00mmol/l in all extract groups) and Low Density Lipoprotein (1.65±0.02mmol/l, 1.59±0.0100mmol/l, and 1.65±0.03mmol/l respectively) levels in the in extracts treated groups when compared to the control group, thus showing that the extract was capable of reducing the lipid profiles. The reduction in HDL and LDL were in line with the findings of Visser *et al*., (2013); Warjri *et al*., (2016). This could however be attributed to a number of reasons such as altered lipid metabolism. Plasmodium infection has been reported to have the ability to disrupt

normal lipid metabolism in the host organism (Kluck *et al*., 2019). This disruption may lead to an imbalance in cholesterol transport and metabolism, resulting in altered levels of HDL and LDL. The ethanol extract of neem leaves, when administered, may help restore lipid homeostasis by regulating key enzymes and pathways involved in cholesterol metabolism, thus influencing HDL and LDL levels (Sharma *et al*., 2014).

Inflammatory response is another probable verified reason. Since malaria infection has been reported to trigger inflammatory response of the host's immune defense mechanism (Kalia *et al*., 2021), chronic inflammation can therefore lead to alterations in lipoprotein metabolism, including a decrease in HDL levels. The ethanol extract of neem leaves, with its anti-inflammatory properties, may help attenuate the excessive inflammation associated with the infection. By reducing inflammation, neem leaves extract could potentially normalize lipoprotein levels (Sharma *et al*., 2014).

CONCLUSION

From the result of this study, it could be concluded that leave extract of neem exhibited potentials in lowering lipid profiles of the experimental animals and consequently may aid in ameliorating lipid-related disorders.

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