Biochemical studies on the effect of *Toxoplasma* infection on liver and kidney functions in mice  

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**SUMMARY**

The present study was carried out to study the efficacy of acute toxoplasmosis on liver and kidney functions of mice and the effect of azithromycin on treatment the infection and restore the normal values levels of liver and kidney functions to its normal values.

Forty albino mice were used in this work. Liver functions were evaluated by estimation of serum total protein, serum albumin, aspartate aminotransferase (AST) and alanine aminotransferase activities (ALT). Kidney functions were also evaluated by estimation of creatinine and urea levels.

Serum total protein, ALT and AST activities were increased and albumin level decreased in infected non treated mice compared with control group (non infected non treated mice).

It was also noticed decrease in levels of serum T. protein, ALT and AST activities in infected and treated mice with azithromycin as compared with infected non treated mice and showed proximate level in non infected treated mice with azithromycin. Albumin level showed increase in infected and treated mice with azithromycin compared with infected non treated mice while the level also of proximate level in non infected and treated with azithromycin.

There was increase in level of urea of infected non treated mice as compared with control group (non infected non treated mice) while there was no noticed a significant differences between levels of creatinine in all groups of study.

It was concluded that toxoplasmosis affects liver and kidney functions which were characterized by increase levels of T. protein, ALT, AST activates and urea and decrease of most parameters of infected treated mice into normal levels that indicated the treatment with azithromycin improve the immune system of the treated mice.

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasite in the family of Apicomplexa. It is one of the most common parasites of animals being found worldwide in a large range of warm blooded vertebrates including human at a very high prevalence (Boothroyd et al., 1997). The parasite causes infection that can be either acute or chronic (Macquardt et al., 2000). The most common way of transmissions are through contact with feces of an infected cat (Montoya and Lieschenfeld, 2004). The only form of a direct contact of the parasite occurs from animals mother to fetus during pregnancy (Torda, 2001). Diagnosis of toxoplasmosis based on clinical sign and supporting laboratory analysis including blood and urine tests and visualization of the organism in body tissues (Calderaro et al., 2009).

The diagnosis of infection can be made directly by identifying the parasite in tissue sections or in body fluid or indirectly by serological and biochemical techniques (Yano and Nakabavashi 1980). The infection may cause elevated alanine transferase, elevated creatinekinase, elevated alkaline phosphatase, hyperbilirubinemia and hyperproteineemia (Abdul–Ridha, 2000).

Azithromycin is one of the world's antibiotics and is derived from erythromycin; Azithromycin is used to treat or prevent certain bacterial infections, most often those causing middle ear infections, tonsillitis, throat infections, laryngitis, bronchitis, pneumonia, Typhoid and sinusitis. In recent years it has primarily been used to prevent bacterial infections in infants and those with weaker immune systems. It is also effective against certain urinary tract infections and venereal diseases, such as non-gonococcal urethritis, chlamydia, gonorrhea and cervicitis. Recent studies have also indicated it to be effective against late-onset asthma, but these findings are controversial and not widely accepted (Klausner et al., 1998).

Aim of the proposed work: 1. detection of the effect of toxoplasmosis infection on liver and kidney functions of infected mice. 2. The therapeutic effect of the antibiotic azithromycin on infected mice.

MATERIAL AND METHOD

1- Experimental animals:
A total of 40 Swiss albino mice were used in the work (20 infected and 20 controls), they were males of 6 weeks old. The mice were divided into 4 groups each of 10 mice and were: non infected non-treated, infected non-treated, infected and treated and non infected and treated with azithromycin antibiotic.
2- The antibiotics:
Azithromycin (Zithromax) (2R,3S,4R,5R,8R,10R,11R,12S,13S,14S)-11-((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)-2-ethyl-3,4,10-trihydroxy-13-((2S,4R,5S)-5-hydroxy-4-methoxy-4-methyltetrahydro-2H-pyran-2-yloxy)-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one), is an azalide, a subclass of macrolide antibiotics. Azithromycin was used orally in the treated mice at dose: 250 mg/kg/day for 3 days.

