Effect of Pomegranate Peel Extract Alternatives to Oxytetracycline on Growth Performance and Physiological Homeostasis in Rabbits

Asmaa M. Abdel-Samad, Abdelkarim I. M. El-Sayed, Ahmed A. Radwan, Tharwat A. Imbabi
Department of Animal Production, Faculty of Agriculture, Benha University, Egypt
Corresponding author: Asmaa.samady@gmail.com

Abstract
The current study focused on evaluation of pomegranate peel extract (PPE) and the oxytetracycline as antioxidant and antiapoptotic potentials on New Zealand White rabbits (NZW) performance, physiological homeostasis, hematobiochemical indices and stabilized DNA structure resembling in 8-hydroxy-2-deoxyguanosine (8OH2dG) level. For this purpose, four groups (each one comprise of 6 animals) of weaned NZW rabbits aged four weeks were randomly divided as follows: The 1st group preserved as normal control (C) treated with tap water; 2nd group (OXY) treated with daily oral dose of Oxytetracycline 200 mg/kg BW/day; 3rd group (PPE) treated with daily oral dose of 130 mg/kg BW/day of PPE; 4th group OXY + PPE treated with daily oral dose of 100 mg/kg BW/day of OXY + 65 mg/kg BW/day of PPE. The experiment lasted for 8 weeks. Concerning growth parameters, results showed that treatment with PPE and its combination with OXY enhanced body weight and ameliorate the negative effect on liver and kidney function with minimal side effects. In addition, PPE elevate antioxidant parameters. However, OXY decreases the growth parameters (live body weight and body weight gain), total protein, albumin, globulin and A/G ratio levels and induces markedly increases in AST, ALT, creatinine, cortisol, and liver 8OH2dG concentrations. In conclusion, present study aimed to investigate the common side effects of oxytetracycline when compared with natural pomegranate peel extract and suggests that PPE and its combination with OXY at half dose of each ameliorate side effects of oxytetracycline, accelerate body weight and normalize physiological homeostasis.

Keywords: Pomegranate peel extract, Oxytetracycline, Antioxidants, New Zealand White rabbits.

Introduction
Oxytetracycline (OXY) is a type of antibiotic called a tetracycline which commonly used as antibiotic for the treatment of Anthrax, Chlamydia, Cholera, Lyme disease, Typhus, Relapsing Fever, Tularemia, Malaria, Plaque, Syphilis, Respiratory infection, Mycoplasma, Rickettsiae, Streptococcal infection and Acne1. High doses of OXY are generally regarded as toxic; they produce a fairly large number of adverse effects, some of which can be life threatening (Brunton et al., 2005). Several lines of evidence show that OXY produces severe macrovesicular steatosis of the liver in human and it has been reported that excessive dose of OXY produce hepatic damage (Saraswat et al., 1997). Oxytetracycline works by interfering with the ability of bacteria to produce essential proteins. Without these proteins, the bacteria cannot grow, multiply and increase in numbers. Oxytetracycline therefore stops the spread of the infection and the remaining bacteria are killed by the immune system or eventually die. Oxytetracycline is also used to treat flare-ups of chronic bronchitis, due to its activity against the bacteria usually responsible, Haemophilus influenzae (Kahsay et al., 2013).

The pomegranate (Punica granatum), is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer’s disease, arthritis, and obesity (Julie Jurenka, 2008). Negi et al. (2013) and Imbabi et al., (2021) showed that the pomegranate peel extracts have both antioxidant and antimutagenic properties and may be exploited as bio preservatives in food applications and neutraceuticals. Pomegranate peel extract contains substantial amounts of polyphenols such as tannins, ellagic acid and gallic acid. It has been used in preparation of tinctures, cosmetics, therapeutic formulae and food recipes (Nasr et al., 1996). Guo et al. (2003) found that pomegranate peel had highest antioxidant activity among the peel, pulp and seed fractions of 28 kinds of fruits commonly consumed in China.

The aims of present study were to investigate the common side effects of oxytetracycline (OXY) in comparing with natural remedy pomegranate peel extract (PPE).

