Safety evaluation of auraptene in rats in acute and subacute toxicity studies

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1. Introduction

In recent decades, there has been an increasing interest in the use of alternative medicines and natural products for the treatment of a variety of disorders. Medicinal plants are the main source of these natural products (Yamada et al., 2011; Zheng et al., 2002; Krishnan et al., 2009; Tribble, 1999).

Citrus spp. (Rutaceae) are an important class of plants with remarkable anti-inflammatory, antioxidant, anti-ischemic, anticarcinogenic, cardioprotective and hepatoprotective effects (Tanaka et al., 1999, 2010; Tanaka et al., 2000; Kohn et al., 2006). Auraptene (7-geranyloxycoumarin) is a naturally-occurring monoterpenic coumarin ether found in many genus of the Rutaceae family, in particular Citrus spp. (Curini et al., 2006; Epifano et al., 2008; Ogawa et al., 2000). It is the most abundant prenyloxyocoumarin in nature and a common component of Citrus spp. (Curini et al., 2006; Epifano et al., 2008). Auraptene (AUR) has been reported to possess several interesting biological functions that are of therapeutic importance (Curini et al., 2006; Epifano et al., 2008; Takahashi et al., 2011; Sekiguchi et al., 2012; Prince et al., 2006; Murakami et al., 1997; Marquis et al., 2012). The most frequently documented activity of AUR is its powerful cancer chemoprevention effect, which has been confirmed by several studies (Kohn et al., 2006; Tanaka et al., 1998). In addition to this effect, AUR has been reported to have antioxidant, antigenotoxic, anti-inflammatory, anti-inflamatory, immunomodulatory, hepatoprotective neuroprotective, antibacterial and anti-hypertension properties (Becks et al., 2010; Curini et al., 2006; Hayashi et al., 2007; Soltani et al., 2010; Imenshahidi et al., 2013). Pharmacokinetic and bioavailability of AUR in rat were investigated recently (Ye, X. et al., 2016). The oral bioavailability was about 8.5%.

To the best of our knowledge, the toxicological properties of this compound have not been thoroughly investigated.

In the present study, we have evaluated the acute and subacute toxicity and the immune-toxicity of AUR. We have looked into several parameters, including body weight changes, food and water consumption, haematological and biochemical parameters, and the histopathology of various organs. Assessments of immune-toxicity were conducted through two tests, a delayed-type hypersensitivity response and a humoral antibody titre, and to complete the assessment, analysis of the white blood cell (WBC) differential count and an exploration of histopathological changes in spleen tissue were conducted.
2. Material and methods

2.1. Animals

The experiments were performed under the Animals’ (Scientific Procedures) Act of 1986 and conformed with the institutional (Mashhad University of Medical Sciences) and National Institutes of Health guidelines for the use of experimental animals. This study was carried out on male and female Wistar rats, of 200–300 g, which were purchased from the Small Animal Breeding Station, Mashhad University of Medical Sciences, Mashhad, Iran.

The rats were housed in well-ventilated sterile polypropylene cages. Each cage contained six rats of the same sex. They were maintained at a controlled temperature of 22 ± 2 °C with relative humidity of 60 ± 10% and were provided with 12 h light/dark cycles. Experiments started after the rats had been acclimatized for one week. They were fed with normal pelleted rat chow (Javane Khorasan Ltd.) and water ad libitum. The composition of the food is maize 36%, rice bran 34%, rice polish 4.97%, cotton seed extract 4%, soybean meal 10%, groundnut extract 4%, dried yeast 2%, salt 5%, mineral mixture 0.025% and vitamin 0.01%, mean energy (kcal/kg) is 3600 and pellet size 12 mm.

2.2. Drugs

Ketamine 10% and heparin (5000 IU/ml) were obtained from Merck (Rotexmeica, Germany), and xylazine 2% from Alfasan (Woerden, The Netherlands), respectively. AUR was obtained from the Golestirpars Company, and 2-mercaptoethanol was used as an emulsifier with sodium to prepare the AUR in an emulsion with normal saline.

