



# International Journal of Life Sciences Biotechnology and Pharma Research





Research Paper

## AMELIORATIVE EFFECTS OF ETHANOL LEAF EXTRACT OF MORINGA OLEIFERA ON THE LIVER AND KIDNEY MARKERS OF MALARIA INFECTED MICE

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The ameliorative properties of ethanol leaf extract of *Moringa oleifera* on the malaria infected liver and kidney injuries of mice were 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 results showed that kidney marker of serum creatinine increased significantly ( $p < 0.05$ ) in group 1 (positive control) compared to group 6 (negative control) and other groups. Group 6 (negative control) showed a non-significant difference ( $p > 0.05$ ) in serum urea compared to group 1 (positive control) and other groups. Liver marker of total bilirubin (TB) increased significantly ( $p < 0.05$ ) in group 1 (positive control) and group 2 (45mg/kg body weight of the extract) compared to group 6 (negative control) and other groups. Alanine aminotransferase (ALT) also, significantly increased ( $p < 0.05$ ) in group 1 (positive control) and group 2 (45mg/kg body weight of the extract) when compared to group 6 (negative control). Group 6 (negative control) showed no significant difference ( $p > 0.05$ ) in aspartate aminotransferase (AST) compared to group 1 (positive control) and other groups. Alkaline phosphatase (ALP) activity of mice significantly increased ( $p < 0.05$ ) in group 1 (positive control) and group 4 (180mg/kg body weight of the extract) compared to group 6 (negative control) and other groups.

**Keywords:** Ameliorative effects, *Moringa oleifera*, Liver marker, Kidney marker, Malaria

### INTRODUCTION

Malaria is a mosquito borne infectious disease of humans and other animals caused by eukaryotic protists of the genus plasmodium (Sarita et al.,

2012). The protists first infect the liver, act as parasites within the red blood cells, causing symptoms that typically include fever and headache which, could lead to death and coma

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in severe cases (Idro *et al.*, 2005). It is widespread in tropical and sub-tropical regions, including much of sub-sahara Africa, Asia and the Americans (Clark and cowden, 2003).

Human infections are initiated when the sporozoites are injected with the saliva during mosquito's feeding. The sporozoites enter the circulatory system and within 30-60 minutes invades the liver cells (Mueller *et al.*, 2007). After, invasion of the hepatocyte, the parasites undergo asexual replication. This replicative stage is often called exoerythrocytic or pre-erythrocytic schizogony. The progeny called merozoites are released into the circulatory system following the rupture of the host hepatocyte (Idro *et al.*, 2005). In *Plasmodium vivax* and *Plasmodium ovale* some of the spozoitites do not immediately undergo asexual replication, but enters a dormant phase known as the hypnozoite. This hypnozoite can reactivate and undergo schizogony at a later time resulting in a relapse (Idro *et al.*, 2005).

After the initial success of malaria eradication programmes through vector control by DDT spraying and chemoprophylaxis, there have been a resurgence of the disease as well as a change in the virulence worldwide (Wyler, 1983). Severe malaria of the kidney could lead to both tubulointerstitial damages as well as glomerulonephritis (Rajapurkar, 1994). In India, two species of plasmodia namely *Plasmodium falciparum* and *Plasmodium vivax* have been implicated with malaria. Renal lesions are commonly caused by *Plasmodium falciparum* and *vivax* (Mahakur *et al.*, 1983). The most common renal lesion of malaria is acute renal failure due to acute tubular necrosis. This is seen in around 1% of all the *Plasmodium falciparum* infected patients (Rajapurkar, 1994). The incidence of acute renal

failure rises to as high as 60% in patients having heavy parasitaemia (more than 10% *Plasmodium falciparum* infested RBCs) (Rajapurkar, 1994).

Acute kidney injury (AKI) is one of the most dreaded complications of severe malaria. (Saroj and Bhabani, 2008) reported that acute renal failure occurs as a complication of *Plasmodium falciparum* malaria in less than 10% of cases, but the mortality rate in these cases may be up to 45%. It is more common in adults more than children. Renal involvement varies from mild proteinuria to severe azotemia associated with metabolic acidosis.

