Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 10 (6): 281-289, 2015
ISSN 1815-9923 / DOI: 10.3923/ajb.2015.281.289

Anti-Malarial and some Biochemical Indices of the Ethanol Extract of *Zapoteca portoricensis* Root on Malaria-Infected Mice

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ABSTRACT

The study investigated the effect of ethanol extract of *Zapoteca portoricensis* roots on malaria-infected mice. The effect of ethanol extract on malaria infected mice was studied by assaying percentage parasitaemia, some liver marker enzymes and kidney function markers. The acute toxicity study showed that the ethanol extract had LD_{50} above 2900 mg kg^{-1} b.wt. Malaria infected mice treated with the ethanol extract had significant ($p<0.05$) decrease in the mean percentage parasitaemia when compared with group 2 mice (malaria untreated). The AST activity showed significant ($p<0.05$) increase in all the test groups when compared to normal control on day 14 but significantly ($p<0.05$) reduced in all the treated groups when compared to group 2 mice on day 28. The ALT and ALP activities decreased significantly ($p<0.05$) in all the test groups when compared to group 2 mice. The urea concentration significantly ($p<0.05$) decreased in the normal control, group 4 and 5 when compared with group 2 mice. Creatinine concentration decreased significantly ($p<0.05$) in the normal control, group 3 and group 4 when compared to group 2 mice on day 14, however, on day 28, no significant ($p>0.05$) differences were observed in the creatinine concentrations in all the groups when compared to normal control. The results suggest that the ethanol extract of *Zapoteca portoricensis* roots is relative safe has no negative effects on liver and kidney with huge potentials in the management of malaria.

Key words: Percentage parasitaemia, liver marker enzymes, kidney markers, acute toxicity

INTRODUCTION

Malaria parasites belong to the genus *Plasmodium* (phylum Apicomplexa). In humans, malaria is caused by *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* (Collins, 2012). Among those infected, *P. falciparum* is the most common species identified (~75%) followed by *P. vivax* (~20%) (Nadjm and Behrens, 2012). *Plasmodium falciparum* accounts for the majority of deaths, (Sarkar *et al.*, 2010) non-*falciparum* species have been found to be the cause of about 14% of cases of severe malaria in some groups (Nadjm and Behrens, 2012). *Plasmodium vivax* proportionally is more common outside of Africa (Arnott *et al*., 2012).

The signs and symptoms of malaria typically begin 8-25 days following infection (Fairhurst and Wellems, 2010). However, symptoms may occur later in those who have taken antimalarial medications as prevention. According to Nadjm and Behrens (2012), initial manifestations of the disease common to all malaria species are similar to flu-like symptoms (Bartoloni and Zammarchi, 2012) and can resemble other conditions such as septicemia, gastroenteritis and viral diseases. The

classic symptom of malaria is paroxysm which is a cyclical occurrence of sudden coldness followed by rigour and then fever and sweating, occurring every 2 days (tertian fever) in *P. vivax* and *P. ovale* infections and every 3 days (quartan fever) for *P. malariae*. *Plasmodium falciparum* infection can cause recurrent fever every 36-48 h or a less pronounced and almost continuous fever (Ferri, 2009).

Severe malaria is usually caused by *P. falciparum* (often referred to as *falciparum malariae*). Symptoms of falciparium malaria arise 9-30 days after infection (Bartoloni and Zammarchi, 2012). Splenomegaly, severe headache, hepatomegaly (enlarged liver), hypoglycemia and haemoglobinuria with renal failure may occur. Renal failure is a feature of black water fever, where haemoglobin from lysed red blood cells leaks into the urine (Bartoloni and Zammarchi, 2012).

Traditional methods of treatment and control of malaria could be a promising source of potential anti-malaria drugs (Ugwu *et al*., 2013; Sumalatha and Sreedevi, 2012; Venkat *et al*., 2011; Wright and Phillipson, 1990). More than 80% of the world's population relies on traditional medicine for their primary healthcare needs (WHO., 2008). In developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infectious diseases. These plants are ingested as decoctions, teas or juice preparations (Gonzalez, 1980).