3. Parasitological techniques:
Toxoplasma gondii trophozoites were maintained by infection of mice intraperitoneally by 3 x 10^6 trophozoites for each mouse. This was also applied for infection of mice of the infected group (Eid, 2004). Toxoplasma trophozoites were obtained from the peritoneal washing of infected mice, 3 days post-infection.

4. Biochemical tests:
Liver function was evaluated by estimation levels of serum total protein (Cannon et al., 1974), serum albumin (Doumas et al., 1971), and activates of aspartate aminotransferase (AST/ALT) and alanine aminotransferase (ALT/ALT) (Reitman and Frankel, 1957). Globulins were estimated by subtracting the values of serum albumin from the serum total proteins, A/G ratio was estimated mathematically.

Kidney function was evaluated by estimation of creatinine (Siest et al., 1985) and urea by modified urease-Berthgot method (Batton and Crouch, 1977).

5. Statistical analysis:
Data were analyzed using ANOVA and Duncan Multiple Range test, P value was considered significant when it was < 0.05 according to SPSS 14 (2006).

RESULTS
The results of T. protein, Albumin, GPT and AST of all mice groups were observed in Table (1). It was noticed an elevation in levels of T. protein and activities of GPT, and AST in G2 (infected non treated mice) compared with G1 (non-infected non-treated) while there was decrease in albumin level in G2 (infected non treated mice) as compared with control group G1 (non infected non treated mice). Activates of GPT, AST and T. protein of G3 (infected and treated with azithromycin) showed to be decreases compared with G1 (infected non treated mice) and of proximate to G4 (non infected and treated with azithromycin), albumin level increased in G3 (infected and treated with azithromycin) compared with...
G2 (infected non treated mice) while the level also of proximate to G4 (non infected and treated with azithromycin).

Table (1): Liver function tests in *Toxoplasma* infected, treated and controls mice.

<table>
<thead>
<tr>
<th>Item</th>
<th>T. protein</th>
<th>Albumin</th>
<th>ALT</th>
<th>AST</th>
<th>Globulin</th>
<th>A:G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/dl</td>
<td>g/dl</td>
<td>U/L</td>
<td>U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 infected non treated</td>
<td>C</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>non treated</td>
<td>7.42</td>
<td>4.86</td>
<td>9.60</td>
<td>10.60</td>
<td>2.56</td>
<td>1.89</td>
</tr>
<tr>
<td>±0.12</td>
<td>±0.09</td>
<td>±0.26</td>
<td>±0.12</td>
<td>±0.03</td>
<td>±0.03</td>
<td></td>
</tr>
<tr>
<td>G2 infected non treated</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>G3 infected and treatedº</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>and treatedº</td>
<td>8.38</td>
<td>3.8</td>
<td>10.6</td>
<td>10.0</td>
<td>4.58</td>
<td>0.83</td>
</tr>
<tr>
<td>±0.06</td>
<td>±0.05</td>
<td>±0.12</td>
<td>±0.16</td>
<td>±0.03</td>
<td>±0.02</td>
<td></td>
</tr>
<tr>
<td>G4 Non infected and Treatedº</td>
<td>C</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>7.44</td>
<td>4.88</td>
<td>9.20</td>
<td>10.0</td>
<td>2.56</td>
<td>1.96</td>
</tr>
<tr>
<td>±0.11</td>
<td>±0.13</td>
<td>±0.29</td>
<td>±0.16</td>
<td>±0.08</td>
<td>±0.10</td>
<td></td>
</tr>
</tbody>
</table>

treated with azithromycin
# Significant different using ANOVA test at P < 0.05.
A, B, C insignificant differences between similar litter using Duncan Multiple Range test.

Table (1) showed increase level of globulin in G1 and G3 compared with group G2 and G4 and accordance the increase in globulin level in G1 and G3; the A:G ratio showed the same behavior.
Table (2): kidney function tests in *Toxoplasma* infected; treated and controls mice.

<table>
<thead>
<tr>
<th>Item</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 non infected non treated</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>12.20 ±0.19</td>
<td>1.07 ±0.04</td>
</tr>
<tr>
<td>G2 infected non treated</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>97.00 ±3.19</td>
<td>1.12 ±0.06</td>
</tr>
<tr>
<td>G3 infected and treated</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>14.20 ±0.19</td>
<td>1.16 ±0.05</td>
</tr>
<tr>
<td>G4 Non infected and treated</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>13.20 ±0.49</td>
<td>1.06 ±0.04</td>
</tr>
</tbody>
</table>

*# Significant different using ANOVA test at P < 0.05. A, B, C insignificant differences between similar litter using Duncan Multiple Range test.*

It was also shown to increase the level of urea in G1 compared with G2 while not noticed a big differences between levels of creatinine of G1, G2, G3 and G4 groups (table 2).