Materials and methods
1. Experimental animals and management:
A total of twenty-four weaned NZW were used in this study. Rabbits were obtained from a local farm (Nahya), Egypt. This study was carried out in summer during the period from 01/06/2018 to 30/07/2018. Rabbits were supplied with adequate standard diet pellets purchased from IBEX company and water provided ad libitum. After adaptation period (3 days), the experimental treatment started directly with animals aged about 32 days with average body weight of 500 ± 25g. Animals were housed in cages with wire-mesh bases constructed of galvanized steel. Dimensions of cages were 60 × 40 × 40 cm. All animals were housed in a room with controlled lighting (14 h/day), ambient temperature ranged from 16 to 20°C, relative humidity from 55 to 65% and natural ventilation. These animals were supplied with adequate standard diet pellets ingredient composition designed from Nutrition Requirement Center (N. R. C. 1998). The animals were randomly divided into four groups each one comprise 6 animals. The 1st group of rabbit served as normal control.
to (Svobodová et al., 1991). Hemoglobin was determined by spectrophotometer according to (Drabkin and Austin, 1932). Serum total protein and albumin were determined according to Gornall et al. (1949) and Doumas et al. (1971) respectively. However, serum globulin was calculated by subtracting albumin values from total protein of each corresponding sample. The Albumin to Globulin ratio (A/G) was calculated by dividing A/G values. Aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) were determined calorimetrically according to the method of (Reitman and Frankel, 1957). Creatinine was determined spectrophotometrically according to the method of (Schirmeister et al., 1964). Shidyaxy2’deoxyguanosine was determined by HPLC according to (Abd-Elrazek and Ahmed-Farid, 2017).

3.4.3. Antioxidant parameters:
- The thiols compounds of oxidized and reduced glutathione (GSH) were detected by High Performance Liquid Chromatography (HPLC) using the method of (Jayatilleke and Shaw, 1993). Glutathione (oxidized and reduced) reference standard purchased from Sigma Chemical Co. Malondialdehyde (MDA) standard was prepared according to (Karatepe, 2004). The samples were analyzed according to (Karalas et al., 2002).

3.4.4. Hormones analysis:
- For determination of serum cortisol by ELISA (Enzyme Linked Immunosorbant Assay), the kit was obtained from Zoetis, New Jersey, USA, all procedures were conducted according to (Olayemi, 2007).

Statistical analysis:
- Statistical analysis of the obtained data was performed using the General Linear Model (GLM) produced by Statistical Analysis Systems Institute (SAS, 2004). Significant differences among means were evaluated using (Duncan, 1955). The following linear model was applied for body weight, body weight gain and all tissue and blood parameters measured after rabbit's decapitation (MDA, GSH, 8OH2dG, AST, ALT, protein fraction, creatinine, cortisol and hemoglobin):
- \[ y_{ij} = \mu + \alpha_{i} + \epsilon_{ij}, \quad y_{ij} = \text{Observation measured, } \mu = \text{Overall mean, } \alpha_{i} = \text{Effect of the } i^{\text{th}} \text{ treatment, } \epsilon_{ij} = \text{Experimental error assumed to be randomly distributed with } IND \approx (0, \sigma_{e}^{2}) \]

Results:

| Table 1. Effect of OXY compared with PPE and their combination on body weight and body weight gain of weaned New Zealand White rabbit during experimental period: |
|-------------|--------------|--------------|
| Treatment (T) | Body weight (g) after 8 weeks of treatment | Body weight gain (g/day) at 4-12 weeks |
| Control | 1840.8\textsuperscript{b} | 22.17\textsuperscript{b} |
| OXY | 1791.5\textsuperscript{b} | 21.27\textsuperscript{b} |
| PPE | 1924.3\textsuperscript{a} | 23.58\textsuperscript{*} |
| OXY + PPE | 1994.6\textsuperscript{a} | 24.86\textsuperscript{a} |
| MSE | 25.36 | 0.45 |

- Data are expressed as Mean ± MSE for 6 rabbits/group.
- \* Means having different superscript in the same column are significantly different at P<0.05.
the final rabbit body weight and the average body weight after 8 weeks of age compared with OXY and control groups. However, OXY treated group did not show any significant changes compared with control group after 8 weeks of age for each parameter.

