Original Research Article

Effect of *AzadirachtaIndica* Leaf Extract on Some Biochemical Parameters in Wistar Albino Mice Infected with *Plasmodium berghei*

ABSTRACT

The use of medicinal plants in combating many tropical diseases including malaria is gaining wide acceptance owing to their many bioactive compounds. 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 *AzadirachtaIndica*leaf extract on some biochemical 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 *AzadirachtaIndica* 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. Activities of ALT, AST, ALP were determined using standard assay kits and concentrations of bilirubin (total and direct) as well as urea, creatinine, sodium, potassium, chloride and bicarbonate levels were carried out using standard methods. Results showed a significant increase ($p<0.05$) in serum activities of ALT and AST in extract treated animals when compared with the untreated control while there was significant decrease ($p>0.05$) in the serum activities of ALP in extract treated animals when compared with the untreated control. Results further showed an increase in the bilirubin (total and direct) levels of the extract treated groups as compared with the untreated control. Similar trends were observed for the levels of urea, creatinine, sodium, potassium and chloride. It could therefore be concluded that administration of *AzadirachtaIndica* leaf extract, though potent in fighting against malarial infection, could pose a threat to the liver and other organs of the body if not properly monitored.

Keywords: Medicinal plant, *AzadirachtaIndica, Plasmodium berghei,* Malaria, Health.

INTRODUCTION

Malaria is a significant public health issue around the world, especially in developing African countries, causing 90% morbidity and mortality [1], owing to difficultyin its treatment

strategydue to multi-drug resistant parasites[2]. This implies that the African continent is the most endemic continent in the world. Malaria is an infection whose vector is the infected female Anopheles mosquito which spreads this disease to humans and other animals[3]. Plasmodium protozoan is specifically the parasite that causes malaria [3], through the bite of infected mosquitoes. Since severity of malaria varies according to the severity of bite from plasmodium species, studies have shown that *Plasmodium falciparum* is responsible for the highest mortality rate in malaria cases whereas *Plasmodium ovale, Plasmodium malariae* and *Plasmodium vivax,* which are other types of the protozoa, generally cause a milder form of malaria [4]. Another specie (*P. knowlesi*), have been reported to rarely cause malaria disease in rodents but rarely in the human populace [3, 5]. *P. berghei* and *P. falciparum* are having similarities in many biochemistry and genetic relationships, for this reason *P. berghei* has been a very useful model system in studying malaria Infection[6].

Patients initially experience fever, chills, sweating, headache, weakness, and other symptoms similar to a "viral syndrome." Severe disease may develop later, resulting in an abnormal level of consciousness, severe anaemia, renal failure, and multisystem failure.

Malaria has been treated for many years with various conventional drugs and medicinal plants; many of which works by clearing the parasite load in the host body system. Despite all efforts by various scientists in malaria prevention and treatment, the recent widespread resistance of *Plasmodium falciparum* to currently available anti-malarial drugs; the resistance of mosquito vectors to currently available conventional insecticides; the serious setback in the development of malaria vaccines especially in African countries; and the rampant adverse reactions to some conventional anti-malarial drugs pose some challenges to the breakthrough. As a result, the plant kingdom offers a better chance for the discovery of lead compounds and new drugs with resistance to *Plasmodium falciparum* in order to treat this debilitating parasitic disease.

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care [7]. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and easily accessible [7]. According to the World Health Organization [1], the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times. The use of plants including *Azadirachtaindica*for healing purposes predates human history and forms the origin of most modern medicine.

Azadirachtaindica, 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 [8], and grow well in tropical and sub-tropical regions around the world [9], with ability to withstand many adverse environmental conditions such as drought, infertile soil, shallow or acidic soil [9]. *Azadirachtaindica*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 [8]. Despite the widespread usage of this plant in combating tropical diseases, there is a dearth reports on its effect on biochemical processes and parameters. Hence, it is important that research focuses on the effects that continuous administration of this plant may pose to biochemical processes; thus, evaluating the effect of *Azadirachtaindica* leaf extract on some biochemical parameters in wistar albino mice infected with *plasmodium berghei* remain crucial.