2.3. Acute toxicity study and determination of LD50

Initial studies were conducted to assess whether AUR produced any mortality or other adverse reactions when given at the maximum recommended concentration. To determine LD50 (lethal dose 50), mortality or other adverse reactions when given at the maximum recommended concentration, five groups of rats were used. Each group consisted on five male Wistar Rats of similar weight. The LD50 of a coumarin derivative—500 mg/kg—was chosen as the base administration dose. Subsequently, according to ICH and OECD protocols, doses that included 125, 250, 1000 and 2000 mg/kg were selected, prepared and gavaged to rats. Rats were monitored for two days. Mortality and behavioural symptoms were noted.

2.4. Subacute toxicity study

For evaluation of subacute toxicity, 60 healthy rats were divided into six groups. Two doses of 125 mg/kg b.w/day and 250 mg/kg b.w/day of AUR were selected to administer. The emulsion of AUR was prepared in 0.5 ml of normal saline to administer once daily, in the morning, by oral gavage for 28 days in the treatment groups. To the control groups, 0.5 ml normal saline was administered orally. The animals were monitored for two days. Mortality and behavioural symptoms were noted.

2.4.1. Food and water consumption

The quantity of food and water consumed was recorded for each group of animals every four days during the course of the experiment.

2.4.2. Body weight

The weight of each rat was recorded on the first day and at four-day intervals throughout the course of the study and mean body weights were calculated.

2.4.3. Haematology and biochemical analysis

Blood was collected in EDTA tubes and analysed for haematological parameters. Total white blood cells (WBC), total red blood cell count (RBC), haemoglobin content (Hb), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (Plt) were measured using a specific veterinary cell counter, Nihon Kohden, calibrated for rats. For differential leukocyte counts, a blood smear was prepared on a clean glass slide, stained with Leishman’s stain, and various types of cells were counted manually, using a microscope. A portion of the blood was collected in non-heparinized tubes, and the serum was separated after centrifugation at 5000 rpm for 10 min, which was used for the biochemical analysis.

2.4.4. Histopathological analysis

Portions of the selected organs (liver, kidney, lung, heart, bone marrow and spleen) from both the control and treated groups were fixed in 10% neutral buffered formalin. Embedded organ tissue samples were cut into slices of 2–4 μm and stained with haematoxylin-eosin and the sections were observed under a light microscope (40×). Histopathological changes were described by grading the inflammation and/or necrosis of the organ tissue (Table 1).

2.4.5. Delayed-type hypersensitivity response

Delayed-type hypersensitivity response (DTH) was performed as a generalized assessment of cellular immunity. The DTH response was determined for treatment and control groups using the method of Fararjeh et al. (2008) and Raisuddin et al. (1993) with a slight modification. AUR was administrated daily at 250 mg/kg dose by oral gavage to the treatment group. On the 23rd day of the treatment, animals were immunized by intraperitoneal injection (i.p.) of 1 × 10⁸ sheep red blood cells (SRBCs), in incomplete Freund’s adjuvant (sensitization phase). Five days after immunization, all animals were again challenged with a booster dose of 1 × 10⁸ SRBCs in complete Freund’s adjuvant, in the left hind footpad (effector phase). On days 29 and 30 swelling of the left footpad was measured and compared with the right one. The percentage of swelling in the treatment group was measured and compared with that in the control groups (Riahi et al., 2010).

2.4.6. Serum antibody titre: haemagglutination (HA) titre assay

To evaluate humoral immunity, a haemagglutination test (HA) was performed. AUR was administrated daily at 250 mg/kg dose by oral gavage to the treatment group. Four days before ending the treatment period, the rats were immunized by i.p. injection of 5 × 10⁸ SRBCs in complete Freund’s adjuvant. At the termination of the experiment (day 28), after preparing serum from peripheral blood samples, aliquot (25 μl) of two-fold diluted serum in phosphate-buffered saline (PBS) was challenged with 25 μl of 1% v/v SRBCs suspension in glass tubes. The tubes were incubated at 37 °C for 1 h and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre (Gokhale et al., 2003; Riahi et al., 2010).