**DISCUSSION**

*Toxoplasma* infection affect both liver and kidney functions which was clear in group 2 (infected non treated group) as compared with group 1 (non infected non treated). All studied parameters which included Total protein, ALT, AST activities and urea levels increases, while there was decrease in level of albumin with disturbance of globulin and A/G ratio. This results in agree with Suzuki (1971) and Portugal et al. (2004) who revealed increase in level of AST and ALT and disturbance in level of total protein, albumin and globulin at 3 days post infections but, these data did not agree with what found by El-Shazly et al., (2001) and Ustun et
al. (2004) who recorded non-significant differences in severity of liver damage between the infected and non-infected rats.

Extensive and progressive damage in liver and changes of protein fractions AST, ALT, urea, creatinine in sera were observed by Blais and Chamberland (1993) and Calderaro et al. (2009).

Boothroyd et al. (1997) observed an increase of enzymes activities in rats serum and higher actives of AST and ALT. In consequence, the degree of damage in the liver was much less in the acute stage of infection (Suzuki, 1973). As for the serum protein, alpha, beta globulin and globulin increase with subsequent globulin and decrease of albumin in the acute stage which indicates decrease in protein metabolism or increase catabolism (Boothroyd et al., 1997). The accompanied titers appeared to rise almost parallel with the raise in serum gamma globulins (Torda, 2001). The system of body reaction might reflect the differences resistance to toxoplasmosis (Mordue et al., 2001).

Toxoplasmosis causes extensive and progressive damage to the liver, remarkable proliferations of organisms such damage in the liver brought about changes in the liver metabolism (Montoya and Liep-essenfeld, 2004). Changes of protein fractions, AST, ALT varied according to the qualitative difference in intensity of inflammation by strains of Toxoplasma and host (Khan et al., 1997).

Although the relation of enzymatic host - cell and organism has been stated in varies reports (Elamin et al., 1992). Remarkable changes of enzymes in sera showed a tendency to increase after infection which might reflect the degree of damage of liver, albumin production in the reticuloendothelia tissue of the liver, kidney and gamma globulin in some tissues (Pinon et al., 1995). Globulin increased mechanisms associated with mechanisms of antibody against toxoplasmosis in acute case. The body defense mechanism is extremely complicated. The prognosis would be poor with low level of globulin (Couper et al., 2005 and Yarim et al., 2007).

It was found in the present work a significant decreases in AST and ALT, total protein and urea in Group3 (infected and treated with azithromycin compared with G2 (infected and non-treated groups) and G1 (control group), no statistical decrease of creatinine, albumin and A/G ratio, although treatment restore actives of liver cell to its normal values. Decrease in the most parame-
ters especially ALT AST, T. protein and globulin indicated that the treatment improve the immune system and slow rate of the hepatocyte metabolism either by increase anabolism and decrease catabolism, although Azithromycin known to prevents bacteria from growing by interfering with their protein synthesis. Azithromycin binds to the 50S subunit of the bacterial ribosome, and thus inhibits translation of mRNA. Nucleic acid synthesis is not affected (Chisholm et al., 2009), but the relation between the antibiotic and hepatocyte protein metabolism is need further examinations (Piao et al., 2005 and Serensen et al., 2005).

In consequence with results in table 1, the results of table 2 in where both urea and creatinine levels increased only in G2 (infected non-treated) as compared with G1 (non infected non treated mice). Although Rocha et al. (1993) used Toxoplasma gondii tachyzoites from a virulent strain, to subcutaneously infect mice. All died 7 to 9 days after infection during acute phase infection. Eighty per cent eliminated T. gondii forms by urine. Interstitial interbular hemorrhage was the more frequently observed lesion in renal histology. Tissue cysts were found in kidneys of mice at each killing time (Dubey, 1997). Histopathological changes induced by the parasite (Merogonic stages of Toxoplasma gondii) in different organs observed in kidneys (Fayed et al., 2004). Hammouda et al. (2006) observed that antigens of Toxoplasma gondii (T. gondii) in different mice tissue were detectable in kidney by double antibody sandwich enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) technique.

