In vivo antioxidant and biochemical evaluation of Sphenocentrum jollyanum leaf extract in P. berghei infected mice

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Abstract: Recent approach in treatment and drug development suggested that the control of oxidative stress in malarial infected patients may be an added advantage. In this study, effect of methanolic leaf extract of Sphenocentrum jollyanum pier (S. jollyanum) on liver damage, markers of oxidative stress and alteration in lipid profile in P. berghei infected mice was assessed. Oxidative stress was induced by intravenously inoculation of mice with 1 × 10^7 sporozoites P. berghei. Treatment of parasitized mice with leaf extract of S. jollyanum had a significant (p<0.05) reductions in elevated levels of total protein, globulin, AST, ALT, ALP, GGT and total bilirubin, serum, kidney and liver malondialdehyde (MDA) concentrations, but caused a significant (p<0.05) increased in the activities of serum and liver catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) level when compared with parasitized non-treated group (PNT). The extract treated group also showed significant (p<0.05) improvement in the levels of HDLc, total cholesterol, LDL and reduction in triglyceride compared with parasitized non treated group. Our results revealed that the protective capacity and antioxidant activity of the extract is dose dependant. The findings suggest that antioxidant property of Sphenocentrum jollyanum leaf extract might be an added advantage to it anti-malarial activity.

Keywords: Sphenocentrum jollyanum leaves, Plasmodium berghei, antioxidants, oxidative stress.

INTRODUCTION

Malaria is a life-threatening disease characterized mainly by fever, headache and other flu-like symptoms. It infects and destroys red blood cells (anaemia) and other vital organs (WHO, 2011). Malaria is one of the most devastating and prevalent disease of human, infecting more than 200 million people worldwide of which 1-2 million die annually (Devendra et al., 2005; Postma et al., 1996). Oxidative stress, occasioned by imbalance in free radical generation has been reported as one of the major mechanisms of malaria pathogenesis (Nathawut et al., 2004; Potter et al., 2004). Depleted antioxidants status (Das et al., 1993), increased plasma lipid peroxidation and altered fluidity of erythrocyte membrane (Sibmoo et al., 2000) are common features in acute malaria infection. It is well documented that intra-erythrocytic parasite's metabolic processes and host immune reaction (Nathawut et al., 2004; Potter et al., 2004) contributed greatly to increased free radicals generation in malaria infected cells. It’s worth mentioning, depending on the amount and place of production, free radical generation in malaria infected cells can be both beneficial and pathological (Gora et al., 2006; Postma et al., 1996). Postman et al. (1996) revealed that increased ROS generation are involved in pathological changes in the host tissue. Contrary to this, newly developed antimalarial drugs such as artemisinin and its derivatives employed the mechanism of enhanced ROS generation to inhibit intra-erythrocytic growth both in vitro and in vivo(Postma et al., 1996). Another common occurrence in the pathogenesis of malaria infection is oxidation of low density lipoprotein. Oxidized LDL enhanced endothelial expression of adhesion molecules in malaria infected patients and is considered as a critical event in the progression of the disease (Nathawut et al., 2004).

In Africa, a large percentage (80%) of the population still relies on medicinal plants to treat malaria and other diseases (Agbedahunsi, 2000) due to their affordability and accessibility. One of such popularly used medicinal plants is Sphenocentrum jollyanum. Burkhill, (1985) and Iwu, (2003), described Sphenocentrum jollyanum as a small erect sparsely branched shrub, of 1.5m in height. They reported that the plant is commonly found in Southwestern part of Nigeria and its various parts are used in the treatment of malaria.

Olorunnisola and Afolayan (2011) reported for the first time the antiplasmodia activity of the leaf extract. They revealed that the leave extract of the plant is more potent as antimalarial agent than the root plant which is traditionally employed in the treatment of malaria infection. This study is design to elucidate the possible role of the leaf extract on antioxidant status and lipid profile in P. berghei infected mice malaria parasites.

MATERIALS AND METHODS

Plant material

Fresh S. jollyanum leaf was collected in November 2008 from University of Ibadan Botanical Garden and authenticated by Professor AO Adebesi of Department of Agricultural Science. A voucher specimen of the plant

was deposited in the Faculty of Agricultural Sciences University of Ibadan, Ibadan, Oyo State, Nigeria (Olorunnisola et al., 2011).

**Animals and parasite**

Forty males and females mice of 8 weeks divided into five groups were used in the study. Observation protocols and method used for maintaining and inoculation of ANKA strains of *Plasmodium berghei* in our laboratory has been previously described (Olorunnisola et al., 2011).