<table>
<thead>
<tr>
<th>Grade scale</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No changes seen</td>
</tr>
<tr>
<td>1</td>
<td>Mild changes</td>
</tr>
<tr>
<td>2</td>
<td>Moderate changes</td>
</tr>
<tr>
<td>3</td>
<td>Severe changes</td>
</tr>
<tr>
<td>4</td>
<td>Acute and toxic changes</td>
</tr>
</tbody>
</table>

Table 1

Grade scaling of histopathological changes.
2.4.7. White blood cell differential count analysis

To complete the immune system assessment, comparisons between WBC differential counts of all groups studied were undertaken.

2.4.8. Statistical analysis

Data were statistically analysed using Student’s t-test to determine significant differences in the data of the various groups. P values of less than 0.05 were considered significant. The values are expressed as means ± SD.

3. Results

3.1. Acute toxicity study and determination of LD50

No significant changes were observed in the physical and behavioural parameters of all groups of rats on the day of acute administration or throughout the 48 h of monitoring. The administration of AUR at different doses did not cause any mortality. Our experimental results show that AUR in high values, up to 2 g/kg, has no lethal toxicity, therefore, no LD50 values were considered for this compound.

3.2. Subacute toxicity study

3.2.1. General conditions and behaviour

General conditions and behaviour of rats were not adversely affected by the AUR administration at doses of 125 or 250 mg/kg body weight (b.w.) for 28 days. There was no mortality in any of the groups. The animals were healthy and no signs of toxicity were observed during the period of study.

3.2.2. Body weight

Administration of AUR for 28 days did not lead to any meaningful abnormal changes in the body weight of male and female rats when compared to the control groups (Fig. 1).

3.2.3. Food and water consumption

Administration of AUR did not make any difference to the food consumption of male and female rats in most groups as compared to the control groups. However, in male group 125 only, the food consumption and body weights were abnormal and unexpectedly small in comparison to other groups of the same age. The average food intake of male rats was nearly 19 g/day/animal and of the females was 10 g/day/animal. Similarly, the water consumption did not alter in the treated groups in comparison to the control animals (Fig. 2).

3.2.4. Necropsy

Observations from the necropsy of AUR-treated animals were found to be normal, with no apparent pathological abnormalities in any of the treated groups.