Materials and Methods

Preparation of Plant Material

*Azadirachtaindica*leaf were collected from the environment of NnamdiAzikiwe University, Awka, Anambra State, and were identified at the Herbarium unit, Botany Department, NnamdiAzikiwe 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 laboratorygrinder and the fine powder obtained was stored in an airtightcontainer 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 solutionwas 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 housingconditions of 12:12 light: dark cycles. They were fed with standardgrower's mash pellets and water *ad libitum.*All the experimental proceduresand protocols used for this study were in accordance with theguidelines and principles of Animal Research Ethics Committeeof NnamdiAzikiwe 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 *Azadirachtaindica* was prepared with distilled water in three divided dose (100, 200, and 400) mg/kg, Lonart (4 mg/kg) was used as a referencedrug, 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* leafextract
E (Treatment)	Infected plus 200 mg/kg *A. indica* leafextract
F (Treatment)	Infected plus 400 mg/kg *A. indica* leafextract

Collection of Blood Sample for Bioassay

At the end fifth day, the experimental animals wereanaesthetized with chloroform vapor, and sacrificed. A 5 ml sterilesyringe with needle was used for collection of blood via cardiacpuncture and the sera obtained were used for bioassay studies.

Biochemical assays

Alanine aminotransferase (ALT), aspartate transaminase (AST), Alkaline phosphatase (ALP), activities were assayed using standardenzyme kitssourced from Randox Laboratories Ltd., BT29 4QY, United Kingdom with strict adherence to manufacturer's instructions while the concentrations of direct and total bilirubin were determined usingthe method of Garber[10]while Kidney function assessment (Urea, creatinine, bicarbonate, sodium, potassium,) were determinedusing the method of Bergmeyer and Bernt [11].

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

The table below shows the results of the malaria test after induction using *Plasmodium berghei*. The table gives an overview of the average number of malaria parasites seen before commencement of treatment. This shows a successful malaria induction.

Table 2: Malaria parasite results after induction with *Plasmodium berghei*

Groups	Average number of malaria parasites seen
Normal control	0.00±0.00
Untreated	12.40±1.43
Standard drug	12.33±1.56
100mg/kg extract	10.00±0.97
200mg/kg extract	9.33±1.23
400mg/kg extract	10.67±1.14

Values are mean±standard deviation of triplicate determination.

Figure 1 presents the effect of *AzadirachtaIndica*leaf extract on alanine aminotransferase (ALT) activities in mice infected with *Plasmodium berghei.* Results showed a significant increase ($p<0.05$) in the ALT activities of the extract treated group when compared with the untreated control.