**Animal grouping and extract administration**

The mice were divided randomly into groups of eight mice each. Different doses of the leaf, extract (100 and 200 mg/kg) were orally administered respectively to mice in parasitized extract-treated (PET) groups 1 and 2 respectively. 10 ml/kg of distilled water were given to mice in none parasitized non–treated-(NPNT) and parasitized non–treated [PNT] Groups 3 and 4 respectively. The animals were fed with pelleted grower mash from commercial feed vendor (Bova Jay Nig. Ltd, Ogbomoso) and water *ad libitum*.

**Tissue extracts preparation and assays**

At the end of the experiment (30 days), the mice were anaesthetized with Halothane and blood collected by cardiac puncture into sample tubes from where plasma used for assay was harvested. Tissue preparations and homogenate were obtained using method described by Oyedemi et al. (2010). The supernatant was collected and various markers oxidative stress parameters such as glutathione [GSH] (Ellman, 1951), catalase [CAT] (Aebieh, 1982), superoxide dismutase [SOD] (Sun et al., 1998) and lipid peroxidation (Yagi, 1998) was monitor using various activity assay. Blood samples collected were allowed to clot. Serum was separated by centrifuging at 3000 rpm for 10 min and analysed for various biochemical parameters such as Alkaline phosphatase [ALP] (Mallay and Evelyn, 1937) and total bilirubin [TB] (Kind and King, 1954) and total serum protein [TSP] total serum protein (TSP) (Lowry et al., 1951), LDLc, total cholesterol, HDLc, VLDL and triglyceride were analysed using commercially available kit (Randox Laboratories Ltd., Ardmore, Co. Antrim, United Kingdom). The experiment was carried out after its approval by the Ethics Committee of the Ladoke Akintola, University of Technology in accordance with the recommendations of the proper care and use of laboratory animals.

**STATISTICAL ANALYSIS**

An ANOVA followed by Dunnett’s test was used to compare treated groups to a control group. Values were considered significant if \( P < 0.05 \). Each sample was run in duplicate. All data were expressed as mean ± standard deviation [SD].

**RESULTS**

**Effect of the extract on markers of liver damage**

The results of the present study showed a significant \( (p<0.05) \) increase in the level of total protein, globulin, AST, ALT, ALP, GGT, total bilirubin, malondialdehyde (MDA) but produced no significant change in the level of albumin in parasitized non treated (PNT) mice when compared with non-parasitized non treated (NPNT) control group (table 1).

**Effect of extract on Lipid profile**

The present study revealed a remarkable elevated serum TG, VLDL and decreased HDL, TC and LDL in parasitized non-treated group compared with the positive control group (table 2).

**Effect of extract on tissues antioxidant enzymes**

Assessment of oxidative stress in serum, liver and kidney of *P. berghei* infected mice also revealed depleted

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NPNT</th>
<th>PNT</th>
<th>PET 100mg/kg</th>
<th>PET 200mg/kg</th>
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</thead>
<tbody>
<tr>
<td>Total proteins (g/L)</td>
<td>59.03±1.52^c</td>
<td>75.39±2.10^a</td>
<td>65.32±0.15^b</td>
<td>60.43±0.30^b</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>31.25±2.51^b</td>
<td>29.15±2.30^a</td>
<td>30.31±2.01^b</td>
<td>30.59±2.01^b</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>27.78±1.80^c</td>
<td>47.15±1.33^a</td>
<td>30.55±1.56^a</td>
<td>27.79±2.43^a</td>
</tr>
<tr>
<td>AST</td>
<td>26.02±2.11^b</td>
<td>31.43±0.15^a</td>
<td>28.15±1.22^b</td>
<td>26.89±1.72^b</td>
</tr>
<tr>
<td>ALT</td>
<td>29.54±1.35^c</td>
<td>33.32±2.01^a</td>
<td>30.25±0.11^b</td>
<td>29.59±1.50^b</td>
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<tr>
<td>ALP</td>
<td>106.0±1.29^b</td>
<td>132.8±1.73^a</td>
<td>120.5±1.12^c</td>
<td>110.2±1.15^b</td>
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<tr>
<td>GT</td>
<td>12.58±1.88^b</td>
<td>15.20±1.30^a</td>
<td>13.41±1.21^b</td>
<td>12.69±1.42^b</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.15±0.05^c</td>
<td>0.71±0.13^a</td>
<td>0.39±0.22^b</td>
<td>0.32±2.14^b</td>
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ALT – alanine amino transferase, AST – aspartate amino transferase, ALP – Alkaline phosphatase, YGT – Gamma glutamy transferase. NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract. Mean + SD triplicate determinations (n = 6). Values in same row with different alphabet are significantly different (\( p<0.05 \)).