Zapoteca portoricensis belonging to the family Fabaceaeare traditionally used as antidiarrhoel, anti canvulsant, antispasmodic and in the treatment of tonsillitis. Terpenoids and steroids obtained from the column fractions of the root extracts are proved to be responsible for the production of significant anti-inflammatory activity (Agbo *et al*., 2010). This study was designed to investigate the antimalarial properties of the ethanol extract of *Zapoteca portoricensis* root.

MATERIALS AND METHODS

Plant material: The roots of *Zapoteca portoricensis* (Elugelu) were collected from Umabor-Ehalumona in Nsukka Local Government Area of Enugu State, Nigeria. The plant was identified by Mr. A. Ozioko of Bioresources Development and Conservation Programme Research Centre Nsukka.

Animals: Thirty six Wistar albino mice weighing 20-40 g were used for the study. The mice were obtained from the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized for 1 week, under a standard condition with 12 h light and dark conditions with free access to food and water before the commencement of the experiments.

Extraction procedure: The roots of *Zapoteca portoricensis* were collected, washed with distilled water and then dried under room temperature for 4 weeks. The dried roots were pulverized into powdered form with a high speed milling machine. The powdered sample (500 g) was macerated in 2.5 L absolute ethanol for 24 h. After that, the resulting extract was filtered using Wattman No 1 filter paper. The resulting filtrate was concentrated to dryness using rotary evaporator at temperature of 60. The concentrated extract was stored in the refrigerator and used for the study.

Experimental design: Thirty six Wistar albino mice weighing 20-40 g were housed in separate cages, acclimatized for 1 week and then divided randomly into 6 groups of 6 mice each. The animals were allowed free access to water and feed. The acclimatisation of the experimental animals lasted for 7 days (starting from 1st July-7th July, 2014) and the malaria passage was

carried out on the day 8 while treatment with the extract was started on the day 13 and lasted for 28 days (i.e. from July 13-August 10, 2014). The route of administration (treatment) was oral with the aid of an oral intubations tube. The animals were grouped, inoculated and treated as follows:

- Group 1: Normal control which was not inoculated with malaria parasite and was treated with 5 mL kg^{-1} b.wt., of distilled water
- Group 2: The positive control inoculated with malaria parasite (mp^{+}) and was treated with 5 mL kg^{-1} b.wt., of distilled water
- Group 3: Inoculated with malaria parasite (standard control) and was treated with 28 mg kg^{-1} b.wt., of artemeter and lumenfantrine
- Group 4: Inoculated with malaria parasite and treated with 100 mg kg^{-1} b.wt., of the ethanol extract of *Zapoteca portoricensis* roots
- Group 5: Inoculated with malaria parasite and treated with 200 mg kg^{-1} b.wt., of the ethanol extract of *Zapoteca portoricensis* roots
- Group 6: Inoculated with malaria parasite and treated with 300 mg kg^{-1} b.wt., of the ethanol extract of *Zapoteca portoricensis* roots

The animals in group 2-6 were inoculated with malaria parasite as shown above and confirmed positive on the 7th day before treatment commenced. The experiment lasted for 28 days during which blood samples were collected through occular puncture on days 0, 7, 14 and 28 in EDTA bottles and non-heparinised tubes for the analysis.

Determination of percentage yield of the extract: The percentage yield of the extract was determined by weighing the pulverized dry roots and the concentrated extract obtained after extraction and then calculated using the following equations:

$$\text{Yield (\%)} = \frac{\text{Weight of the extract (g)}}{\text{Weight of pulverized roots (g)}} \times 100\%$$

Determination of percentage parasitaemia: The determination of malaria parasitaemia (mp+) was carried out according to the method of Dacie and Lewis (2000).

