Melatonin and Vitamin C Administration Alone or as a Combination Ameliorative Role on Acrylamide Hematotoxicity Effects in Wistar Male Rats

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This study focuses on evaluating the ameliorative effect of melatonin and vitamin C administration alone or in combination on haemotoxicity of acrylamide in male rats. Sixty adult male Wistar rats divided into six groups of (10 animals in each group) divided randomly into two periods, two groups in first period C (control group), 20 male rats given 5ml of distal water and Acr (ACR group), forty male rats given ACR (0.5µg/kg BW/day) for 60 days. After 60 days the groups in the first period divided into five groups, C1 (reasonable control) give distal water orally, Acr-group, given distal water orally, Mel (5mg/kg BW/day), Vit.C-group (200 mg/kg BW/day), Mel+Vit.C-group (5+200 mg/kg BW/day) were administered by gavage once daily. At the end of the administration period, the rats were anesthetized, then the blood samples collected and analyzed. The results appeared the enhancing role of melatonin to the toxicity induced by acrylamide more than to other treatment groups in both RBCs and WBCs parameters.

Keywords: Melatonin, Vit C, Acrylamide, hematological parameters.

Introduction

Acrylamide is a chemical material used in the industries to prepare polyacrylamide, it has harmful effect and causes cancer [1]. Acrylamide used in industries such as, in pulp and paper processing and water and wastewater treatment. The primary source of dietary acrylamide is food cooked at high temperatures. Acrylamide has neurotoxic effects according to the dose (38.27 mg/kg dose for 10 days) and prolonged exposure [2].

Many gaps identified on knowledge concerning the formation, dietary exposure, and potential for adverse health risks of it. ACR is reliable for odorless white crystalline material at room temperature [3].

Acrylamide has a potentially toxic, which is derived mainly from heat-induced reactions between the free amino acid (the amino group of asparagine) and glucose and fructose (carbonyl groups) in plant-derived starchy foods such as potatoes, cereals, and other [4]. When acrylamide enter the body, it is easily absorbed and binds to hemoglobin, and it is distributed through different organs by body fluid [5]. Glycidamide is an oxidized form of acrylamide by its oxidizing agent CYP450 2E1 especially in rats, mice, and humans [6].

Melatonin, along with its metabolites, has been shown to scavenge reactive oxygen species (ROS) or reactive nitrogen species (RNS) [7]. The principal secretory product of the pineal gland is Melatonin, and it produced during the circadian cycle in the dark phase. When the retina exposed to the light causes a reduction in the amount of serotonin which metabolized to Mel by neural pathways connecting the retina to the pineal gland, so the amount of Mel secreted by the pineal gland into the plasma is dependent
on the length of exposure to darkness [8]. The highly effective antioxidant for melatonin is due to this cascade reaction in protecting cells from oxidative stress [9].

Most animals can synthesize vitamin C from glucose. However, humans, other primates, guinea pigs and fruit bats lack the last enzyme involved in the synthesis of vitamin C (gluconolactone oxidase) and so it requires the presence of the vitamin C in their diet. Ascorbic acid (vitamin C) is a micronutrient water-soluble required for multiple biological functions. Ascorbic acid works as a cofactor for several enzymes participating in the post-translational hydroxylation of collagen and the biosynthesis of carnitine, in the conversion of the neurotransmitter dopamine to norepinephrine, in peptide amidation and tyrosine metabolism. Besides, vitamin C is an essential regulator of iron uptake [10].

This study aimed to evaluate the ameliorative effect on the administration of melatonin and vitamin C alone and its combination together on the acrylamide hematotoxicity in male rats.

**Material and Methods**

**Animals and housing:** Sixty adult Albino Wister rats weighing 250-300 gm and aged 8-10 weeks, used for this study. The animals kept in Veterinary college animal house under standard conditions for one week for acclimatizing before the beginning of the experiment and it have kept in polyethylene plastic cages. Water and Food provided throughout the experiment.

Acrylamide (ACR 99.9% purity) obtained from Sigma Chemical Co.

**Study Design:** This study divided into two periods, in the first period, fifty adult male rats were divided into two groups randomly as follows,

- **Control group (C I) (n=10):** adult male rats administered distilled H\textsubscript{2}O daily by gavage for 60 days.

- **Acr I (n=40):** adult male rats administrated ACR (5mg/kg BW/day) for 60 days by gavage to induce oxidative stress as (Acr corresponding to the LD\textsubscript{50}, 150 mg/kg BW) [11].