Table 1: Effect of oral treatment of methanolic leaf extract of *Sphenocentrum jollyanum* on biochemical parameters in *P. berghei* infected mice
Table 2: Effect of oral treatment of methanolic leaf extract of *Sphenocentrum jollyanum* on serum lipid in *P. berghei* infected mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NPNT</th>
<th>PNT</th>
<th>PET 100mg/kg</th>
<th>PET 200mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>94.32 ± 2.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.42 ± 3.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.21 ± 2.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.12 ± 1.00&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>TG</td>
<td>75.14 ± 1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.31 ±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.21 ± 1.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.98 ± 2.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDLc</td>
<td>15.33 ± 0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.66 ± 2.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.01 ± 2.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.87 ± 1.76&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>HDLc</td>
<td>30.37 ± 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.23 ± 1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.54 ± 2.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.62 ± 1.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>VLDL</td>
<td>13.99 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.49 ± 1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.99 ± 1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.98 ± 1.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

TC- Total cholesterol, TG- triglyceride, LDLc – Low density lipoprotein, HDLc – High density lipoprotein, NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract, Mean ± SD triplicate determinations (n = 8). Values in same row with different alphabet are significantly different (p < 0.05).

Fig. 1: Effect of oral treatment of methanolic leaf extract of *S. Jollyanum* on serum antioxidant enzymes in *P. berghei* infected mice.
SOD - Superoxide dismutase, CAT - NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract. Mean ± SD triplicate determinations (n = 8). Mean values with the same letter subscripts within the same group are not significantly different (p<0.05).

Fig. 2: Effect of oral treatment of methanolic leaf extract of *S. Jollyanum* on serum MDA and GSH in *P. berghei* infected mice.
NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract. Mean ± SD triplicate determinations (n = 8).

Fig. 3: Effect of oral treatment of methanolic leaf extract of *S. Jollyanum* on Superoxide dismutase and catalase in the liver of *P. berghei* infected mice.
NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract Mean ± SD triplicate determinations (n = 8)

Fig. 4: Effect of oral treatment of methanolic leaf extract of *S. Jollyanum* on liver MDA and GSH in *P. berghei* infected mice.
NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract. Mean ± SD triplicate determinations (n = 8)

antioxidant status (figs. 1-6). Figs. 1, 3 and 5 showed low (p<0.05) serum, liver superoxide (SOD), catalase (CAT) activities in parasitized non treated group (PNT) compared to non-parasitized-non-treated (NPNT) and parasitized extract treated group (PET). Also figs. 2, 4 and 6 revealed a significant high (p<0.05) concentration of MDA and low GSH. We observed that parasitemia decreased activities of serum, liver SOD, CAT and GSH but caused significant (p<0.05) increase in serum, liver and kidney MDA. The results demonstrated that treatment
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Fig. 5: Effect of oral treatment of methanolic leaf extract of S. jollyanum on antioxidant enzymes (Superoxide dismutase and catalase) in the kidney of P. berghei infected mice. NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract. Mean ± SD triplicate determinations (n = 8).

Fig. 6: Effect of oral treatment of methanolic leaf extract of S. jollyanum on antioxidant enzymes (Superoxide dismutase and catalase) in the kidney of P. berghei infected mice. NPNT-non-parasitized-non-treated, PNT- parasitized non-treated, 100 and 200 mg/kg of the extract. Mean ± SD triplicate determinations (n = 8).

d of parasitized mice with S. jollyanum leaf extract reduced (p<0.05) elevated serum, kidney and liver MDA but increased the activities of SOD, CAT and glutathione to near normal when compared to PNT (figs. 1-6).