*Moringa oleifera* was massively grown and promoted by the local media in Uganda in the 1980s as a plant which is capable of curing a number of diseases, including malaria, and relieving some symptoms of HIV/AIDS. *Moringa oleifera* is referred to as a MIRACLE TREE (Fuglie, 2001). This is due to its socio-economic, nutritional, pharmacological and industrial benefits (Makkar and Becker, 2007., Afsar *et al.*, 2012. and Mousmi and Handique, 2013). *Moringa* tree is mainly grown in the semi-arid tropical and sub-tropical areas. It grows best in dry sandy soil and can tolerate any other type of soil. It is a fast growing drought-resistant tree that is native to the Southern foothills of Himalayans in Northern India. It is considered as one of the world's most useful tree, as almost every part of the plant can be used for food or has some other beneficial properties (Anamika *et al.*, 2010). In the tropics, it is used as forage for livestock and in many countries as vegetables that has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care.

This study was designed to determine the ameliorative effects of ethanol leaf extract of *Moringa oleifera* on the liver and kidney markers of malaria infected 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 Herbarium 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.

### Chemicals/Reagents

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, 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°C-35°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 h. 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 between 40 and 45°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 20-34g 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. The experimental animals were treated for five days as follows:

**Group 1** was the (positive control) inoculated with malaria parasite (Mp<sup>+</sup>) 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.

$$\text{Percentage (\%)} \text{ yield} = \frac{\text{Weight (g) of concentrated extract}}{\text{Weight (g) of ground Moringa leaves}} \times 100$$

### Determination of Total Bilirubin

Total bilirubin concentration was determined using the methods of Jendrassik and Grof (1938) as outlined in the Randox kit.

### Determination of Serum Urea Concentration

The concentration of serum urea was determined using the method of Tietz (1994) as outlined in Radox kits, UK.

### Determination of Serum Creatinine Concentration

The concentration of serum creatinine was determined using the method of Tietz (1994) as outlined in Radox kits, UK.

### Determination of Aspartate Aminotransferase (AST) Activity

The activity of aspartate aminotransferase was assayed by the methods of Reitman and Frankel (1957) as outlined in Randox kit.

### Determination of Alanine Aminotransferase (ALT) Activity

The activity of alanine aminotransferase was assayed by the methods of Reitman and Frankel (1957) as outlined in Radox kits, UK.

### Determination of Alkaline Phosphatase (ALP) Activity

The activity of alkaline phosphatase (ALP) was assayed by the method of Klein *et al* (1960) as outlined in Radox kits, UK.

## RESULTS

### Percentage Yield of the Extract

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

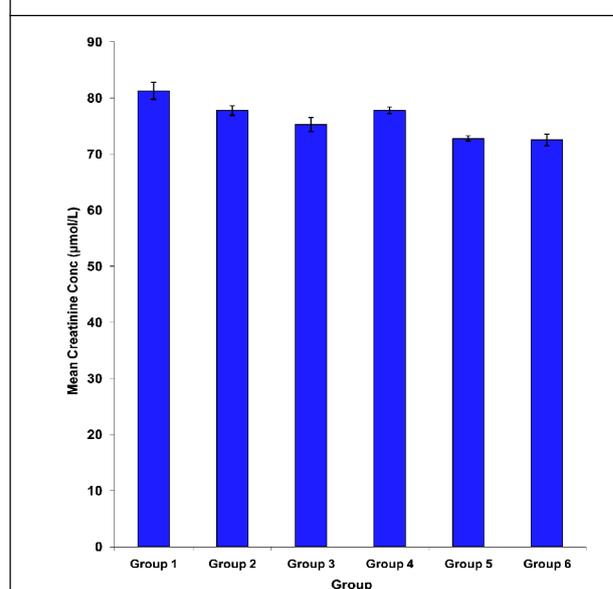
### Effect of Ethanol Leaf Extract of *Moringa oleifera* on Serum Creatinine Concentration in Mice

Figure 1 Shows that on day 28 of post treatment the mean serum creatinine concentration of mice in groups 2,3,4,5 and 6 (negative control) were significantly ( $p < 0.05$ ) lower than that of the group 1 (positive control). Also, the mean serum creatinine concentration of groups 5 and 6 mice were similar when compared.