Azithromycin was restore both parameters to it is normal level either in infected treated group G3 which indicated that this antibiotic has significant and pronounced effect on toxoplasmosis infections and on non infected and treated mice G4 which indicated also the this antibiotic in therapeutic dose has not influenced on the urinary tissues in normal health state Azithromycin was one of family of Lonomycin which A at various concentrations was tested for its inhibitory effect on Toxoplasma multiplication in host cells demonstrated a high degree of antitoxoplasma activity with complete inhibition of Toxoplasma multiplication in the host cells (Miyagami et al., 1983). Lymphokines, a supernatant produced from spleen cells of mice infected chronically with Toxoplasma gondii, inhibited Toxoplasma multiplication in mice macrophage and kidney cell monolayers. However, lonomycin A inhibited completely Toxoplasma multiplication in non-
specific cells, i.e. not only in mice macrophages and kidney cells but also in cells of human and other animal species.

Final conclusions: Toxoplasmosis affects liver and kidney functions which were characterized by increase levels of T. protein, ALT, AST activates and urea and decrease of most parameters of infected treated mice into normal levels that indicated the treatment with azithromycin counter act the infections so advised to used as treatment of the disease in acute stage and improve the immune system of the treated mice.

REFERENCES


دراسة بيوكماتية عن تأثير العدوى بالتوكسوبلازما على وظائف الكبد والكلى في الجزائر

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الملخص العربي

اجريت الدراسة الحالية لاختبار مدى تأثير الإصابة بداء المقومات الحادة على وظائف الكبد والكلى في الفئران المعدية بالفتكل والكويت قرار الأزيثروميسين على مستوى إنزيمات ووظائف الكبد والكلى. اربعون فأرًا أُصيبوا تم تقدم نشاط إنزيمات ووظائف الكبد والبروتين الكلي والألبومين في المصل وأيضاً قيمته ووظائف الكلي وهما الكرياتينين واليوريا. وقد وجد أن هناك زيادة في مستويات البروتين الكلي، ونشاط الألانين أسبارتاتيت والألانين ترانسفيريز في الفئران المصابية غير المقارنة بالفإنار الغير مصابية والغير المقارنة، في حين كان هناك انخفاض في مستوى الألبومين في الفئران المصابية غير المقارنة مع مجموعة الضابط (الفإنار الغير المصاربة والغير المقارنة).

ولوحي أن هناك انخفاض في مستوى البروتين الكلي والألبومين، الألانين أسبارتاتيت والألانين ترانسفيريز في الفئران المصاربة والمعالجة بالأزيثروميسين مقارنة بالمجموعة المصاربة والغير مالجة بالفتكل، بينما كان مستويات متقاربة مع الفئران غير المصاربة والمعالجة بالأزيثروميسين، وظهرت أيضاً زيادة في مستوى الألبومين في الفئران المصاربة والمعالجة بالأزيثروميسين مقارنة مع الفئران المصاربة غير المصاربة في حين كان مستوى ألبومين في مصل الفئران الغير مصاربة والمعالجة بالأزيثروميسين. وتبين أيضاً أن هناك زيادة في مستوى اليوريا في الفئران المصاربة غير المصاربة في حين لم يلاحظ وجود اختلافات كبيرة بين مستويات الكرياتينين في جميع مجموعات الدراسة.

تم استنتاج أن داء المقومات تأثيراً على وظائف الكبد والكلى عن طريق زيادة مستوى كل من البروتين الكلي والألانين أسبارتاتيت والألانين ترانسفيريز، واليوريا ولاحظ انخفاض في مستويات تلك الإنزيمات في مجموعات الفئران التي عولجت بالعقار وهذا يدل على أن العلاج بعقار الأزيثروميسين ساهم في تحسين فاعليّة الكبد ووظائفه وانتعاش ذلك على الجهاز المناعي للفئران المصاربة والمعالجة به بالمقارنة مع المجموعة الضابطه.

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