Table 2. Effect of OXY compared with PPE and their combination on blood biochemical and hematological parameters of weaned New Zealand White rabbit during experimental period:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment (T)</th>
<th>OXY</th>
<th>PPE</th>
<th>OXY + PPE</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.23</td>
<td>7.14</td>
<td>7.30</td>
<td>7.21</td>
<td>0.05</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.20</td>
<td>4.24</td>
<td>4.23</td>
<td>4.24</td>
<td>0.04</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>3.02</td>
<td>2.90</td>
<td>3.07</td>
<td>2.97</td>
<td>0.07</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.40</td>
<td>1.47</td>
<td>1.39</td>
<td>1.45</td>
<td>0.04</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>39.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.17</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>34.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± MSE for 6 rabbits/ group.
- <sup>a,b</sup> Means having different superscript in the same row are significantly different at P<0.05.

Table 3. Effect of OXY compared with PPE and their combination on different liver and kidney antioxidant parameters of weaned New Zealand White rabbit during experimental period:

<table>
<thead>
<tr>
<th>Treatment (T)</th>
<th>MDA (µmol/g tissue)</th>
<th>GSH (µmol/g tissue)</th>
<th>8OH2dG (pg/g tissue)</th>
<th>Cortisol (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.90&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.26&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>143.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>OXY</td>
<td>20.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PPE</td>
<td>16.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>143.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.62&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>OXY + PPE</td>
<td>15.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>143.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MSE</td>
<td>1.32</td>
<td>0.33</td>
<td>4.59</td>
<td>0.1</td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± MSE for 6 rabbits/ group.
- <sup>a,b</sup> Means having different superscript in the same column are significantly different at P<0.05.

The results indicate that OXY treatment decreased body weight, immunological parameters and increase oxidant reactive parameters. Hematological results revealed reduction in oxygen carrying capacity hemoglobin and increase hormonal stress marker (cortisol). Also, OXY decrease liver and kidney function. These results reflect the hepatic damage caused by OXY treatment. The increase in serum AST levels mirrors the injury of cell membrane and leakage of cytoplasmic enzymes to the blood (Janbaz <em>et al.,</em> 2004). Moreover, the reduction in serum albumin and total protein concentrations indicates failure of protein synthesis by hepatocytes (Zimmerman, and Seeff, 1970). These functional alterations were long-established histopathologically. The hepatic structural changes prompted by OXY were diffuse hepatic vacuolation, mononuclear cell infiltrates in portal areas and hepatic necrosis. OXY induced hepatic damage may be due to inhibition of β oxidation of free fatty acids and lipoprotein secretion in liver and increased cholesterol and triglyceride biosynthesis (Ibrahim and Abdel-Daim, 2015). Neither antibiotics nor OXY produced a clinical evidence of toxicity in rats.

Pomegranate peel extract (PPE) treatment appeared to be beneficial in weaned rabbit’s terms of accelerated body...
weight, average body weight, immunological parameters, and increase oxidonitrosative parameters. Also, reduced liver and kidney alteration accelerated by OXY. The consumption of PP under hot conditions helps the animal to improve body weight and decrease oxidative stress through antioxidant properties of pomegranate peel. Digestive disorders of weaned rabbits were prevented by pomegranate peel, moreover severity of diarrhea was not observed by pomegranate peel inclusion in the experiment (Zewel et al., 2016 and Imbabi et al., 2021). The protective effect of PPE in recovering the induced reduction in hepatic function in the current study is indicated by the significant reduction in ALT and AST activities. PPE decreased 8OH2dG concentration which is due to reduce the DNA-damaging effect of doxorubicin, H2O2 and spermine (Fimognari et al., 2008). The mechanism of anticholinesterase activity of PPE may be due to antioxidant power of its bioactive compounds such as flavonoids (Amri et al., 2017). The main aim of this study was to evaluate the protective effects of PPE against OXY side effects in weaned male rabbits. These protective effects might depend on decreased MDA levels and increase endogenous antioxidant activity, which implied anti-oxygenation activity may be the possible mechanism of PPE in liver and kidney (Wei et al., 2015). The increased levels of aminotransferase are the results of leakage from damaged hepatic cells and are used as markers of liver injury. These results provided evidence that the PPE able to improve hepatic-steatosis in rabbits.

