ABSTRACT

Schistosomiasis is one of the most prevalent parasitic diseases in tropical and subtropical areas. Praziquantel is the drug of choice but this drug showed its efficacy against adult only with no effect on juvenile stage and there was drug resistance. Natural drugs are safe. In this study phytol was used to evaluate its efficacy on schistosoma mansoni infected mice. Mice were grouped into 4 groups: Control healthy group included 15 mice, Positive control group included 15 infected animals and received no drugs. Group infected and treated with 40 mg/kg and group treated with 80 mg/kg for two successive days at 6th week post infection Serum and liver homogenate were taken for all, Biochemical, Hematological and Serum hepatic enzymes. This study revealed that phytol administration improved liver enzymes and decreased Tumor necrosis factor-alfa (TNF- α) in dose dependent manner.

INTRODUCTION

Schistosomiasis is the most common disease, it affects over 240 million people about the world with almost 800 million at risk of infection (Steinmann et al., 2006).

The major etiological agent of human schistosomiasis is S. mansoni and intestinal schistosomiasis caused by this species is present in Africa, the Middle East, the Caribbean, and South America. Typically, the morbidity associated with schistosomiasis results from the immunological reactions in response to parasite egg deposition in the liver and other host tissues (Gryseels et al., 2006).

The used drugs for management of schistosomiasis are Praziquantel (PZQ) and Oxamnique. However, any parasitic treatment based on the use of a single drug possesses serious concerns regarding the onset of resistance (Castro et al., 2013).

Plants have always been used as a common source of medicine, both for traditional remedies and in industrialized products (Kayser et al., 2003) (Newman and Cragg, 2012). Chlorophylls, found in all green vegetables, constitute an important source of an isoprenoid component, phytol (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol) (Vetter et al., 2012). Phytol is an aromatic ingredient used in many fragrance compounds and it may be found in cosmetic and non-cosmetic products (McGinty et al., 2010).
In medicinal fields, phytol has shown antioxidant activities (Santos et al., 2013) as well as anti-inflammatory and anti allergic effects (Ryu et al., 2011). Recent studies have revealed that phytol is an excellent immune stimulant, superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity (Lim et al., 2006). Additionally, phytol and its derivatives have no cumulative inflammatory or toxic effects even in immunocompromised mice (Chowdhury and Ghosh, 2012). Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* (Rajab et al., 1998) (Saikia et al., 2010).

Drugs of natural origin have already been used to treat parasitic diseases. In this regard, the search for antischistosomal compounds from natural sources, mainly from plants, has been intensified (Allegritti et al., 2012) (Moraes, 2012). We have been specifically interested in phytol since it has well-characterized mechanisms of toxicity, is structurally simple, easily available, and cost-effective. Additionally, phytol is a common food additive and, thus, should be well tolerated by the body (Vetter et al., 2012).

Cytokines play important role in immunological pathogenesis of schistosomiasis which is associated with imbalanced in inflammatory cytokines that lead to a decrease of T helper (Th) 1 and an increase of Th2 cytokine secretion (Yu et al., 2012). Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine and important mediator of severity for periportal fibrosis (Oliveira et al., 2015). The current study targets to evaluate the effect of PYT co-administration to *S. mansoni* infected mice and to evaluate if there is dose dependency of this effect.

**MATERIALS AND METHODS**

**Drugs**

Phytol was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was suspended in 3.7 ml of phosphate buffered saline (PBS) to be ready for oral administration. Dose was adjusted to receive either 40 or 80 mg/kg, according to pre-procedural animal weight.