Biochemical assay

Assay of aspartate aminotransferase: A Randox Commercial Enzyme kit according to the method of Reitman and Frankel (1957) was used to assay for the activity of aspartate aminotransferase.

Assay of alanine aminotransferase activity: The activity of alanine aminotransferase was assayed by the method of Reitman and Frankel (1957) as outline in Randox kit.

Assay of alkaline phosphatase activity: The activity of alkaline phosphatase (ALP) was assayed by the method of Klein *et al.* (1960) as outline in DCA kit.

Determination of serum urea concentration and creatinine concentration: The concentration of serum urea and creatinine were determined using the method of Tietz (1994) as outlined in Randox Kits, UK.

RESULTS

Percentage yield: From the result in Table 1, percentage yield of the ethanol extract of *Zapoteca portoricensis* roots was 3.18%.

Figure 1, shows significant ($p<0.05$) increase on day 14, in the aspartate aminotransferase activity (AST) in all the groups compared to AST activity of group 1 (normal control). Non-significant ($p>0.05$) different was observed in groups 2 (malaria untreated), 3 treated with 28 mg kg^{-1} b.wt., of the artemether and lumenfantrine, 5 and 6 administered 200 and 300 mg kg^{-1} b.wt., of the extract, respectively. On day 28 the mean values of AST activity shows significant ($p<0.05$) decrease in groups 3 treated with 28 mg kg^{-1} b.wt., of the artemether and lumenfantrine, 4 and 5 administered 100 and 200 mg kg^{-1} b.wt., of the extract, respectively compared to group 2 (malaria untreated).

Figure 2 shows significantly ($p<0.05$) increase in the alanine aminotransferase activity (ALT) in group 2 (malaria untreated) compared to ALT activity in group 1 (normal control). Non-significant ($p>0.05$) difference was observed in groups 3 treated with 28 mg kg^{-1} b.wt., of the artemether and lumenfantrine, 4, 5 and 6 administered 100, 200 and 300 mg kg^{-1} b.wt., of the extract, respectively. On day 28 the ALT activity shows significant ($p<0.05$) decrease in groups 3, 4, 5 and 6 administered 28 mg kg^{-1} b.wt., of the artemether and lumenfantrine, 100, 200 and 300 mg kg^{-1} b.wt., of the extract respectively compared to group 2 mice (malaria untreated) on day 28.

Figure 3, shows significant ($p<0.05$) increase on day 14 in group 2 (malaria untreated) and group 3 treated with 28 mg kg^{-1} b.wt., of the artemether and lumenfantrine compared to group 1 (normal control). Significant ($p<0.05$) reduction was observed in the alkaline phosphatase (ALP) activity in groups 3, 4, 5 and 6 administered 28 mg kg^{-1} b.wt., of the artemether and lumenfantrine, 100, 200 and 300 mg kg^{-1} b.wt., of the extract respectively compared to 2 (malaria untreated) on day 28.

Table 1: Percentage yield of the ethanol extract of *Zapoteca portoricensis* roots

Parameters	Values
Weight of pulverized root (g)	500
Weight of the concentrated extract (g)	15.9
Yield (%)	3.18

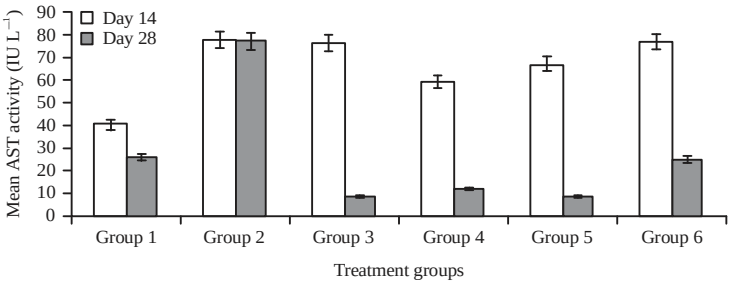

Fig. 1: Effect of ethanol extract of *Zapoteca portoricensis* roots on aspartate aminotransferase activity in malaria-passaged mice, Group 1: Normal/Negative control, Group 2: Positive control (Malaria-passaged), Group 3: Standard control (Malaria-passaged+28 mg kg^{-1} of Artemether and Lumefetrine), Group 4: Malaria-passaged+100 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root, Group 5: Malaria-passaged+200 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root, Group 6: Malaria-passaged+300 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root