**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

**Figure 1: Effect of Ethanol Leaf Extract of *Moringa oleifera* on Creatinine Concentration in Mice**

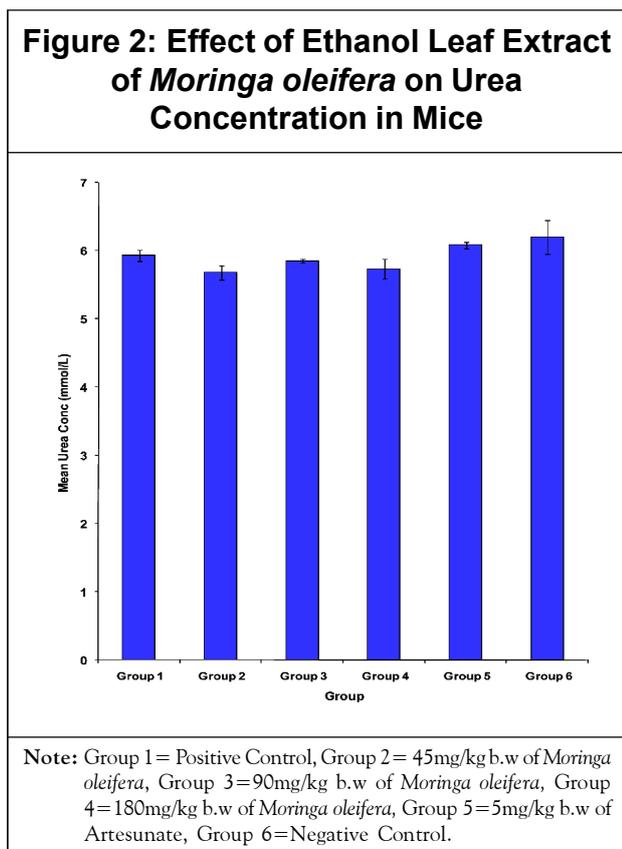


Note: Group 1 = Positive Control, Group 2 = 45mg/kg b.w of *Moringa oleifera*, Group 3 = 90mg/kg b.w of *Moringa oleifera*, Group 4 = 180mg/kg b.w of *Moringa oleifera*, Group 5 = 5mg/kg b.w of Artesunate, Group 6 = Negative Control.

### Effect of Ethanol Leaf Extract of *Moringa oleifera* on Urea Concentration in Mice

Figure 2 shows that on day 28 of post treatment

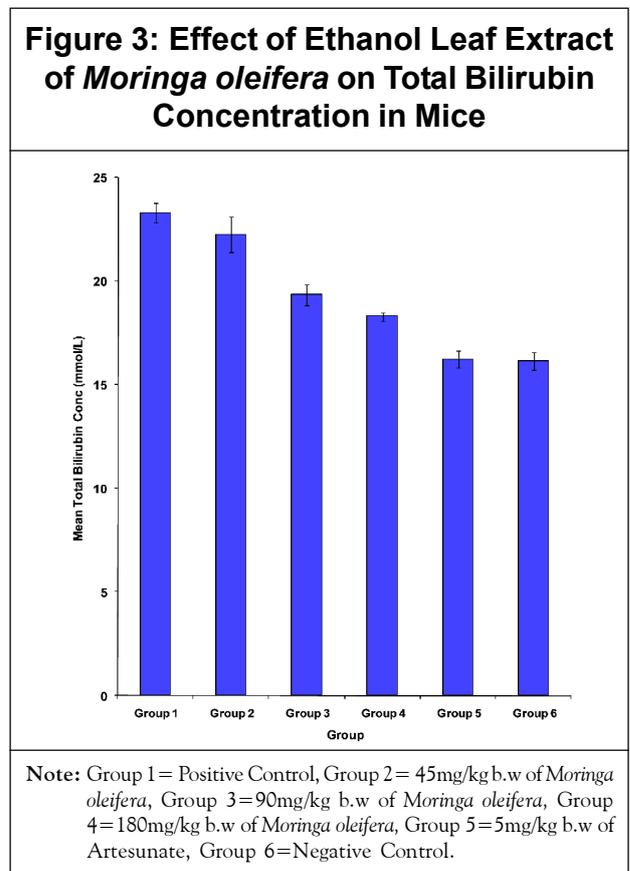
mean values for urea concentration of mice in groups 2, 3, 4, 5 and 6 (negative control) were not significantly ( $p > 0.05$ ) different from the value obtained for mice in group 1 (positive control). But the values obtained for mice in groups 2 and 4 were significantly ( $p < 0.05$ ) lower than that for mice in group 6 (negative control). Also mean values for urea concentration in groups 1 (positive control) and 3 were similar when compared.