**Parasites:**

**Animals and experimental design:**

The study comprised 60 male albino mice of CD strain, to spare any possible effect of pregnancy hormones in females, weighting 20–25g, obtained from the Schistosome Biological Supply Programmes (SBSP), Theoder Bilharz Institute, and maintained on standard diet and free water supply till the start of study regimens. Each mouse was subjected to subcutaneous injection with 60±10 cercariae (Peters and Warren, 1969). Animals were divided into 3 groups + control healthy group (non infected non treated)

1. Control group included 15 uninfected animals (healthy).
2. Positive control group included 15 infected animals (infected non treated).
3. PYT-40 group included 15 infected animals and received PYT 40 mg/kg once daily for two successive days at 6th week post infection.
4. PYT-80 group included 15 infected animals and received PYT 80 mg/kg twice daily for two successive days at 6th week post infection. Mice were sacrificed 7th week post infection.
Evaluation of drug efficacy:

Hematological examination:

Whole blood samples were collected from retro-orbital venous plexus of mice in EDTA tube for determination of erythrocytic count (RBCs), haemoglobin concentration (Hb), haematocrit value (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration(MCHC), total (TLC) and differential leucocytic count according to Feldman et al., (2000).

Biochemical Analysis

Serum and liver homogenate were taken for all measurements. Serum samples were collected and stored at - until used. The liver was dissected out, washed in ice-cold saline, blotted dry, and weighed. Then homogenate was prepared in phosphate buffer 0.1 M, pH 7.4 and used for the biochemical analysis.

Serum hepatic enzymes

Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assessed according to Reitmans & Frankel (1957). alkalinephosphatase (ALP) was assayed by the kinetic methods of human kits (Germany) according to EDKC (1972). Activities expressed as IU/L. Total protein and albumin were measured according to Doumas et al., (1981).Serum globulin was calculated by subtracting the obtained albumin value from the total protein as described by Doumas & Biggs (1972). Tumor necrosis factor-alfa (TNF-α) was assayed using a commercial ELISA kit.

Statistical analysis

Laboratory data are presented as mean±SD and analyzed statistically by One-Way ANOVA test. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

RESULTS

Hematological evaluation of studied animals showed non-significant (p>0.05) difference regarding all parameters. Total leucocytic count was non-significantly (p>0.05) elevated with evident increases of lymphocyte and monocyte counts in blood of infected untreated animals than animals of other groups. Impact of PYT co-administration was evident on estimated hematological parameters but the difference between the used doses was non-significant (p>0.05), despite being in favor of 80 mg dose (Table 1).

Estimated serum levels of liver enzymes showed significant (p<0.05) variance among studied groups and were highest in serum of animals of positive control group. Moreover, serum albumin levels were lowest in infected untreated animals with non significant (p>0.05) variance among groups. On the other hand, infected treated animals showed lessened impaired liver function tests than infected untreated animals with significant (p<0.05) difference in favor of PYT-80 animals (Table 1).

Estimated serum TNF-α were significantly (p<0.05) higher in all infected animals compared to negative control animals and in infected untreated animals compared to treated animals with significant difference between both treated groups in favor of PYT-80 group (Table 1, Fig. 1).
Table (1): Effect of *S. mansonia* infection and PYT co-administration on hematological variables, liver function tests and serum TNF-α