DISCUSSION

Reactive Oxygen species has been reported to play an important role in malaria infection pathology (Nikhat, and Pandey, 1996). To back up this claim, increased hepatic xanthine oxidase activity and lipid peroxidation was reported in P. berghei infected mice (Nikhat, and Pandey, 1996). Although, reactive oxygen species might be deleterious to tissues and organs, several studies have equally showed that increase free radical generation occasioned by P. berghei infection is beneficial to the host in combat against intra-erythrocytic parasite growth (Nikhat, and Pandey, 1996). This theory was employed in pro-oxidant treatment against malaria infection (Nikhat, and Pandey, 1996). Although, pro-oxidant (artemisinin and it derivatives) treatment is effective against malaria infection (Hunt and Stocker, 1990), It’s also (Hunt and Stocker, 1990). Hence, it was suggested that antimalarial drugs with antioxidant properties might be more effective in combat against malaria infection and is complications. This observation is consistence with previous report (Iyawe and Onigbinde, 2009). Administration of methanolic leaf extracts S. jollyanum at 100 mg/kg and 200mg/kg doses significantly (p<0.05) decreased the elevated total protein, Albumin, Globulin, AST, ALT, ALP, γ-GT and total bilirubin. The increased serum total protein, globulins, ALT, AST, ALP and γ-GT in the parasitized non-treated mice was suggested be due to cellular response to hyper-parasitemia (Orhue et al., 2005). Malaria parasite infections is accompany by cellular mobilization of T. cell and its complements with a resultant synthesis and secretion of antibody molecules leading to elevated globulin in parasitized non-treated mice (Orhue et al., 2005). The increased activities of serum AST, ALT, ALP and γ-GT in the liver and blood of P. berghei infected mice may be due to hepatic dysfunction (George et al 2011; Guthrow et al., 2007) or hepatic damage. The observed increase in activities of markers enzymes of hepatic damage is agreement with the report of Uzuegbu and Emeka, (2011). However, oral administration of S. jollyanum for 30 days resulted in the near normalisation of total protein, globulin and the activities of AST, ALT, ALP and γ-GT in the serum and liver of infected treated mice. The activity of the plant extract is dose dependant with strongest activity at 200 mg/kg. The results also suggested that the extract may possess hepatoprotective agent.

Beside, enhanced intra-erythrocytic (Atamma and Ginsburg, 1993), extra-erythrocytes and host response induced oxidative stress (Das et al., 1993; Descamps-Latasca et al., 2004), malaria infection also induced oxidative modification of lipoproteins thereby contributing to oxidative stress, progression and complications of malaria infections (Nathawut et al., 2004; Krishna et al., 2009). Derangement in lipid status in malarial infection may contribute to depletion of natural antioxidants, increased free radicals generation and alteration of structure and functions of biological molecules like lipids, proteins, carbohydrates, DNA (Krishna et al., 2009; Veerapan et al., 2004). The alteration in lipid metabolism has been attributed to acute phase response to the infection (Memo et al., 2000). Our results are in agreement with several other reports (Krishna et al., 2009; Khovidhunkit et al., 2000; Das et al., 1996; Davis et al. 1993 and Memon et al., 2000). Treatment with S. jollyanum caused a significant (p<0.05) reduction in the elevated serum concentration of total cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL) and significantly (p<0.05) stabilized level of HDL and LDL in parasitized treated mice. The leaf extract normalised these abnormalities in concentration dependant manner. The result suggested
that the plant may possess antilipidemic agents or may be due to its antiplasmodium (Olorunnisola and Afolayan, 2011) effect which leads to reduction in parasite density and free radical generation.

This observation is in agreement with various reports on effect of malaria infection on antioxidant enzymes (Nikhat and Pandey, 1996; Iyawe and Onigbinde, 2009). The decrease in hepatic, kidney and serum SOD, CAT and intracellular GSH in the current study might be due to heavy parasite burden which lead to increase superoxide radical generation \( \text{O}_2^- \) (Nikhat and Pandey, 1996). The negative effect of increase \( \text{O}_2^- \) production is responsible for the elevated hepatic lipid peroxidation and a concomitant impaired membrane functions, decreased fluidity and inactivation of membrane bound enzymes (Nikhat and Pandey, 1996). Results of our study revealed a significant increase in malondialdehyde (MDA) level an indicator of lipid peroxidation in the liver and serum of parasite infected non treated mice. Administration of the extract efficiently lowered lipid peroxidation; restore intracellular GSH, SOD and CAT activities to near normal thus protecting tissue against \( P. \text{berghei} \) induced oxidative damage.

CONCLUSION

The results of this study have showed that leaf extract of \( Sphenocentrum \text{jollyanum} \) might contain hepatoprotective compound(s), anti-lipidemic agents and may serve as a source of potential antioxidant. This is in agreement with the hepatoprotective ability of the plant against carbon tetrachloride induce toxicity (Olorunnisola and Afolayan, 2011). The observed antioxidant activity of the plant extract might be due to the presence of phytochemical constituents (Olorunnisola and Afolayan, 2011). Although, this present work does not assess the phytochemical constituents of the plant, it has earlier been reported that the plant is rich in phytochemicals with high antioxidant activities (Moody et al., 2006, Nia et al., 2005).

REFERENCES


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