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

Figure 3 shows that on day 28 of post treatment mean values for total bilirubin concentration of mice in groups 3, 4, 5 and 6 significantly decreased ( $p < 0.05$ ) in a dose-dependent pattern when compared to the mean total bilirubin concentration of mice in groups 1 (positive control) and 2. The mean value for total bilirubin

concentrations for mice in group 5 and 6 (negative control) were similar when compared.



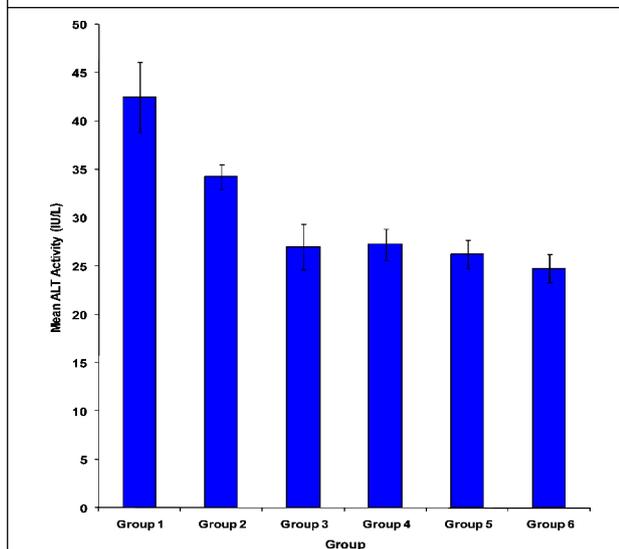
**Effect of Ethanol Leaf Extract of *Moringa oleifera* on Alanine Aminotransferase Activity in Mice**

Figure 4 Shows that on day 28 of post treatment mean values for ALT activity for mice in groups 3, 4, 5 and 6 (negative control) were significantly ( $p < 0.05$ ) lower than that for groups 1 and 2. Meanwhile, the mean value for ALT activity of mice in group 2 was significantly ( $p < 0.05$ ) lower than that for group 1. But the mean ALT values for groups 3, 4 and 5 were essentially similar.

**Effect of Ethanol Leaf Extract of *Moringa oleifera* on Aspartate Aminotransferase Activity in Mice**

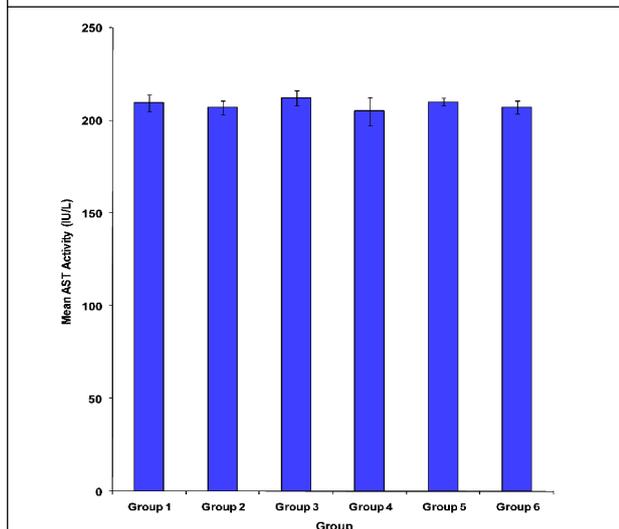
Figure 5 Shows that on day 28 of post treatment mean values for AST activity in mice for groups

**Figure 4: Effect of Ethanol Leaf Extract of *Moringa oleifera* on Alanine Amino-transferase Concentration in Mice**



Note: 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.