At the end of the experimental period, the animals of each group a divided into the following groups:

1- The C I (control group) (n=10) administrated distal water by gavage
2- The ACR group (n=40) divided into four equal subgroups:
   - ACR+distal water (Acr), administrated distal water.
   - b- G Mel, administrated Mel 5mg/kg BW/day [12].
   - c- G Vit.C, administrated Vit.C (200 mg/kg BW/day) [13].
   - d- G Mel+Vit.C, administrated both Mel and Vit.C in the same dose cited above of each one by gavage.

The experimental period extended for 21 days.

**Collecting blood samples and hematological indices**

At the end of the treatment period, the animals were anesthetized using diethyl ether and sacrificed. The blood samples were collected directly by cardiac puncture into vials containing the anticoagulant, ethylene diamine tetra-acetic acid. Total erythrocytes (RBC), total leukocyte count (WBC), packed cell volume (PCV), hemoglobin (Hb), Mean corpuscular volume (MCV), MCH and MCHC estimated by using the blood-analyzer (count60) (Genix-USA).

**Statistical analysis**

As the mean ± standard division of the mean (M ± SD), the values for hematological indices reported obtained from triplicates. The data analyzed by using One-way ANOVA by SPSS (Special Program for Statistical System) version 21.0 of variance uses the least significant difference test (LSD) used to determine the differences between groups in ANOVA-test at significance level (p<0.05) [14].

**Results**

**Effects of melatonin and vitamin C alone and combination together on blood picture evoked by sub acute acrylamide toxicities of Wistar rats, On RBC Parameters:**

The results of the RBC count showed significant (p<0.05) differences between the Acr group and CI and Mel groups. While statistically (p<0.05) decrease in RBC count in groups Acr, Vit.C, and Mel+Vit.C (Table 1). Similarly, there were no significant differences between the control and Mel treated groups except the Acr
group significantly (p<0.05) decreased in Hb concentration. Whereas, MCV in the groups CI, Mel and Mel+Vit.C were significantly lower than in the Acr and Vit.C groups. Likely, no significant differences recorded between the control and Mel groups in terms of MCH and MCHC but significantly higher than the control in groups Vit.C and Mel+Vit.C (Table 2).

Table 2 shows the effect of Acr, Mel, and Vit.C treatment on the total leukocytes count of mature male rats. Changes in the Wbc were also observed, including significant increases (p<0.05) in leukocyte, lymphocyte, and monocyte counts and decreases in eosinophil count in rats treated with Acr at 5 mg/kg BW than other treated groups and CI.

**TABLE 1. Effects of melatonin and vitamin C alone and its combination on RBC counts evoked by subacute acrylamide toxicities of Wistar rats.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RBCs X 10^6 mm^3</th>
<th>Hb</th>
<th>PCV</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI (control)</td>
<td>6.22 ± 0.25^a</td>
<td>11.33 ± 0.45^a</td>
<td>32.88 ± 1.72^a</td>
<td>53.21 ± 2.89^a</td>
<td>0.82 ± 17.98</td>
<td>34.21 ± 2.09^a</td>
</tr>
<tr>
<td>ACR</td>
<td>7.20 ± 0.62^A</td>
<td>9.91 ± 0.69^C</td>
<td>35.05 ± 5.03^A</td>
<td>57.28 ± 4.54^A</td>
<td>19.80 ± 1.35^A</td>
<td>34.01 ± 1.65^A</td>
</tr>
<tr>
<td>Mel</td>
<td>6.51 ± 0.44^a</td>
<td>12.01 ± 1.33^A</td>
<td>32.76 ± 1.13^a</td>
<td>55.83 ± 2.44^a</td>
<td>18.43 ± 0.98^a</td>
<td>35.95 ± 1.38^a</td>
</tr>
<tr>
<td>Vit.C</td>
<td>5.86 ± 0.86^C</td>
<td>11.41 ± 0.89^B</td>
<td>28.41 ± 3.13^B</td>
<td>54.75 ± 2.79^B</td>
<td>19.63 ± 1.39^A</td>
<td>35.81 ± 1.32^B</td>
</tr>
<tr>
<td>Mel+Vit.C</td>
<td>5.51 ± 0.44^C</td>
<td>10.91 ± 0.98^B</td>
<td>32.87 ± 1.89^C</td>
<td>53.21 ± 2.89^C</td>
<td>19.80 ± 1.35^A</td>
<td>33.87 ± 1.89^A</td>
</tr>
<tr>
<td>LSD</td>
<td>0.70</td>
<td>1.10</td>
<td>4.33</td>
<td>4.06</td>
<td>1.38</td>
<td>N.S</td>
</tr>
</tbody>
</table>