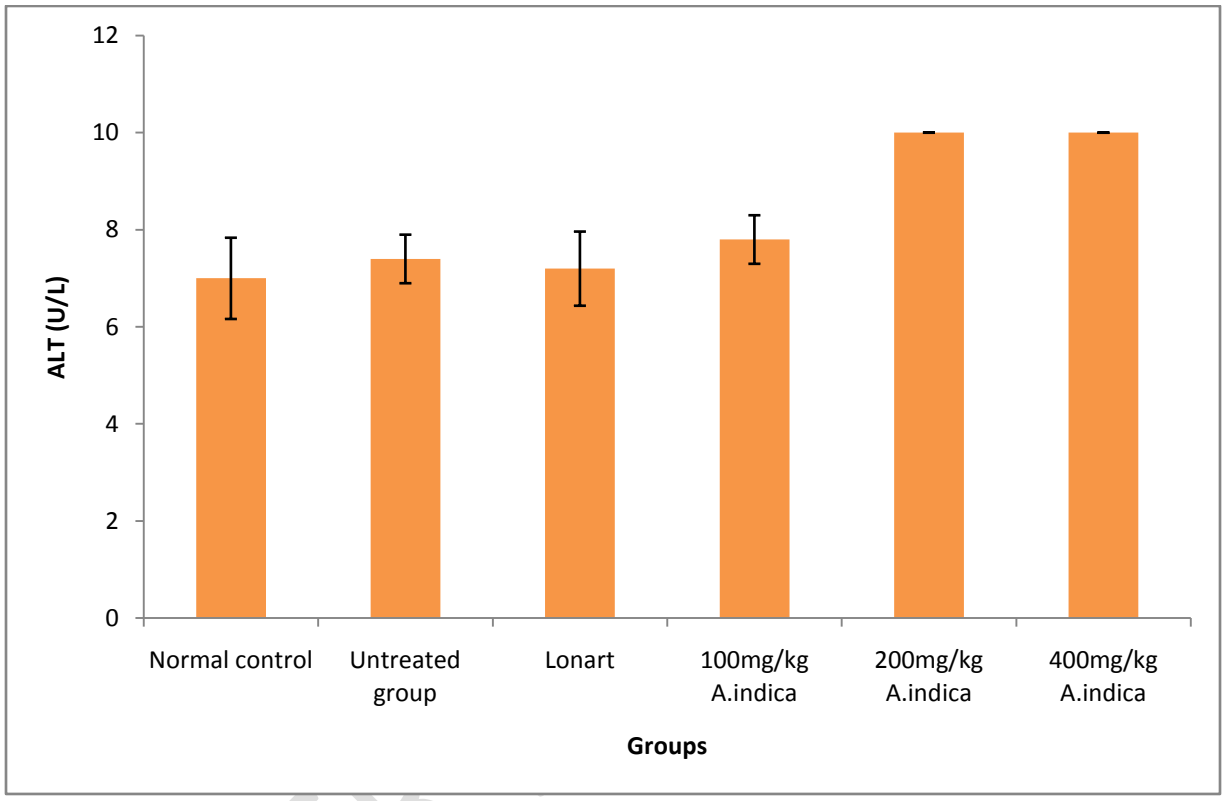

Figure 1: Effect of *AzadirachtaIndica*leaf extract on Alanine aminotransferase (ALT) activities in mice infected with *Plasmodium berghei.*

Figure 2 presents the effect of *AzadirachtaIndica*leaf extract on aspartate aminotransferase (AST) activities in mice infected with *Plasmodium berghei*. Results showed a significant increase ($p<0.05$) in the AST activities of the extract treated groups (200 mg/kg and 400 mg/kg) doses when compared with the untreated control.