**Figure 5: Effect of Ethanol Leaf Extract of *Moringa oleifera* on Asparate Amino-transferase Concentration in Mice**



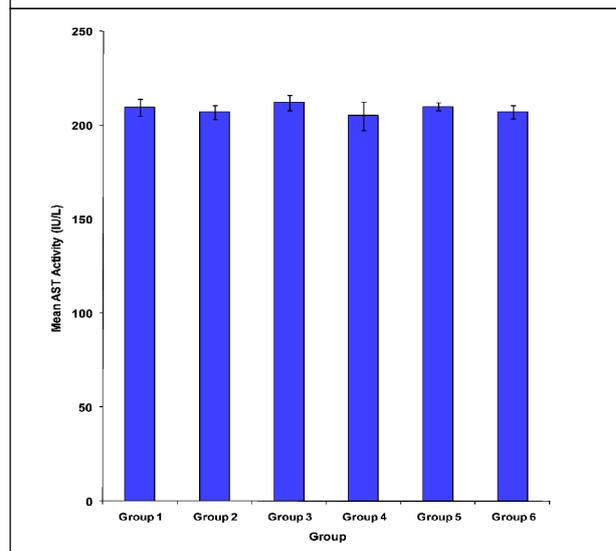
Note: Group 1= Positive Control, Group 2= 45mg/kg b.w of *Moringa oleifera*, Group 3=90mg/kg b.w of *Moringa oleifera*, Group 4=180mg/kg b.w of *Moringa oleifera*, Group 5=5mg/kg b.w of Artesunate, Group 6=Negative Control.

2, 3, 4 and 5 were similar with the values for mice in groups 1 (positive control) and 6 (negative control).

**Effect of Ethanol Leaf Extract of *Moringa oleifera* on Alkaline Phosphatase Activity in Mice**

Figure 6 Shows the effect of ethanol leaf extract of *Moringa oleifera* on alkaline phosphatase activity in mice. On day28 of post treatment shows that the mean ALP values for mice in groups 2, 3, 5 and 6 were significantly ( $p < 0.05$ ) lower than that for mice in groups 1 (positive control) and 4. But the ALP values for mice in groups 4 and 1 (positive control) were similar when compared.

**Figure 6: Effect of Ethanol Leaf Extract of *Moringa oleifera* on Alkaline Phosphatase Concentration in Mice**



Note: 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.

## DISCUSSION

The liver is a vital organ in vertebrates and other animals. It is used in the elimination and

detoxification of harmful biochemical waste products and toxins. It plays key role in the synthesis of biochemicals that are very vital in body metabolism. The kidney is an important organ of regulation in mammals. They are essential in the urinary and homeostatic function. Liver and acute kidney injuries could occur as a result of dreaded complications of severe malaria (Idro *et al.*, 2005) and (Saroj and Bhabani, 2008).

The results of ethanol leaf extract of *Moringa oleifera* on serum creatinine concentration in mice showed a significant decrease ( $p < 0.05$ ) in serum creatinine concentration in groups (2,3,4,5 and 6) treated with 45,90,180 mg/kg body weight of the extract, 5mg/kg body weight of artesunate and 5mg/kg body weight of distilled water respectively were compared to group 1 (positive control). This showed that the ethanol leaf extract of *Moringa oleifera* has reduced the level of serum creatinine in group 2 (45 mg/kg body weight of the extract), group 3 (90 mg/kg body weight of the extract) and group 4 (180 mg/kg body weight of the extract) thereby ameliorating the effects of malaria parasitaemia on the kidney. There was no significant difference ( $p > 0.05$ ) in serum creatinine when group 4 (180 mg/kg body weight of the extract) was compared to group 3 (90 mg/kg body weight of the extract) and group 2 (45 mg/kg body weight of the extract). This is in line with the discovery of Mazumder *et al.*, (1999) who showed the hepatorenal function of *Moringa oleifera* on mice.

