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Acrylamide Toxicity and Therapeutic Effect of Vitamin C on Albino Rats Immunological Parameters

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ABSTRACT

Acrylamide (ACR) is a widespread industrial chemical with recognized adverse effects not only to humans but to other organisms in the environment as well, Acrylamide forms during the heating of starchy foods at high temperature, and is regarded as a potential genotoxic carcinogen. However, with the worldwide concern about the carcinogenicity of ACR, how to reduce the toxicity of ACR has become a hot research topic. In this study, albino rats were divided into three groups; control rats, rats treated with acrylamide and rats treated with ACR and Vit C. Results revealed that significant changes were observed in immunological parameters where there was no significant decrease ($P > 0.05$) in level of TNF-α in rats treated with ACR,there significant increase in the mean level of- TNF-α in rats treated with ACR, treated with Vit C, and there was significant decrease ($P < 0.0001$) in level of IL-17in rats treated with acrylamide there significant decrease ($P < 0.05$) in the mean level of IL-17 in rats treated with ACR, treated with Vit C with compared with control group , Therefore, our investigation revealed that Vit C appeared to be a promising agent for protection against ACR -induced toxicity. This study aims to clarify the therapeutic role of Vit C to avoid exposure to ACR, which can come from several sources, and the role of Vit C in influencing some indicators of immunity in the body.

الآثار العلاجية لفيتامين ج على سمية الاكرييلاميد في جرذان الألبينو على بعض المتغيرات المناعية

فايزة اللافي منى الفاخري

مادة الأكريلاميد (ACR) هي مادة كيميائية صناعية واسعة الانتشار لها آثار ضارة معروفة ليس فقط على البشر ولكن على الكائنات الحية الأخرى في البيئة أيضًا ، وتتشكل مادة الأكريلاميد أثناء تسخين الأطعمة النشوية عند درجة حرارة عالية ، وتعتبر مادة عالية السمية ومسرطنة للجينات. ونظرا لأهمية تأثير هذه المادة في جميع أنحاء العالم بشأن التسرطن من الاكريلاميد أصبحت كيفية الحد من سميتها موضوع بحث جدير بالدراسة . في هذه الدراسة تم تقسيم الجرذان البيضاء إلى ثلاث مجموعات. المجموعة الضابطة ومجموعة الجرذان المعالجة بالأكريلاميد والجرذان المعالجة بالأكريلاميد وفيتامين ج , أظهرت النتائج أن هناك تغيرات معنوية لوحظت في المؤشرات المناعية , حيث لم يكن هناك انخفاض معنوي ($P> 0.05$) في مستوى TNF-α في الجرذان المعالجة بالأكريلاميد ،بينما كانت هناك زيادة معنوية في مستوى TNF-α في الجرذان المعالجة بالأكريلاميد المعالج بفيتامين ج. وكان هناك انخفاض معنوي($P <0.0001$) في مستوى IL-17 للجرذان المعالجة بالأكريلاميد ، وكان هناك انخفاض معنوي ($P <0.05$)في مستوى IL-17 في الجرذان المعالجة بالأكريلاميد و المعالجة بفيتامين ج بالمقارنة مع المجموعة الضابطة ، وهنا أثبتت الدراسة أن فيتامين ج أظهر تأثيرا واضحا للحماية من السمية التي يسببها الأكريلاميد.