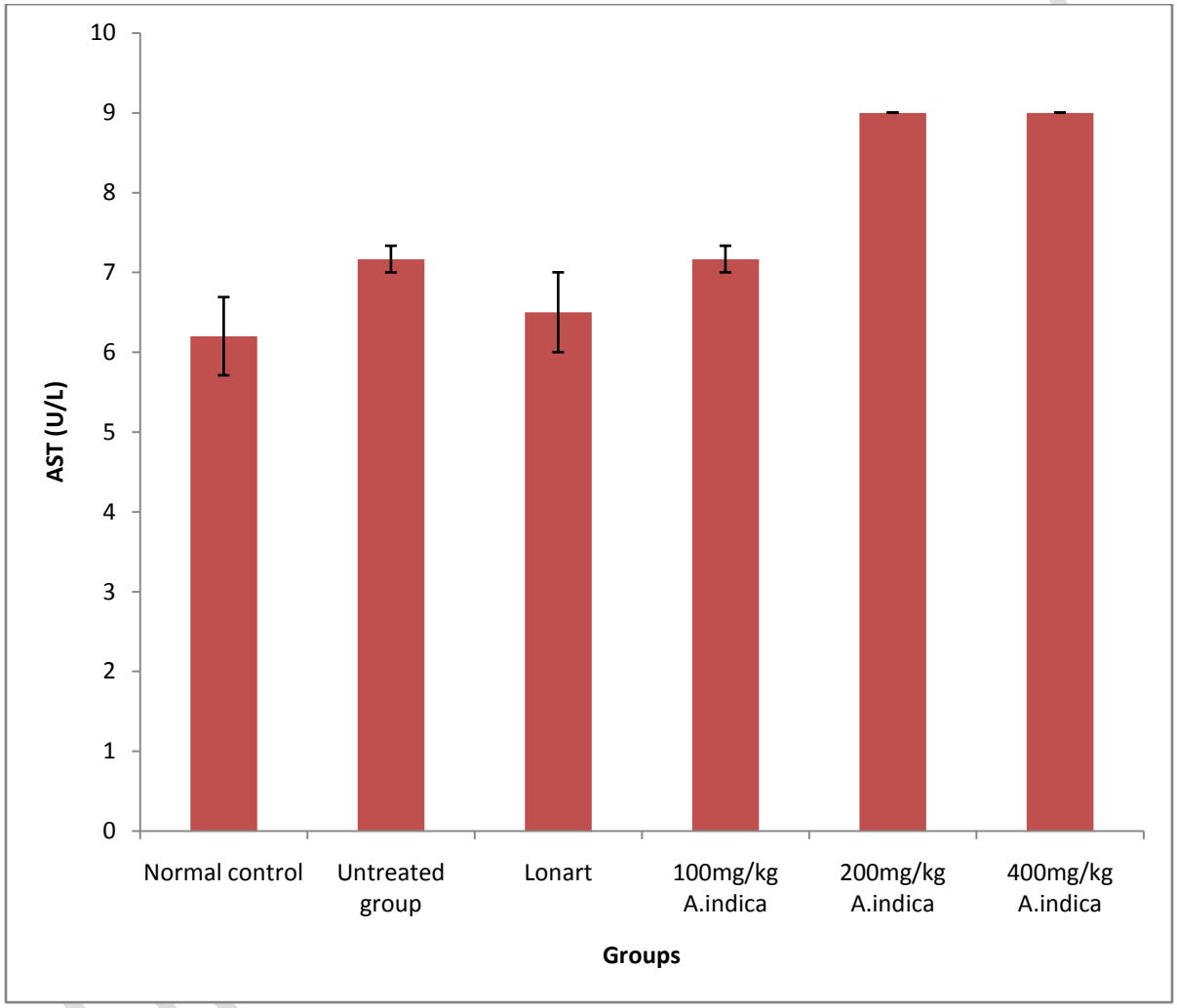

Figure 2: Effect of *AzadirachtaIndica*leaf extract on Alanine aminotransferase (AST) activities in mice infected with *Plasmodium berghei.*

Figure 3 presents the effect of *AzadirachtaIndica*leaf extract on Alkaline phosphatase (ALP) activities in mice infected with *Plasmodium berghei.* Results showed a significant decrease ($p>0.05$) in the ALP activities of the extract treated groups (200 mg/kg and 400 mg/kg) doses when compared with the untreated control.