3.2.5. Haematological parameters

The Student’s t-test showed meaningful differences between groups in PCV, Hb, RBC and MCHC (Table 2). The PCV, RBC and haemoglobin counts in treated male groups were significantly lower than in the untreated groups (p < 0.05). In the females, the MCHC in group 250 was significantly lower than in group 125 (p = 0.012) and in the controls (p = 0.004). The platelet counts in both male and female treated groups were lower than in the untreated groups (p < 0.05). No significant differences were observed between the treated groups and the control groups in the other haematological parameters.
Data presented as mean ± SD for N = 10.
Significantly different from control: *P < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups (n = 10)</th>
<th>Control vehicle (saline 0.9%) M</th>
<th>AUR 250 mg/kg M</th>
<th>AUR 125 mg/kg M</th>
<th>Control vehicle (saline 0.9%) F</th>
<th>AUR 250 mg/kg F</th>
<th>AUR 125 mg/kg F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td></td>
<td>43.74 ± 2.39</td>
<td>38.08 ± 3.82*</td>
<td>34.16 ± 2.85*</td>
<td>38.04 ± 2.85</td>
<td>37.4 ± 3.46</td>
<td>35.79 ± 3.1</td>
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<tr>
<td>Hb (g/dl)</td>
<td></td>
<td>16.78 ± 0.85</td>
<td>14.68 ± 1.52*</td>
<td>13.12 ± 0.98*</td>
<td>14.88 ± 1.13</td>
<td>13.97 ± 1.48</td>
<td>13.74 ± 0.99</td>
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<tr>
<td>MCHC (g/dl)</td>
<td></td>
<td>38.36 ± 0.45</td>
<td>38.49 ± 0.81</td>
<td>38.83 ± 1.05</td>
<td>39.12 ± 0.59</td>
<td>37.22 ± 1.19*</td>
<td>38.57 ± 0.87</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td></td>
<td>52.14 ± 1.36</td>
<td>51.79 ± 1.82</td>
<td>51.88 ± 1.89</td>
<td>51.22 ± 0.54</td>
<td>49.65 ± 2.02</td>
<td>47.89 ± 0.69</td>
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<tr>
<td>Hb (g/100 ml)</td>
<td></td>
<td>9.67 ± 1.14</td>
<td>7.67 ± 2.63</td>
<td>6.72 ± 2.04</td>
<td>8.17 ± 1.65</td>
<td>7.33 ± 2.5</td>
<td>6.64 ± 2.16</td>
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<tr>
<td>PLT (× 10^6/μl)</td>
<td></td>
<td>7.84 ± 0.81</td>
<td>6.06 ± 2.27*</td>
<td>5.19 ± 1.25*</td>
<td>8.47 ± 2</td>
<td>4.28 ± 1.66*</td>
<td>5.91 ± 2.89*</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD for N = 10.
Significantly different from control: *P < 0.05.

### Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups (n = 10)</th>
<th>Control vehicle (saline 0.9%) M</th>
<th>AUR 250 mg/kg M</th>
<th>AUR 125 mg/kg M</th>
<th>Control vehicle (saline 0.9%) F</th>
<th>AUR 250 mg/kg F</th>
<th>AUR 125 mg/kg F</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/l)</td>
<td></td>
<td>57.4 ± 5.1</td>
<td>53.2 ± 7.3</td>
<td>48.8 ± 3.1</td>
<td>62.45 ± 4.39</td>
<td>58.55 ± 4.3</td>
<td>67.19 ± 13.92</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td></td>
<td>85.2 ± 14.18</td>
<td>81.6 ± 21.06</td>
<td>57.6 ± 6.32</td>
<td>62.8 ± 23.08</td>
<td>81.83 ± 11.71</td>
<td>83.6 ± 16.19</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td></td>
<td>24.6 ± 5.2</td>
<td>19.1 ± 2.2</td>
<td>20.1 ± 0.31</td>
<td>31.44 ± 4.42</td>
<td>25.1 ± 3.19</td>
<td>40.11 ± 5.71</td>
</tr>
<tr>
<td>Tp (g/dl)</td>
<td></td>
<td>6.76 ± 0.27</td>
<td>6.98 ± 0.7</td>
<td>6.8 ± 0.31</td>
<td>7.16 ± 0.33</td>
<td>7.07 ± 0.46</td>
<td>6.77 ± 0.24</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td></td>
<td>4.0 ± 0.07</td>
<td>3.76 ± 0.32</td>
<td>3.43 ± 0.17</td>
<td>4.1 ± 0.14</td>
<td>3.28 ± 0.36</td>
<td>3.18 ± 0.13</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td></td>
<td>23.27 ± 2.47</td>
<td>24.48 ± 30.18</td>
<td>23.45 ± 3.66</td>
<td>24.48 ± 1.88</td>
<td>20.8 ± 2.14</td>
<td>22.28 ± 1.14</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td></td>
<td>0.34 ± 0.04</td>
<td>0.45 ± 0.09*</td>
<td>0.49 ± 0.05*</td>
<td>0.28 ± 0.04</td>
<td>0.43 ± 0.08*</td>
<td>0.41 ± 0.07*</td>
</tr>
<tr>
<td>Chol (mg/dl)</td>
<td></td>
<td>92.4 ± 8.7</td>
<td>84.2 ± 8.06*</td>
<td>83.3 ± 9.19*</td>
<td>101.8 ± 9.62</td>
<td>66.6 ± 7.22*</td>
<td>83.4 ± 10.78*</td>
</tr>
<tr>
<td>TG</td>
<td></td>
<td>98.6 ± 43.85</td>
<td>61.7 ± 9.22*</td>
<td>53.84 ± 22.71*</td>
<td>60.4 ± 10.06</td>
<td>60.6 ± 10.7</td>
<td>59.1 ± 12.1</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>135.8 ± 11.4</td>
<td>199.5 ± 37.88*</td>
<td>143.7 ± 10.6</td>
<td>136.4 ± 11.26</td>
<td>221.07 ± 58.64*</td>
<td>152.53 ± 18.9</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD for N = 10.
Significantly different from control: *P < 0.05.