INTRODUCTION

Acrylamide is a toxic chemical substance found in baked and fried foods such as potato chips, cookies, and biscuits. When starch-rich foods are fried at high temperatures (Dutta *et al* 2015). Acrylamide is a vinyl monomer having high water solubility. It is used in the manufacturing polyacrylamides, in cosmetic industries such as lotions, cosmetics, deodorants (Rawi *et al* 2012). ACR is formed in the presence of the amino acid asparagine and compounds with carbonyl moiety, such as starch. ACR arises at temperatures above 120°C. ACR has toxicity effects on tissues and causes irritation of the skin, eyes, and airways ((Marquez and Anon 1989; Anese *et al* 2011). ACR toxicity results from dermal absorption rather than from inhalation routes (Forstova *et al* 2014 ; Hagmar *et al* 2001). Pervious evidences showed that ACR derived during food processing may induce neurotoxic, genotoxic, and carcinogenic effects (Spencer and Schaumburg 1974 – Bartkiene *et al* 2013). ACR has also been classified as a potential human carcinogen. In addition, dietary exposure of ACR can increase health risks and some diseases' severity (Chen *et al* 2014 ; Chen *et al* 2015). Orally consumed ACR is absorbed into the circulation then distributed to various organs, and reacts with DNA, neurons, hemoglobin, and essential enzymes (Parzefall 2008 ; Soliman and Ghada 2019).

Vitamin C (ascorbic acid) is a required nutrient for a variety of biological functions (Gann 2009). Vitamin C is an essential cofactor for many enzymatic reactions including HIF1-alpha and collagen synthesis, and constitutes a primary line of defense against reactive oxygen species and lipid peroxidation (Long *et al* 2003). Humans, primates and a few other animals (e.g., guinea pigs) depend on the diet as a source of Vit C to prevent the Vit C deficiency disease, scurvy, and to maintain general health (Anitra *et al* 2015).

MATERIALS AND METHODS

Experimental rats model

Obtained Wester albino albino rats (male and female rats weighing about 80-120 g) from Animal House College of Science University Sebha. Animals were maintained on a standard diet and housed, in polystyrene cages in a room free from any source of chemical contamination, artificially lit (12 h dark / light cycle) and thermally controlled (25 ± 2° C). All animals received human care according to the guidelines of the Ethics Committee. Experiments began after the animals were allowed to adapt for four weeks.

Rats were divided randomly into 3 groups as follows:

G1: (control group): include 7 males and 7 females in separate cages were fed in normal diet.

G2: (Acrylamide group): include 7 males and 7 females separately and were administered daily ACR in drinking water (50mg /kg body weight) for 4 weeks.

G3: (ACR and Vit C group): include 7males and 7 females separately. Acrylamide was administered daily in drinking water as the above and vitamin C was given daily in drinking water for 4 weeks. Dissolved 5000 mg of effervescent Vit C tablets produced by the Chemical Industries Development Company (CID) in 500 ml of water and each 1 ml of water contains 2 mg of Vit C and given 20 ml of the solution for the male cage and 30 ml for the female cage

Blood sampling

After 4 weeks, from the beginning of the experiment, rats of all group were sacrificed and blood sample were collected as follow: Rats were anesthetized with chloroform, Blood samples were collected directly form heart by 5ml syringe. Blood was collected in heparinized tubes from heart directly under deep an aesthesia with chloroform. The collected blood samples were used for plasma preparation, Plasma was obtained from centrifugation for 10 min, 3000 r.p.m. Plasma was stored in -20 ºC until used.

Data were expressed as mean ± SEM of different treated groups compared to control ones. Normal distribution of all parameters was tested. The results were analyzed using one way analysis of variance (ANOVA). $P<0.05$ was considered significant. All statistical analyses were performed using XLSTAT program.

RESULTS AND DISCUSSION

The results postulated in Table1 revealed that revealed that administration of ACR had a significant effect on rats with its clear effect on the immune activities of animals, in addition to that, the treatment of Vit C for this toxic effect.

1. Tumor Necrosis Factor (TNF-α)

The mean level of- TNF-α in the control group was (8.02±0.84 pg/ml) and in rats treated with ACR, the level was (8.13±0.914 pg/ml). Moreover, the levels were (10.81±3.46 pg/ml) in rats treated with ACR, treated Vit C. Statistical analysis (one-way ANOVA) indicated that, there was high significant difference in TNF-α level ($p < 0.01$) among all studied groups (Table 1 and Figure 1). Student T-test indicated that, there was no significant

increase (P > 0.05) in level of TNF-α in rats treated with ACR compared with control group (Table 1).In addition, statistical analysis (t-test) it indicates that, the mean level of TNF-α in rats ACR treated with Vit C high significant increase (P < 0.01) compared with control and significant increase (P < 0.05) compared with rats treated with ACR only (Table 1). T-test for the differences between male and female shows that, there was no significant differences in TNF-α between male and female (P > 0.05) in control and ACR groups but there were significant differences in TNF-α between male and female (P < 0.01) in Vit C group; (Table 2).