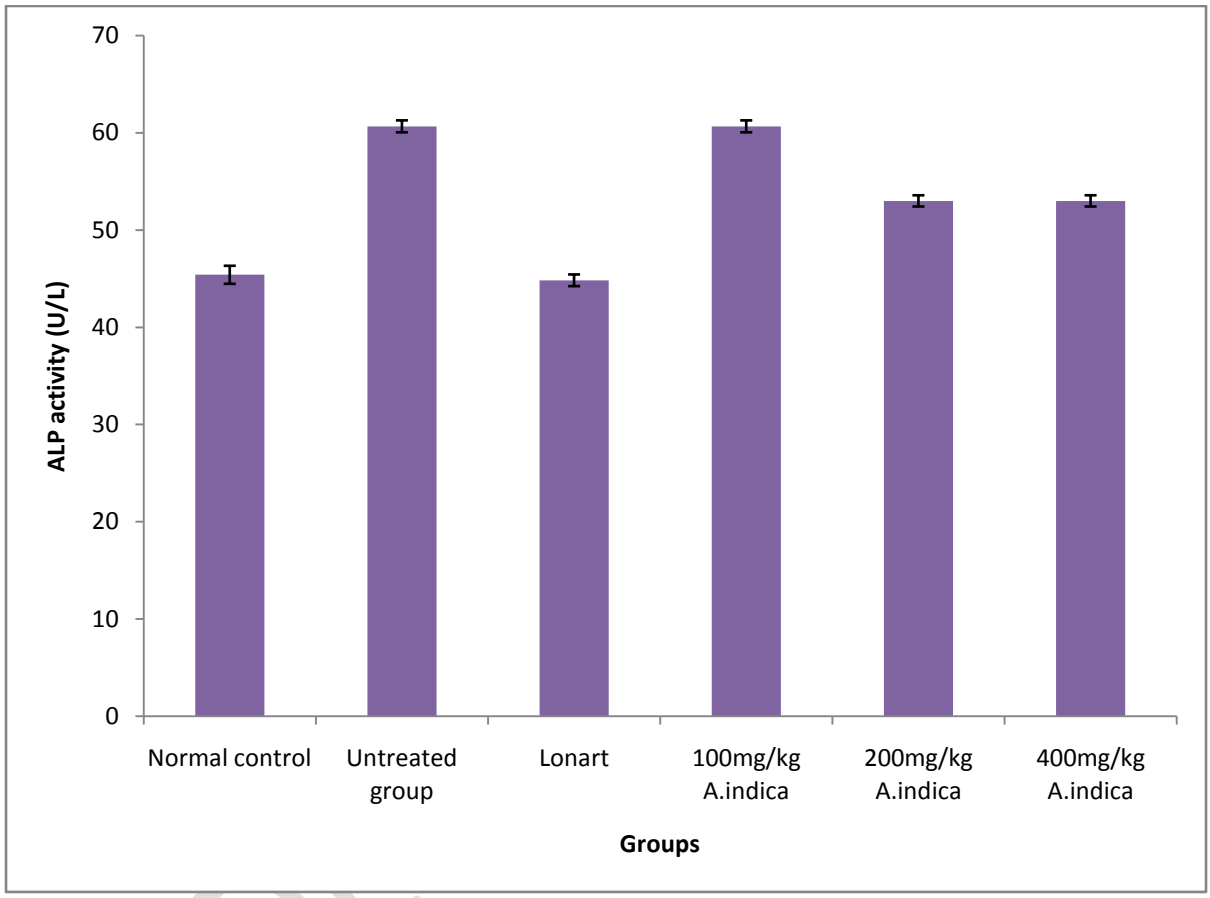

Figure 3: Effect of *AzadirachtaIndica*leaf extract on Alkaline phosphatase (ALP)activities in mice infected with *Plasmodium berghei.*

The effect of *AzadirachtaIndica*leaf extract on the concentration of direct and total bilirubin in mice infected with *Plasmodium berghei*is presented in figures 4 and 5 respectively.Results showed a significant increase ($p<0.05$) in the concentration of direct of the extract treated groups (200 mg/kg and 400 mg/kg) doses when compared with the untreated control with similar trends for total bilirubin.