The effect of ethanol leaf extract of *Moringa oleifera* on urea concentration in mice showed a non significant difference ( $p > 0.05$ ) in urea concentration in groups (2,3,4,5 and 6) treated with with 45,90,180 mg/kg body weight of the extract, 5mg/kg body weight of artesunate and

5mg/kg body weight of distilled water respectively were compared to group 1 (positive control). This showed that malaria had no effect on urea concentration. But there was a significant decrease ( $p < 0.05$ ) in urea concentration when group 2 (45mg/kg body weight of the extract) and group 4 (180 mg/kg body weight of the extract) were compared to group 6 (negative control). This corroborates with the work of Mazumder *et al.*, (1999) showing the potential effects of the ethanol leaf extract of *Moringa oleifera* on ameliorating renal dysfunctions.

The effect of ethanol leaf extract of *Moringa oleifera* on alanine aminotransferase activity in mice showed a significant decrease ( $p < 0.05$ ) in alanine aminotransferase in groups (3, 4, 5 and 6) treated with 90,180 mg/kg body weight of the extract, 5mg/kg body weight of artesunate and 5mg/kg body weight of distilled water respectively were compared to the alanine aminotransferase of group 1 (positive control) and group 2 (45 mg/kg body weight of the extract). Alanine aminotransferase in conjunction with aspartate aminotransferase is usually used to diagnose hepatocellular injury and diseases. Alanine aminotransferase is cytosolic and is present in large concentrations in liver and, in lesser amount in kidney, heart and skeletal muscle (Johnston,1999). It is therefore a more specific liver marker than aspartate aminotransferase (Song *et al.*, 2004). This confirms the damages that were done to the liver as a result of the malaria infection. But 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) all showed no significant difference ( $p > 0.05$ ) in alanine aminotransferase compared to group 6 (negative control). This showed the ameliorative effect of the liver

damages by the *Moringa oleifera* ethanol leaf extract as a result of the malaria parasitaemia. This agrees with the findings of Fakurazi *et al.*, (2008) and Alaaeldin (2009) that showed the preventive and ameliorative effects of *Moringa oleifera* on liver injury and damages. The effect of ethanol leaf extract of *Moringa oleifera* on aspartate aminotransferase activity in mice showed no significant difference ( $p > 0.05$ ) in aspartate aminotransferase when group 6 (negative control) was compared to other groups including group 1 (positive control). This showed that malaria parasitaemia, the extract and artesunate did not affect the aspartate aminotransferase activity in the mice.

The effect of ethanol leaf extract of *Moringa oleifera* on alkaline phosphatase activity in mice showed a significant decrease ( $p < 0.05$ ) in alkaline phosphatase in all the groups compared to group 1 (positive control) and group 4 (180 mg/kg body weight of the extract). Alkaline phosphatase is present in all the tissues throughout the body, but is particularly concentrated in the liver, bile duct, kidney, bone and the placenta. It is therefore not a specific liver marker. This result showed that malaria parasitaemia had effect on the alkaline phosphatase activity in the mice. But, in group 4 (180 mg/kg body weight of the extract) the elevated level of alkaline phosphatase could be as a result of active bone formation occurring as alkaline phosphatase is a by-product of osteoblast activity. But, group 2 (45 mg/kg body weight of the extract) and group 3 (90 mg/kg body weight of the extract) showed a significant reduction ( $p < 0.05$ ) in alkaline phosphatase which corroborated with the findings of Alaaeldin (2009)

and Fakurazi *et al.* (2008) who showed ameliorative effects of *Moringa oleifera* leaf extract on liver injury.

The effect of ethanol leaf extract of *Moringa oleifera* on total bilirubin in mice showed a significant decrease ( $p < 0.05$ ) in total bilirubin concentration of all the groups compared to the total bilirubin concentration of group 1 (positive control) and group 2 (45 mg/kg body weight of the extract). This could be as a result of liver damage by the malaria parasitaemia. But in group 3 (90 mg/kg body weight of the extract) and group 4 (180 mg/kg body weight of the extract) both showed a significant reduction ( $p < 0.05$ ) in total bilirubin thereby ameliorating the effect of malaria on the liver. This agrees with the findings of Alaaeldin (2009) and Pari and Kumar (2002) which showed the protective effects of *Moringa oleifera* extract on the liver.

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