2. Interleukin-17

As shown in (Table 1 and Figure 2), it has been found that, the mean level of IL-17(pg/ml) and in the control group was (342.94±95.92 pg/ml) in rats treated with ACR, the level was (831.16±202.81 pg/ml). Moreover, the levels were (236.46±101.82pg/ml) in rats treated with ACR treated Vit C. Statistical analysis (one-way ANOVA) indicated that, there was an extremely high significant difference in IL-17 level (p < 0.0001) among all studied groups (Table 1 and Figure 2). Student T-test indicated that, there was an extremely high significant increase (P < 0.0001) in level of IL-17in rats treated with ACR compared with control group (Table 1).

In addition, statistical analysis (t-test) it indicates that, the mean level of IL-17 in rats treated with ACR treated with Vit C with an extremely high significant decrease (P < 0.0001) compared with rats treated with acrylamide only; (Table 1). In addition, statistical analysis (t-test) it indicates that, the mean level of IL-17 in rats treated with ACR treated with Vit C with significant decrease (P < 0.05) compared with control group (Table 1). However, statistical analysis for the differences between male and female shows that, there is no significant differences in IL-17 between male and female there is no significant differences (P > 0.05) in all groups of the present study; (Table 2).

Table 1: Tumor Necrosis Factor TNF-α and Interleukin-17 in rats treated with ACR and treated with Vit C

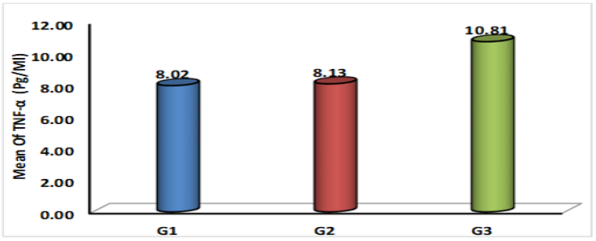
Group	TNF-α			IL-17		
	Mean ± SD		P value	Mean ± SD		P value
	Male	Female		Male	Female	
Con n=12	8.0±1.0	8.3±0.7	> 0.05	407.2±117.3	299.8±52.2	> 0.05
ACR n=14	8.2±0.9	7.7±0.6	> 0.05	802.7±203.3	859.6±214.3	> 0.05
Vit C n=14	8.4±1.0	13.3±3.3	< 0.01**	302.0±96.6	171.0±56.4	> 0.05

*P: compared between male and female; P > 0.05 considered not significant, P < 0.05 considered significant, **P < 0.01 considered high significant, and ***P < 0.0001 considered extremely significant.*

Table 2: statistical analysis of the mean of TNF-α and IL-17 in males and females of the different groups under study

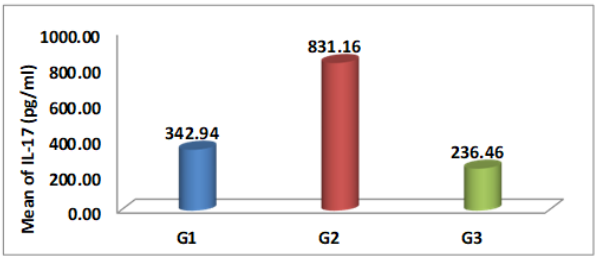
Groups		TNF-α (pg/ml)	IL_17(pg/ml)
Control (G1)	*Mean± SD*	8.02±0.84	342.94±95.92
	Minimum	6.9	228.1
	Maximum	9.7	522.6
Acrylamide (G2)	*Mean± SD*	8.13±0.914	831.16±202.81
	Minimum	6.9	540.6
	Maximum	9.7	1086.0
	Minimum	7.3	281.5
	Maximum	9.2	756.0
Vitamin C (G3)	*Mean± SD*	10.81±3.46	236.46±101.82
	Minimum	7.8	111.0
	Maximum	16.6	489.6
P value (ANOVA), P		*< 0.01***	< 0.0001***
Acrylamide vs Control (t-test) , P1		*> 0.05*	< 0.0001***
Vitamin C vs Control (t-test) , P1		*< 0.05**	*< 0.05**
Acrylamide vs Vitamin C (t-test) , P2		*< 0.01***	< 0.0001***