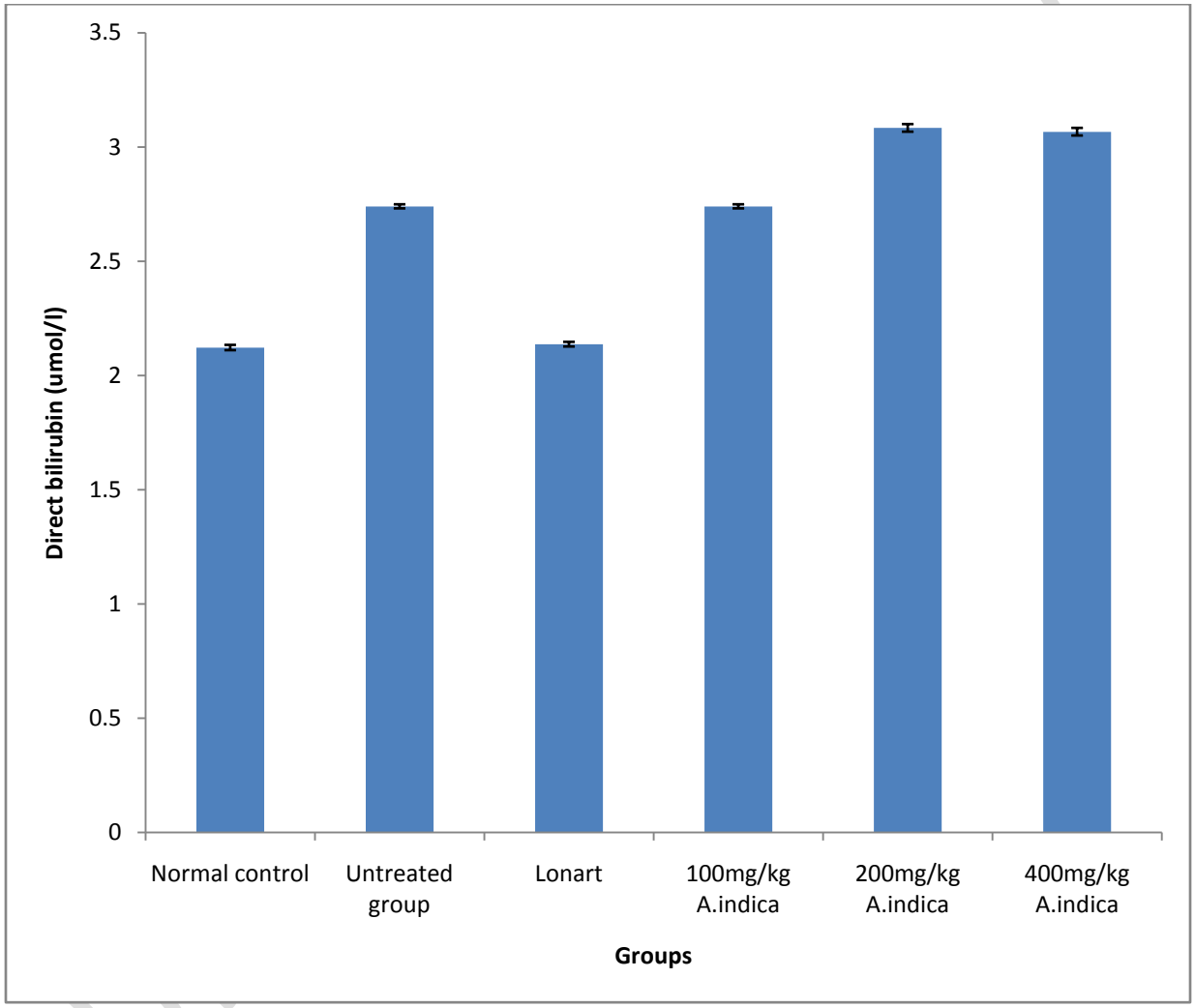

Figure 4: Effect of *AzadirachtaIndica*leaf extract on the concentration of direct bilirubin in mice infected with *Plasmodium berghei.*

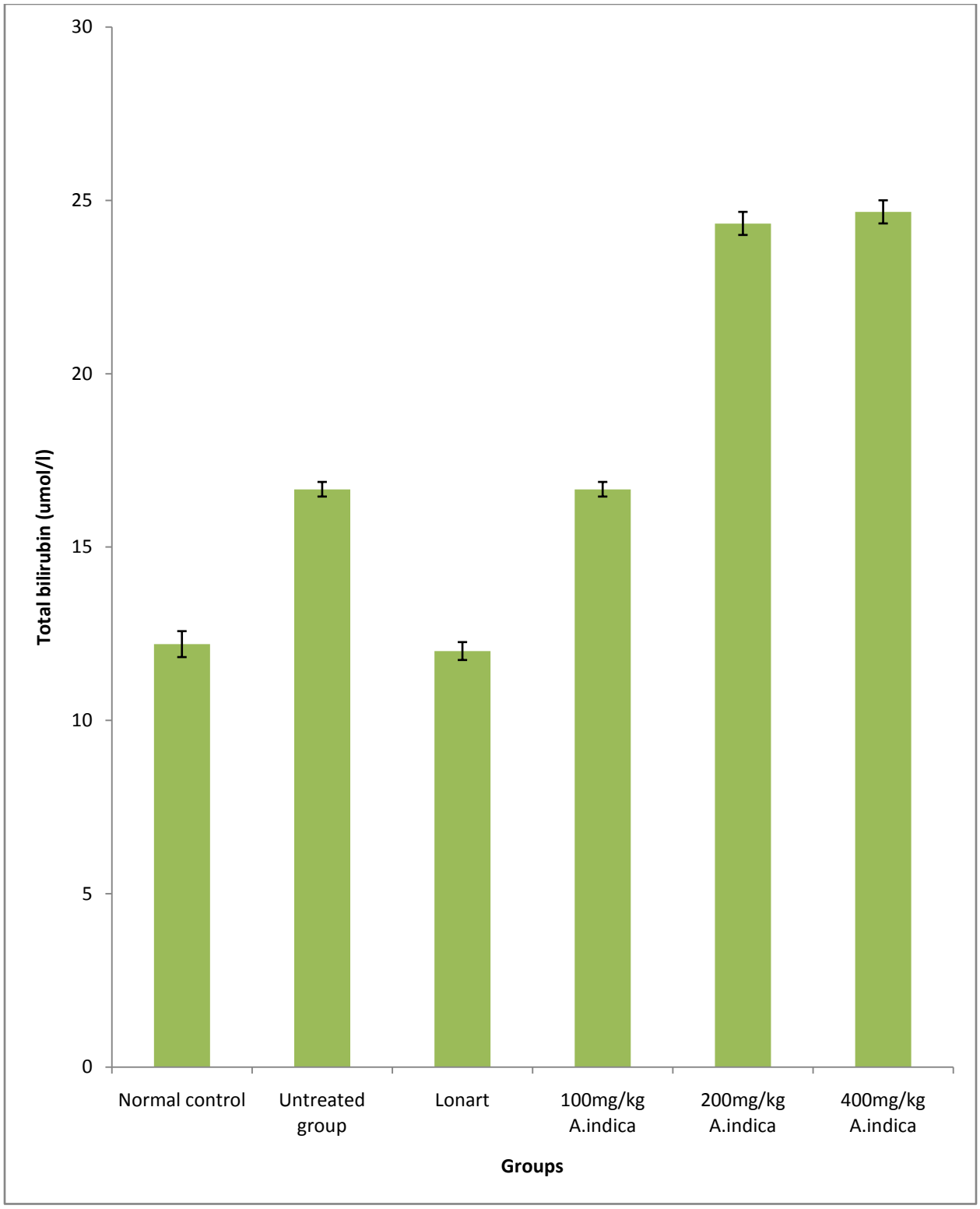

Figure 5: Effect of *AzadirachtaIndica*leaf extract on the concentration of total bilirubin in mice infected with *Plasmodium berghei.*