#### 3.2.6. Biochemical parameters

The Student t-test showed meaningful differences between the groups in glucose, creatinine, cholesterol and triglyceride.

AUR did not cause any significant changes in serum enzymes. Hepatic function parameters, such as ALT, AST, ALP, total bilirubin and total protein, were not altered in the AUR treated groups (Table 3).

Glucose in both male and female 250 groups was more than that in male and female 125 groups. Cholesterol in male and female 250 groups or in the controls (P < 0.05). Triglyceride only showed a significant increase in the male treated groups in comparison with the male control group. In all treatment groups, creatinine was significantly increased in comparison to the creatinine in the control groups (P < 0.05).

#### 3.2.7. Histopathological analysis

Histopathological examinations of organs, such as liver, kidneys, bone marrow, heart and lungs, of animals treated with AUR did not show any changes when compared with those of the control groups. This indicated that subacute AUR treatment up to a concentration of 250 mg/kg b.w. for 28 days did not show any adverse toxicological effects in these organs.

Pathological exploration of spleen tissues also showed no toxic change or any other meaningful differences comparing control groups (see Fig. 3).

#### 3.2.8. Serum antibody titre: haemagglutination (HA) titre assay

Serum anti-SRBC titre did not show any significant difference between AUR treated and control groups.

#### 3.2.9. Delayed-type hypersensitivity response

The DTH test did not show any significant differences between AUR treated and control groups.

#### 3.2.10. White blood cell differential count analysis

Analysis of the WBC differential count showed significant differences in neutrophil count between group 125 mg/kg and two others in treated female groups, and a mild difference in neutrophil count between treated male groups and the male control group (Table 4).

#### 4. Discussion

AUR requires deeper evaluation for its efficacy and safety due to growing demand for it in medicinal uses. Although there are several reports about the toxicity of coumarin compounds, there is no evidence of toxic effects of AUR in the studies. In the experiments relating to anticancer effects of AUR, animals received 100–500 ppm of auraptene mixed in the diet and no sign of toxicity were observed (Krishnan et al., 2009; Tanaka et al., 1998). In our study, we conducted an acute toxicity, a 28-day subacute toxicity and an immune-toxicity study for evaluating the safety of AUR at different doses. The acute oral toxicity study of AUR was done up to 2 g/kg dose. There were no changes in body weight, behaviour, or food and water consumption in comparison to untreated groups. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Tribble, 1999). Since, after administration of AUR for 28 days, there were no significant changes in the general behaviour, body weight, or food and water intake of rats in the treated groups as compared to the control groups, it could be concluded that oral administration of this compound has no effect on the growth and function of rats at the concentrations studied.

The haematopoietic system is one of the most sensitive parameters
for assessing the toxicity of drugs in humans and animals (Rahman, 2007). The present study indicated that there were significant differences in haematocrit, haemoglobin, RBC count, platelet count and MCHC. However, despite these differences, all data were within normal reference ranges (Derelanko, 2000).