P: probability (one-way ANOVA); **P1**: compared with control group; P**2**: compared with acrylamide group; *P > 0.05 considered not significant, * P < 0.05 considered significant, **P < 0.01 considered high significant, and ***P < 0.0001 considered extremely significant.*



Statistical analysis (one-way ANOVA) indicated that, there was significant difference (p < 0.01) among all groups.

Figure (1) Mean of TNF-α (pg/ml) in rats treated with Acrylamide (50mg /kg body weight) and treated with vitamin C.



Statistical analysis (one-way ANOVA) indicated that, there was extremely high significant difference ($p < 0.0001$) among all groups.

Figure (2) Mean of IL-17 (pg/ml) in rats treated with ACR (50mg /kg body weight) and treated with Vit C.

The human body is exposed nowadays to increasing attacks by toxic compounds in polluted air, industrially processed foods, alcohol and drug consumption that increase liver toxicity, leading to more and more severe cases of hepatic disorders(Chen *et al* 2015 , Affordofe and Reginald 2021). Several studies showed that exposure of humans and laboratory animals to monomeric form of ACR causes neuropathies. Indeed, sub chronic low-level work exposure to ACR may bring on ataxia, gait abnormalities, skeletal muscle weakness, skin abnormalities, and numbness of hands and feet. Some toxicological studies suggested that acrylamide vapours irritate the eyes and the skin and cause paralysis of the cerebrospinal system (Soma *el al* 2021- Elham *et al* 2018).

In the current study, results shown that, there was no significant increase ($P > 0.05$) in level of TNF-α in rats treated with ACR compared with control group. Similar results were agerr with (Santhanasabapathy *et al* 2015, Acaroz *et al* 2018 , Jie *et al* 2020 and Ana *et al* 2020) , who found that exposure to acrylamide increases the production of insomnia activating cytokines such as TNF-α . And Seyed *et al* 2021 also indicated that ACR intoxication may have an increasing effect on the production of proinflammatory cytokines such as TNF-α , while results shown that level of TNF-α study reveal the effect of administration of Vit C on immune functions, it was significantly higher of immunomodulatory efficacy compared with acrylamide intoxicated rats . These results agree with Seong *et al* 2011 which showed that treatment with Vit C caused a clear rise in the level of TNF-α,these results suggest the proinflammatory cytokine TNF-α may mediate high homocysteine levels' contribution to the inflammatory process. Vit C provided some protection against the inflammatory reaction in rat.

On the other hand, results shown that level of IL-17 was an extremely significant increase compared with control group. These agree with Seyed *et al* 2021 who found that acrylamide caused in increased IL-17 in rat after 15 days of treatment, also Imen *et al* 2015 was proven that noticed that exposure to ACR leads to a rise in the level of Interleukin-17 after exposure to the toxic substance for 3 weeks .While result shown that level of IL-17 was significantly lower in groups treated with ACR and vitamin C. These result was agree with Ji *et al* 2017 who found that Vit C caused to reduce the expression levels of proinflammatory mediators such as interleukin IL-17 in micezadx . These results suggest that Vit C can affect the expression of IL17 by modulating the histone demethylase activity (Mi *et al* 2017). It could be concluded that, ACR caused many harmful effects on the immune parameters and this was clearly reflected in the high standards of resistance to this toxic substance, while it was found that taking Vit C with acrylamide significantly improves immune disorders as well.

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