The effect of *AzadirachtaIndica*leaf extract on the concentration of urea, creatinine, sodium, potassium, chloride and bicarbonate levels in mice infected with *Plasmodium berghei*is shown in table 3 below.Results showed a significant increase ($p<0.05$) in the concentration of urea, creatinine, sodium, potassium and chloride levels in the extract treated groups (200 mg/kg and 400 mg/kg) doses when compared with the untreated control. Conversely, a significant decrease in the concentration of bicarbonate level was observed for extract treated groups (200 mg/kg and 400 mg/kg) doses when compared with the untreated control.

Table 3: The effect of *AzadirachtaIndica*leaf extract on the concentration of urea, creatinine, sodium, potassium, chloride and bicarbonate levels in mice infected with *Plasmodium berghei*

Groups	Urea (mmol/l)	Creatinine (µmol/l)	Sodium levels (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Bicarbonate (mEq/l)
Normal control	6.16±0.04^{c}	47.00±0.63^{d}	150.80±0.58^{c}	4.28±0.05^{d}	87.60±0.75^{c}	23.40±0.24^{b}
Untreated	6.37±0.02^{b}	52.17±0.48^{c}	150.17±0.17^{c}	4.32±0.02^{c}	100.00±0.00^{b}	24.67±0.21^{a}
Standard drug	6.37±0.02^{b}	52.00±0.45^{c}	150.33±0.21^{c}	4.33±0.02^{c}	100.00±0.00^{b}	24.50±0.22^{a}
100mg/kg extract	6.37±0.02^{b}	52.17±0.48^{c}	150.17±0.17^{c}	4.32±0.02^{c}	100.00±0.00^{b}	24.67±0.21^{a}
200mg/kg extract	6.73±0.07^{a}	64.33±0.67^{b}	155.33±0.33^{b}	4.53±0.03^{b}	105.00±0.00^{a}	23.00±0.00^{b}
400mg/kg extract	6.73±0.07^{a}	67.33±0.33^{a}	157.00±0.00^{a}	5.23±0.03^{a}	105.33±0.33^{a}	23.67±0.33^{b}

Values are mean±standard deviation of triplicate determination.
Columns with different alphabet superscript are significantly different at ($p<0.05$)

DISCUSSION

The use of medicinal plants especially *AzadirachtaIndica*in the treatment of ailments is gaining wide acceptance globally; this has been partly attributed to the many bioactive compounds inherent in them [7].

In the tropical and sub-tropical regions, malaria infection has become a key concern to public health practioners, owing to the menace this has brought to the general wellbeing of the populace. Combating this public challenge will be strategy to improve the health status of the general public. Hence, the need to explore the effects of *AzadirachtaIndica* on some biochemical parameters in Wistar Albino Mice Infected with *Plasmodium berghei*remains crucial.

As depicted in figures 1 and 2, there was a significant increase ($p<0.05$) in the ALT and AST activities in the extract treated groups when compared with the untreated control. The result of this findings is consistent with the report of Stanisic*et al*., [12] who reported significant increase ($p<0.05$) in the activities of ALT and AST in mice infected with *Plasmodium berghei*and treated with aqueous extract of *Azadirachtaindica* leaf extract. ALT and AST are liver specific enzymes whose activities are chiefly expressed in the liver, and therefore serve as reliable determinants of liver parenchyma injury [13]. Over expression of these enzymes in the serum is however a pointer that the liver is under threat owing to alterations in the integrity and/or the permeability of the plasma membrane which has been largely attributed to sarcolemma damage [14].Increase in the serum activities of ALT and AST of these experimental animals could also be plausibly due to oxidative damage. It has been suggested that there is always a surge in the generation of free radicals during parasite development which consequently lead to oxidative damage and ultimately, damage of several organs especially the liver [15].