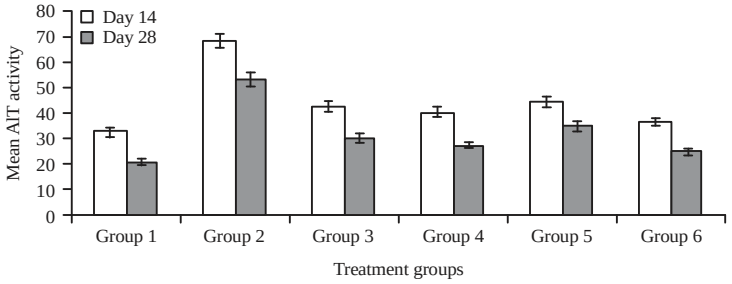

Fig. 2: Effect of ethanol extract of *Zapoteca portoricensis* roots on alanine aminotransferase activity in malaria-passaged mice, Group 1: Normal/Negative control, Group 2: Positive control (Malaria-passaged), Group 3: Standard Control (Malaria-passaged+28 mg kg^{-1} of Artemether and Lumefetrine), Group 4: Malaria-passaged+100 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root, Group 5: Malaria-passaged+200 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root, Group 6: Malaria-passaged+300 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root

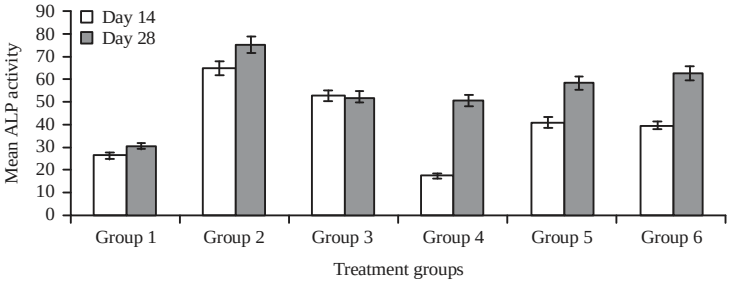

Fig. 3: Effect of ethanol extract of *Zapoteca portoricensis* roots on alkaline phosphatase activity in malaria-passaged mice, Group 1: Normal/negative control, Group 2: Positive control (Malaria-Passaged), Group 3: Standard Control (Malaria-passaged+28 mg kg^{-1} of Artemether and Lumefetrine), Group 4: Malaria-passaged+100 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root, Group 5: Malaria-passaged+200 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root

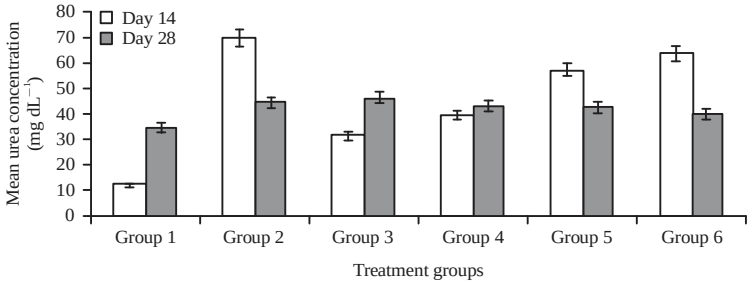

Fig. 4: Effect of ethanol extract of *Zapoteca portoricensis* roots on urea concentration in malaria-passaged mice, Group 1: Normal/negative control, Group 2: Positive control (Malaria-passaged), Group 3: Standard control (Malaria-passaged+28 mg kg^{-1} of Artemether and Lumefetrine), Group 4: Malaria-passaged+100 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root, Group 5: Malaria-passaged+200 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root