**TABLE 2 Effects of melatonin and vitamin C alone and its combination on WBCs counts evoked by sub acute acrylamide toxicities of Wistar rats,**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Wbc X 10^6 mm^3</th>
<th>LYMPH</th>
<th>Mono</th>
<th>Gran.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI (Control)</td>
<td>8.86 ± 0.73^a</td>
<td>58.66 ± 6.70^a</td>
<td>5.01 ± 0.62^h</td>
<td>35.88 ± 6.64^a</td>
</tr>
<tr>
<td>ACR</td>
<td>12.86 ± 2.33^A</td>
<td>69.98 ± 6.01^A</td>
<td>1.55 ± 0.18^A</td>
<td>30.31 ± 5.77^a</td>
</tr>
<tr>
<td>Mel</td>
<td>8.77 ± 0.15^a</td>
<td>55.53 ± 5.40</td>
<td>4.43 ± 0.75^a</td>
<td>37.85 ± 2.37^a</td>
</tr>
<tr>
<td>Vit.C</td>
<td>9.68 ± 1.98^C</td>
<td>60.08 ± 7.73^B</td>
<td>2.01 ± 0.31^B</td>
<td>35.06 ± 5.32^B</td>
</tr>
<tr>
<td>Mel+Vit.C</td>
<td>10.39 ± 0.31^C</td>
<td>65.68 ± 7.66^C</td>
<td>4.39 ± 0.33^C</td>
<td>33.31 ± 5.77^C</td>
</tr>
<tr>
<td>LSD</td>
<td>1.81</td>
<td>9.90</td>
<td>2.40</td>
<td>7.16</td>
</tr>
</tbody>
</table>
Discussion

The results of hematological parameters of the present investigation indicated significant variations in rats exposed to Acr doses for 45 days and then treated with Mel and Vit.C alone, and combination together. The hematological changes considered one of the critical biomarkers in physiological stress [15]. The decline in RBC and Hb was dependent on the dose of Acr administered in this study. Therefore, we can suggest that Acr has toxicity effects on RBCs in male rats’ blood due to an increase in the destruction rate of erythrocytes, which take place in hemopoietic organs.

Under the current investigation demonstrated, the PCV highest decreased under the Acr group, followed by Vit.C. However, the change found to be significant under all the treated groups. These may suggest that PCV is primarily associated with the absorbed nutrients and oxygen transport. Therefore the results were recorded for decreased PCV concentration, the reduction in PCV may be a consequence of severe hemorrhage, and it correlated with RBC count and Hb levels. Raju et al. [16] noted decreases in the RBC, HB, and platelet counts, and an increase in the WBC count detected in the acrylamide group compared to the control group. The decrease in the RBC counts, which indicator for anemia. Anemia was mediated by cytokines produced by inflammatory cells and led to decreased iron availability [17] and also indicated to type of chronic iron-deficient erythropoiesis [16].

Hammad et al. [18] reported that acrylamide and glycidamide (its reactive metabolite) are electrophilic nature and addsucts form with a group of sulphhydryl in Hb resulting in haem degradation part of Hb, reduction in hemoglobin content which another cause of decreasing. Lal et al. [19] showed when given acrylamide 5 mg by gavage to Swiss albino mice, and there was a significant reduction in all hematological parameters and hemoglobin concentrations. Hammad et al. [18] also the result accepted with these results, who signed to that dietary acrylamide in different doses produce structural and functional changes in body organs, e.g., liver, kidneys, spleen, and intestines. There was a significant decrease in Hb concentration in groups with high doses of Acr. The PCV was higher, and MCHC was lower in the test groups than the controls. The Hb and RBCs were higher in rats treated with 10 mg/kg of dietary acrylamide. Significantly MCV values lower record was observed in the group fed on the highest dose (90 mg/kg) dietary Acr. The WBC was significantly higher in all treated groups, the values of Hb and MCH were significantly lower in a group with higher doses of Acr than the control rats.

White blood cells act as part of the immune system. The count of leukocyte was significantly higher in treated rats than in control group which that indicated the activation of immune system by ACR and the presence of inflammation in organs, these results similarly obtained in a previous study such that to the results of Mahmood et al. [17] and Zamani et al. [20]. Benziane et al. [11] reported that Eosinophilic polynuclear cells decrease after administration of acrylamide at (10 mg/L).