The AUR treated groups indicated that subacute administration of AUR probably has a minimum effect on circulating blood cells or on their production. Any damage to the liver results in elevations of both ALT and AST in the blood. In addition, when ALT is found in the serum, it is taken as the first sign of cell and liver damage (Rahman, 2007; El Hilaly et al., 2004). Creatinine is known to be a good indicator of renal function with the result that increases in creatinine mean there is obvious damage to functional nephrons (Rahman, 2007). There were no significant differences in ALP, ALT, AST, blood urea, total bilirubin, total protein or A/G ratio in AUR-treated animals as compared to the controls. There were significant differences in serum creatinine between treated animals and the control group; however, these were still observed to be within normal ranges. These results suggest that acute administration of AUR did not alter hepatic function and probably made minimal changes to renal function. The liver is the site of cholesterol degradation, glucose synthesis and it generates free glucose, which passes into the blood from hepatic glycogen stores (Kaplan et al.,

Fig. 3. Photomicrographs of the heart, kidney, liver, lung, spleen and bone marrow from rat treated with 250 mg/kg in a 28-day subacute oral toxicity evaluation of Auraptene. Cross-sections were stained with hematoxylin and eosin. Observation was made at different amplified levels. The heart cross-section illustrates muscle fibers, connective tissues and nucleus of myocytes mainly well seen at magnification (200 ×). Cross-section of kidney shows at magnification (200 ×) renal corpuscles, tubules and Bowman’s space, all conserved. The cross-section of the lung allowed for the observation of structures such as, bronchioles, bronchiolar lumen, blood vessels, alveolar air space which were all found to be conserved. The liver cross-sections show the hepatic artery, bile duct, central vein, sinusoid, hepatocytes, all clearly conserved. Spleen cross-section shows red pulp and white pulp containing a central artery seen at magnification (200 ×). Cross-section of bone shows the structure of the marrow all conserved. All these structures were also found to be totally conserved. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Table 4

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups (n = 10)</th>
<th>AUR 250 mg/kg M</th>
<th>AUR 125 mg/kg M</th>
<th>Control vehicle (saline 0.9%) M</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTH (%) 24 h</td>
<td>51 ± 12.61</td>
<td>58.6 ± 21.26</td>
<td>–</td>
<td>54.56 ± 15.1</td>
</tr>
<tr>
<td>HA (log titre)</td>
<td>3.4 ± 1.14</td>
<td>4 ± 2.64</td>
<td>–</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>43 ± 12.04</td>
<td>20.44 ± 12.12*</td>
<td>30.8 ± 11.83*</td>
<td>29.8 ± 15.53</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>54.8 ± 11.16</td>
<td>72.6 ± 18.3</td>
<td>61.3 ± 13.24</td>
<td>64.8 ± 14.46</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>Did not have normal distribution</td>
<td>–</td>
<td>–</td>
<td>61.48 ± 15.2</td>
</tr>
<tr>
<td>Monocyte</td>
<td>Did not have normal distribution</td>
<td>–</td>
<td>–</td>
<td>78.4 ± 9.55</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD for N = 10.

1995). High levels of glucose in the male 250 group were maybe due to some disturbances in carbohydrate metabolism that require further exploration and study. A decrease in cholesterol and triglyceride levels in the treated groups, in comparison with those in the control groups, may suggest that AUR had an effect on the lipid and carbohydrate metabolism of the rats as has been reported in other studies (de Medina et al., 2010).

Histopathological investigation showed that there were no analytically significant changes, although a mild degree of organ inflammation and/or necrosis was seen in some specimens of all groups which suggests that other intervening factors including stress because of multiple high volume oral administration and handling probably caused them. There were some differences in the WBC differential count between the treated and control groups. However, there were no meaningful changes in tests evaluating the immune system, DTH and HA. So, despite differences between the groups, because the results fell within the normal ranges for WBC count and for differential count, we can probably consider AUR to be safe for the immune system.

Conflict of interest

The authors declared no conflict of interest.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.10.025.

References