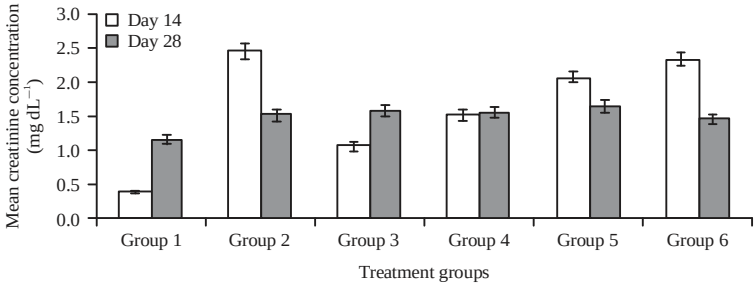

Fig. 5: Effect of ethanol extract of *Zapoteca portoricensis* roots on creatinine concentration on malaria passaged mice, Group 1: Normal/negative control, Group 2: Positive control (Malaria-passaged), Group 3: Standard control (Malaria-passaged+28 mg kg^{-1} of Artemether and Lumefetrine), Group 4: Malaria-passaged+100 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root, Group 5: Malaria-passaged+200 mg kg^{-1} b.wt., of ethanol extract of *Zapoteca portoricensis* root

As shown in Fig. 4, day 14 result showed significant ($p<0.05$) increase in the urea concentration in groups 2 malaria untreated, 3 administered with 28 mg kg^{-1} b.wt., of artemether and lumefantrine, 4, 5 and 6 treated with 100, 200 and 300 mg kg^{-1} b.wt., of the extract, respectively compared to group 1 (normal control). On day 28, non-significant ($p>0.05$) different was observed in groups 2 (malaria untreated), 3 administered with 28 mg kg^{-1} b.wt., of artemether and lumefantrine, 4, 5 and 6 treated with 100, 200 and 300 mg kg^{-1} b.wt., of the extract, respectively compared to group 1 (normal control).

Figure 5, shows significant ($p<0.05$) increase on day 14, in the mean creatinine concentration in all the groups compared to the creatinine concentration in group 1 (normal control). Significant elevation ($p<0.05$) was observed in the creatinine concentration of groups 2 (malaria untreated), 3 administered 28 mg kg^{-1} b.wt., of artemether and lumenfantrine, 4 and 5 administered 100 and 200 mg kg^{-1} b.wt., of the extract respectively compared to group 1 (normal control) on day 28.

DISCUSSION

Most available antimalarials were designed to target the pathogenic blood stages in humans and to address the constant threat of drug resistance (Fidock, 2010). This study on *Zapoteca portoricensis* root has become necessary both to meet the challenges of malaria eradication and to circumvent resistance to most antimalarial drugs. Several medicinal plants have also been used locally to treat malaria infection. Some of such plants are *Enantia chloranta, Nauclea latifolia, Salacia nitida* and *Moringa oleifera.* Their use shows that they ameliorate the effects of malaria parasite as shown by Ugwu *et al*. (2013) and Ogbonna *et al*. (2008).

The result of the effect of ethanol extract of *Zapotecal portoricensis* roots on the mean parasitaemia in mice showed significant ($p<0.05$) reduction in all the groups when compared with group 2 (malaria untreated). The reduction could lead to the destruction of the essential organs such as liver, kidney, blood cells and other organs in the mice. It could also be as a result of the infection of the liver by sporozoites and increase number of meroziotes, this is in consistent with the findings of Ugwu *et al*. (2013) and Trampuz *et al*. (2003). Significant ($p<0.05$) reduction was observed in the parasitaemia of groups 5 and 6 malaria-passaged mice treated with 200 and 300 mg kg^{-1} b.wt., of the extract, respectively compared to the parasitaemia of group 2 (malaria

untreated) mice. This showed that the extract might be effective against malaria parasitaemia due to the reduction of the percentage parasitaemia level in the treatment groups.