<table>
<thead>
<tr>
<th>Variables</th>
<th>Negative control</th>
<th>Positive control</th>
<th>PYT-40 group</th>
<th>PYT-80 group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematological variables</strong></td>
<td></td>
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<tr>
<td>RBC count (10⁶/mm³)</td>
<td>7.1±0.46</td>
<td>6.8±0.39</td>
<td>6.95±0.41</td>
<td>7±0.45</td>
<td>0.755</td>
</tr>
<tr>
<td>Hb. concentration (g/dl)</td>
<td>16.2±0.5</td>
<td>15.7±1</td>
<td>15.8±0.9</td>
<td>16±0.8</td>
<td>0.576</td>
</tr>
<tr>
<td>HCT value (%)</td>
<td>43.2±3.2</td>
<td>41.7±3.8</td>
<td>42.9±3.9</td>
<td>43±2.8</td>
<td>0.834</td>
</tr>
<tr>
<td>Platelet count (10³/mm³)</td>
<td>1297±155</td>
<td>1229±147</td>
<td>1243±133</td>
<td>1280±174</td>
<td>0.391</td>
</tr>
<tr>
<td>WBC (10³/mm³)</td>
<td>11.6±1.2</td>
<td>12.32±1.8</td>
<td>11.88±1.4</td>
<td>11.72±1.46</td>
<td>0.741</td>
</tr>
<tr>
<td><strong>Diff count (%)</strong></td>
<td></td>
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</tr>
<tr>
<td>Esinophils</td>
<td>0.26±0.15</td>
<td>0.35±0.21</td>
<td>0.38±0.2</td>
<td>0.26±0.18</td>
<td>0.607</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.082±0.07</td>
<td>0.081±0.05</td>
<td>0.08±0.06</td>
<td>0.081±0.07</td>
<td>0.869</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.25±0.4</td>
<td>1.36±0.5</td>
<td>1.33±0.49</td>
<td>1.32±0.4</td>
<td>0.511</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>9.75±2.72</td>
<td>8.85±2.26</td>
<td>9.13±2.2</td>
<td>9.37±1.98</td>
<td>0.478</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>88.66±2.8</td>
<td>89±2.6</td>
<td>89.1±2.3</td>
<td>88.9±2.1</td>
<td>0.395</td>
</tr>
<tr>
<td><strong>Liver function tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>20±2.6</td>
<td>29±1.7</td>
<td>26±1.6</td>
<td>24±1.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>20.2±3.1</td>
<td>30±2.3</td>
<td>26.6±2.1</td>
<td>24.5±1.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alk. Ph (IU/l)</td>
<td>72.8±14.4</td>
<td>141.8±14.2</td>
<td>115.4±26</td>
<td>102.6±22.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (g/dl)</td>
<td>4.61±0.39</td>
<td>4.41±0.4</td>
<td>4.52±0.48</td>
<td>4.62±0.64</td>
<td>0.621</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.62±0.33</td>
<td>2.36±0.38</td>
<td>2.49±0.49</td>
<td>2.61±0.64</td>
<td>0.393</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2±0.15</td>
<td>2.05±0.08</td>
<td>2.03±0.11</td>
<td>2.01±0.07</td>
<td>0.374</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.32±0.18</td>
<td>1.15±0.2</td>
<td>1.23±0.26</td>
<td>1.3±0.33</td>
<td>0.882</td>
</tr>
<tr>
<td><strong>Serum TNF-α</strong></td>
<td>95.7±14.7</td>
<td>689.7±105.3</td>
<td>434±57.2</td>
<td>288.5±78.7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD; p value indicates inter-group variance; P<0.05: indicates significant difference; statistical analysis was conducted using One-Way ANOVA test.
DISCUSSION

Phytol is widespread in nature, especially because it occurs ubiquitously as a component of chlorophyll (Vetter et al., 2012). De Moraes et al. (2014) reported that a single oral dose of PYT (40 mg/kg) reduced total and female worm burden in mice infected with adult S. mansoni with reduction of number of eggs and increased proportion of dead eggs in oogram and revealed tegumental damage in adult S. mansoni recovered from mice, especially in female worms. In this study we tried to throw light on its biochemical and hematological effect on schistosoma mansoni infected mice.

The erythrogram, in the present work, showed non-significant (p>0.05) difference regarding all parameters. Our results are in agree with Bugarski et al. (2006) and in disagreement with that obtained by Abd EL-Mottaleb et al. (2008) and Nahla et al. (2008), who recorded a significant decrease in the erythrocytic count and blood indices accompanied with schistosoma infection.

Tumor necrosis factor-alpha (TNF-α) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. Torben and Hailu (2007) stated that increased level of this inflammatory cytokine after egg excretion may be an indication of its effect in complications of Schistosomiasis, it is capable of inducing tissue injury and fibrosis. In line with these findings, Yu et al., (2012) detected increasing levels
of TNF-α and IL-4 with schistosomiasis progression. Etewa et al., (2015) found schistosomal hepatocytes-induced apoptosis was directly proportional to TNF-α serum level, and the protection degree of combined vaccine was inversely proportional with serum TNF-α level and induced apoptosis. Santini-Oliveira et al., (2016) clinically, in volunteers received vaccine prepared of fatty acid-binding protein from S. mansoni (rSm14), detected time-course significant increases in Sm14-specific total and differential IgG antibodies in association with significant increase in CD4+ T cells producing cytokines, particularly TNF-α and IL-2.