Conclusion
In conclusion, present study suggests that PPE and its combination with OXY at half dose of each ameliorate side effects of oxytetracycline on liver and kidney functions, accelerate body weight and normalize physiological homeostasis.

References
Effect of Pomegranate Peel Extract Alternatives to Oxytetracycline on Growth Performance

35


Wei, X.; Fang, R.; Yang, Y.; Bi, X.; Ren, G.; Luo, A.; Zhao, M. and Zang, W. 2015: Protective effects of extracts from pomegranate peels and seeds on liver fibrosis induced by carbon tetrachloride in rats. BMC Complementary and Alternative Medicine, 15: 389.

Annals of Agric. Sci., Moshtohor, Vol. 59 (1) 2021
تأثير مستخلص قشر الرمان مقارنة بالأوكسي تتراسيكلين والخلط بينهما على آداء النمو والالتزام الفسيولوجي في الأرانب

أسماء ماهر عبد الصمد، عبد الكريم إبراهيم السيد، أحمد أبو السعود رضوان، ثروت السعيد إمبابي
قسم الإنتاج الحيواني - كلية الزراعة - جامعة بنها - مصر

أجريت الدراسة لمعرفة تأثير مستخلص قشر الرمان مقابلة بالمضاد الحيوي أوكسي تتراسيكلين والخلط بينهما على آداء الأرانب البيضاء النيوزيلندية، ودراسة تأثيرهما على الإرزان الفسيولوجي، والمؤشرات البيوكيميائية وتاثيرهما على خلايا الكبد والكلى ووظائفهما. ولل достиж هذه الغرض، تم تقسيم الأرانب إلى أربعة مجموعات (كل منها تحتوي على 6 حيوانات) ويبلغ عمرها حوالي أربعة أسابيع وسمت ذكوراً كان الفئة: المجموعة الأولى (ال kontrol) غذيت على علف قياسي + ماء بدون أي معاللات، المجموعة الثانية (أوكسي) غذيت على علف قياسي + أوكسي تتراسيكلين (200 ملغ/كجم وزن جسم) يومياً، المجموعة الثالثة (مستخلص قشر الرمان) غذيت على علف قياسي + مستخلص قشر الرمان (130 ملغ/كجم وزن جسم) يومياً، المجموعات الرابعة: أوكسي + مستخلص قشر الرمان (100 ملغ آكسي تتراسيكلين/كجم وزن جسم + 65 ملغ مستخلص قشر الرمان) غذيت على علف قياسي + نصف الكمية من أوكي تتراسيكلين ومستخلص قشر الرمان يومياً.

برزت النتائج زيادة في مقياس النمو عند المعالمة بمستخلص قشر الرمان، وبدائله مع أوكسي تتراسيكلين حيث أظهرت النتيجة زيادة في الوزن، وزن الجسم، وتوزيع الوزن، مع خفض الأثر الجانبية للمضاد الحيوي إلى الحد الأدنى. كما أظهرت المعالمة بمستخلص قشر الرمان إلى زيادة تركيز مضادات الأكسدة، وانخفض، أدت المعالمة بالأوكسي تتراسيكلين إلى إنخفاض معدلات الفيتامينات (الوزن الحي، وزن الجسم، النروتين الكلي، الألبيومين، الجلوبيولين) مع انخفاض الكرياتينين، الكرياتينين، ALT، AST، الكرياتينين، الكرياتينين، وزائدة معدل كسر الدم في الذكور بشكل ملحوظ.

الخلاصة، من خلال نتائج الدراسة نستنتج أن مستخلص قشر الرمان والخلط مع المضاد الحيوي أوكسي تتراسيكلين يقلل من الآثار الجانبية للأوكسي تتراسيكلين على وظائف الكبد والكلى، ويعود الإرزان الفسيولوجي مما يعود بالفائدة على الآداء الإنتاجي والفسيولوجي للأرانب.

الكلمات الدالة: مستخلص قشر الرمان، أوكسي تتراسيكلين، المضادات الحيوية، الآداء البيضاء النيوزيلندية.