Results appeared inhibitory effects of melatonin on toxic effects of the Acr on blood parameters in rats and the effects of its dependent on the dose of the Acr, according to LD50. These results agreed with Ozmerdivenli et al. [21], who showed that the radiation melatonin administrated rats, no difference significantly seen in the blood parameters than to the group of control. The effects of the melatonin were to reverse the deleterious effects of Acr on the blood parameters in rats. Many mechanisms which included in the prevention of Acr damage to blood cells by melatonin. These mechanisms include oxidative stress preventive activity of melatonin, and antioxidant activity of it is for free radical [22,23]. The results of Ahmed et al. [24] revealed that three doses of melatonin that produced a significant elevation in RBCs count, PCV, Hb concentration, WBC count, and lymphocyte percentage, which due to the improving effect of melatonin on the health and immunity of the chicks. The melatonin action as antioxidative agent this may be mediated by stimulation, activation or enzymes synthesis that metabolizes toxic reactant such as glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase enzymes [25,12,13].

Also, Vit.C significantly reduced serum levels of MDA and attenuated changes in the catalase and superoxide dismutase levels [26, 27]. Results of research by Ferreira et al. [28], who reported that vitamin C administration significantly prevented the increase of lipid peroxidation. Dortaj et al. [29] showed the stereological Survey the effect of Vit.C have beneficial effects on neonatal rat kidney tissue treated with acrylamide.
The effects of combination Mel and Vit.C on blood parameters not studied by another researcher and the results showed these combinations enhance the adverse effect of Acr, but less than treated by Mel alone this may be due to antagonist of these combinations between Mel and Vit.C or formation intermediates have little effects on the toxicity of Acr.

In conclusion, long-term consumption of Acr (45 days) has an adverse effect represented by inducing hemotoxicity damage that affecting RBC and WBC counts. Therefore, irregular consuming of Acr formation in foods or processed food should controlled, and more profound studies and the effect of the Mel and Vit.C uses in the treated the effect of Acr attempted alone each or its combination to find safer and natural food additive for maintaining health status for the human and animals.

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Conflict of Interest
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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References
تأثر الميلاتونين وفيتامين C وحدهما أو معًا على الآثار الجانبية لسموم الأكريلاميد على مستوى صورة الدم في الجرذان المختبرية

نوال عبد الله علوان و رشاد فاضل غضبان

 العراق. - جامعة البصرة - كلية الطب البيطري - فرع الفسلجة والادوية والكيمياء الحيوية - كتلة الطب البيطري - جامعة البصرة - العراق

كان الهدف من الدّرسة الحالية هو تقييم تأثير الميلاتونين وفيتامين C وحدهما والجمع معًا على تسمم الدم في ذكور الفئران المسممة بالأكريلاميد. تم استخدام ستين ذكرًا من الفئران من نوع Wistar المختبري نانسيّ على 5 مجموعات (10 جرذًا في كل مجموعة) للدراسة. ستين ذكرًا جرب طعم تم تقسّمها عشوائيًا إلى فترتين، ومجموعتين في الفترة الأولى (المجموعة الضابتة C)، وأربعة جرف مجمّعة أطعمة الأكريلاميد (Acr) كلكيًا مهمًّا. تم تقسيم المجموعات في الفترة الأولى إلى خمس مجموعات، 10 يومًا. بعد 10 يومًا، تم تقسيم المجموعات في الفترة الأولى إلى خمسة مجموعات، 60 يومًا. بعد 60 يومًا، تم تقسيم المجموعات في الفترة الأولى إلى خمسة مجموعات، 200 يومًا. ملغم / كجم / يوم (، مجموعة الميلاتونين 5 ملغم / كجم / يوم), مجموعة فيتامين C 5 ملغم / كجم / يوم / يوم (، ميلاتونين + Vit.C / يوم), والمجموعة الضابتة C 200 ملغم / كجم / يوم / يوم (، مجموعة الفئران المتسممة بالأكريلاميد). في نهاية فترة المعالجة، تم تخدير الفئران وجمع عينات الدم وتحليلها. كشفت النتائج عن دور الميلاتونين في إزالة تسمم الدم الناجم عن الأكريلاميد أكثر من مجموعات العلاج الأخرى في كل من معايير كريات الدم الحمراء ومعاير كريات الدم البيضاء.

الكلمات المفتاحية: الأكريلاميد، الميلاتونين، فيتامين C، معايير الدم.