Co-administration of PYT significantly ameliorated the effect of bilharzial infection on liver and immune system as manifested by significantly lower serum levels of liver enzymes in treated animals compared to infected non-treated animals with significantly lower total and differential number of leukocytes in association with decreased serum levels of TNF-α.

Similar effects were reported in literature used various drugs or natural extracts having antioxidant and anti-inflammatory properties; where, Liu et al., (2014) reported that boswellic acid-containing extracts attenuate schistosomal egg-induced hepatic granulomas and fibrosis partly due to reduced NF-κB signaling and decreased expression of VEGF, TNF-α, and MCP-1. Thereafter, El-Sayed et al., (2015) detected decreased bilharzial granuloma diameter in silymarin treated animals compared to infected untreated animals through its action on the production of pro-inflammatory cytokines. Recently, Al-laM et al., (2016) experimentally found treatment with ellagic acid augmented IL-10 production and significantly reduced production of interleukin-1β (IL-1β), IL-4, IL-12, IL-13, IL-17A, TNF-α and IFN-γ in response to S. mansoni antigenic stimulation.

The reported ameliorating effect of PYT co-administration could be attributed to previous experimental findings that PYT improves hepatic metabolism through significant suppression of α-Amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD), which plays a key role in regulation of NAD biosynthesis mRNA expression in primary rat hepatocytes and increased blood NAD level (Matsuda et al., 2013). Also, Kim et al., (2015) found PYT induced apoptosis through activation of caspas-9/3 and inhibition of epithelial mesenchymal transition in hepatocellular carcinoma cells lines.

The reported beneficial effect of PYT co-administration was dose-dependent as shown by the reported significant difference between animals received PYT in dose of 80 mg/kg and animals received PYT 40 mg/kg, in favor of dose of 80 mg/kg. Similarly, Matsuda et al., (2013) detected that PYT decreased activity of ACMSD and its mRNA expression in a dose-dependent manner in the liver.

In support of the dose-dependent effect of PYT, Silva et al., (2014) using a mouse model of acute inflammation found PYT (75 mg/kg) inhibited the recruitment of total leukocytes and neutrophils; decreased MPO activity, TNF-α and IL-1β levels and malonaldehyde concentration with increased levels of reduced glutathione.
CONCLUSION

The obtained results illustrated the effect of PYT co-administration with bilharzial infection on both the parasite itself and on the local hepatic inflammation and the systemic immune response. PYT showed dose-dependent anti-inflammatory effect and the used higher dose (80 mg/kg) was safe and effective. However, wider scale studies are mandatory for evaluation of its prophylactic use.

REFERENCES


يقلل إعطاء الفيتول للفئران المصابة بالشيستوسوما ماتسوني من الاستجابة الالتهابية الجهازية وتحسن وظائف الكبد بطريقة تعتمد على الجرعة مروة نجيب- هموت محمد علي قسم الطفيليات - كلية الطب - جامعة بنها

يعد البلهارسيا أحد أكثر الأمراض الطفيلية انتشارًا في المناطق المدارية وشبه المدارية. برازيكوانثيل هو الدواء المفضل ولكن هذا الدواء أظهر فعاليته ضد البالغين فقط دون أي تأثير على مرحلة الأطوار الغير بالغه وكان هناك مقاومة للأدوية. الأدوية الطبيعية آمنة. في هذه الدراسة تم استخدام فيتول لتقييم فعاليته على الفئران المصابة بمرض البلهارسيا. تم تجميع الفئران في 4 مجموعات: وشملت مجموعة مراقبة صحية 15 الفئران، وشملت مجموعة المراقبة الإيجابية 15 الحيوانات المصابة ولم يثق أي أدوية، المجموعة المصابة والمعالجة بالفيتول 40 ميلي غرام لكل كيلوغرام والمجموعة المعالجة بـ 80 ميلي غرام لكل كيلوغرام لمدة يومين متتاليين بعد الأسبوع السادس بعد العدوى تم أخذ مصل الدم والكبد المتجانى من أجل تقييم، الإنزيمات الكبدية الحيوية والكيميائية والمصلية. كشفت هذه الدراسة أن إعطاء الفيتول حسن من أنزيمات الكبد وانخفاض عامل نخر الورم ألفا بطريقة تعتمد على الجرعة.