Alkaline phosphatases (ALPs) are a group of isoenzymes that are located on the outer layer of the cell membrane and they catalyze the hydrolysis of organic phosphate esters present in the extracellular space. Although alkaline phosphatases are present in many tissues of the body, they are present in the canalicular membrane of the hepatocyte [16]. As depicted in figure 3, there was significant decrease ($p>0.05$) in the serum activities of alkaline phosphatase in the extract treated groups especially groups administered with 200 mg/kg and 400 mg/kg doses. It has been established that continuous increase in the serum activities of this enzyme above the reference range (44 IU/L) could lead to cholestatic liver disease [17], liver cirrhosis [18] as well as primary biliary cholangitis [19]. Hence, the ability of the extract to mitigate this continuous increase

suggests that the extract could plausibly protect the liver cell from damage and consequently improve its health. Although the result of this study is consistent with the findings of Fontes-Sousa *et al* [20], it is however in contrast with the report of Cheaveau*et al*., [21] who stated that malaria could only increase total bilirubin levels but not the transaminases and ALP activities.
Bilirubin is a yellowish pigment that is made during the breakdown of red blood cells, secreted by the liver in vertebrates and which gives to solid waste products (feces) their characteristic colour.The results of the total bilirubin levels were similar to that of the direct bilirubin levels where the extract treated groups showed a significant increase in total and direct bilirubin levels (24.33±0.33µmol/l and 24.67±0.33µmol/l) respectively (figures 4 and 5). This however could be due to the cumulative effect of liver damage and increased bilirubin production. Since plasmodium infection could lead to the destruction of red blood cells, resulting in an increased release of hemoglobin whose breakdown produces bilirubin as a byproduct, the infection-induced liver damage can impair the liver's ability to effectively metabolize and excrete bilirubin, thereby leading to its accumulation in the bloodstream [22]. It has been established that malaria infection can cause liver damage due to the destruction of red blood cells and subsequent release of hemoglobin, leading to increased bilirubin production [23].Furthermore, administration of higher doses of *Azadirachtaindica* leaf extract may exacerbate liver damage, and further compromise the liver's bilirubin metabolism and clearance functions.

Urea formed in the body, from protein and amino acid catabolism is eliminated via the urinary system and accounts for about half of the urinary salts. Urea levels have always been known to be important biomarkers of kidney function. The higher levels of urea in the 200mg/kg and 400mg/kg groups (6.73±0.07mmol/l) (table 3), showed an onset of kidney damage due to administration of the extracts. This increased urea level could be attributed plausibly to the impaired renal function resulting from a combination of factors. Chua *et al*., [24] asserted that malaria infection can lead to multiple organ dysfunctions, especially kidney damage. Furthermore, the destruction of red blood cells during the infection may result in the release of hemoglobin, which in turn, can be broken down into heme and globin. The breakdown of this heme produces free iron, which subsequently promote oxidative stress and inflammation andconsequently affecting renal function. Moreover, reports have shown that*Azadirachtaindica*leaf extract may have nephrotoxic effects, leading to additional damage to

the kidneys when administered for an extended period of time. Hence, monitoring renal function while on malaria medication remains crucial.

Creatinine is a waste product generated by the metabolism of creatine in muscle tissue, and it is typically excreted by the kidneys. However, compromised renal function can hinder its excretion, resulting in its accumulation in the bloodstream in higher levels. As shown in table 3, the results followed the same trend as that of urea levels with the higher dosage extract groups, 200mg/kg and 400mg/kg, having higher creatinine levels (64.33±0.67µmol/l and 67.33±0.33µmol/l respectively) which could also be as a result of impaired renal function resulting from a combination of factors [25].

Sodium levels of the experimental animals administered 200mg/kg and 400mg/kg doses of extract were found to be significantly higher (155.33±0.33mEq/l and 157.00±0.00mEq/l) respectively when compared with the untreated control (150.17±0.17). Higher intake of sodium above the reference range (150 mmol/L) has since been attributed to a number of diseases especially hypernatremia. Similar trends were observed for the potassium and chloride levels. Hence, consumption of *Azadirachtaindica*should be strictly monitored as its indiscriminate consumption may pose serious threat to the integrity of the kidney as well as electrolyte levels. It is therefore important to closely monitor fluid and electrolyte balance during the treatment of malaria infection, especially when higher doses of the extract are administered.

CONCLUSION

From the results of this study, it canbe concludedthat the administration of *Azadirachtaindica*leaf extract could help to ameliorate malaria infection especially at a lower dose. However, its continuous usage should be strictly monitored as this could have a devastating effects on biochemical reactions and processes.

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