The observation on the effect of ethanol extract of *Zapoteca portoricensis* root on aspartate aminotransferase activity in mice showed significant increase ($p<0.05$) in all the groups when compared to groups 1 (normal control) and 4 treated with 100 mg kg^{-1} b.wt., of the extract. The liver might have been damaged as a result of the malaria infection. Significant ($p<0.05$) reduction was observed in all the treated groups 4, 5 and 6 administered 100, 200 and 300 mg kg^{-1} b.wt., of the extract, respectively on the day 28, it could be deduced that artemether and lumenfantrine and ethanol extract of *Zapoteca portoricensis* root ameliorated the effect of malaria infection on aspartate aminotransferase activity. Though elevated aspartate aminotransferase activity is not specific for liver damage, it has been used as a cardiac marker as reported by Nyblom *et al.* (2004, 2006).

The result of the effect of ethanol extract of *Zapoteca portoricensis* root on alanine aminotransferase (ALT) activity showed a significant decrease ($p<0.05$) in mice of all the groups compared to group 2 mice (malaria untreated). This could be an indication that the alanine aminotransferase (ALT) activity was affected by malaria parasite. This is in agreement with the findings of Giboney (2005) who reported that increased activities of alanine aminotransferase (ALT) are associated with hepatitis and other liver disorders.

The effects of ethanol extract of *Zapoteca portoricensis* root on alkaline phosphatase (ALP) activity showed significant ($p<0.05$) decrease in the ALP activity in all the groups compared to the ALP activity of group 2 (malaria untreated). An elevated level of ALP occurs in disease that impairs bile formation and many other liver disorders. This is in corroboration with the findings of Uzuegbu and Emeka (2011) who reported significant increase with the malaria patients when compared to non-malaria patients.

The observation on the effect of ethanol extract of *Zapoteca portoricensis* roots on urea concentration showed significant increase ($p<0.05$) in groups 2 (malaria untreated), 3 treated with 28 mg kg^{-1} b.wt., of artemether and lumenfantrine, 4, 5 and 6 treated with 100, 200 and 300 mg kg^{-1} b.wt., of the extract respectively when compared to group 1 (normal control). But on day 28 significant reduction was observed in groups 5 and 6 administered 200 and 300 mg kg^{-1} b.wt., of the extract, respectively. Non-significant difference ($p>0.05$) in urea concentration of groups 3 (treated with 28 mg kg^{-1} b.wt., of artemether and lumenfantrine), 4 and 5 (treated with 100 and 200 mg kg^{-1} b.wt., of the extract, respectively) was observed when compared to group 2 (malaria untreated). This showed the effect of the artemether and lumenfantrine and ethanol extract of the *Zapoteca portoricensis* roots on ameliorating kidney damage.

The effect of ethanol extract of *Zapoteca portoricensis* roots on creatinine concentration showed significant increase ($p<0.05$) in the mean values of serum creatinine concentration of groups 2 (malaria untreated), 5 and 6 (treated with 200 and 300 mg kg^{-1} b.wt., of the extract, respectively) when compared to group 1 (normal control). This could be attributed to the renal destruction by the malaria infection and higher doses of ethanol extract of *Zapoteca portoricensis* roots. No significant difference ($p>0.05$) was observed in group 3 treated with 28 mg kg^{-1} b.wt., of artemether and lumenfantrine and group 4 treated with 100 mg kg^{-1} b.wt., of the extract. This shows that artemether and lumenfantrine and 100 mg kg^{-1} b.wt., of *Zapoteca portoricensis* root ameliorated the effects on the renal function.

CONCLUSION

The findings from this study specify that the ethanol extract of *Zapoteca portoricensis* root has antimalarial properties through the reduction of percentage parasitaemia and the effect on the

activities of liver function enzyme markers showed that the extract might have helped in ameliorating the effects of malaria on the liver. They also support the claim that ethanol extract of *Zapoteca portoricensis* root is effective in folk medicine for the treatment of malaria.

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