

## **AMELIORATIVE EFFECT OF MELATONIN, VIT.C ALONE AND THEIR COMBINATION ON LIVER AND KIDNEY FUNCTIONS IN ACRYLAMIDE INTOXICATED OF ADULT MALE RATS**

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**Key words:** Acrylamide, Melatonin, Vitamin C.

### **ABSTRACT**

The present study was designed to determine the ameliorative effect of melatonin (Mel), vitamin C (Vit.C) alone and their combination on liver and kidney functions in acrylamide (ACR) intoxicated rats (administration ACR for 45 days). Forty eight adult male rats were divided randomly into two main groups. Control group (n=20) subdivided into two groups: group I: ten animals of control administration distal water and group II: ten animals give Mel (5mg/kg BW) for 21 days. second group: the ACR treated group (n=40) subdivided into ACR+distal water orally, ACR+Mel (5mg/kg BW/day), ACR+Vit.C (200 mg/kg BW/day), ACR+Mel (5mg)+Vit.C (200 mg)/kg BW/day for 21 days. The result revealed that no significant differences in serum AST, ALT and ALP enzymes levels between control group treated with Mel and control group. A significant reduction in serum AST, ALT and ALP levels were recorded in all treated groups compared with ACR-non treated group in which the above cited parameters still significantly higher compared with control. No significant differences were recorded in serum total protein, urea and creatinine concentrations between control+Mel treated group compared with control. A significant improvement in serum total protein, urea and creatinine concentrations were recorded in all treated groups compared with ACR-non treated group.

### **INTRODUCTION**

Acrylamide is odorless, white crystalline solid at room temperature and have chemical formula:  $C_3H_5NO$  and structure  $H_2C=CHCONH_2$ , acrylamide is  $\alpha$ ,  $\beta$ -unsaturated vinyl monomer of polyacrylamide ( $CH_2CH-CONH_2$ ), it is a water soluble substance (1 and 2). Acrylamide monomer has been reported to be formed in certain foods cooked in high temperature, levels of acrylamide as 3500  $\mu g/Kg$  have been reported in potato chips and french fries, it was first detected in certain foods in April 2002 and the toxicity studies on animals indicate the results that acrylamide is carcinogenic, in rodent caused toxic effects on the reproductive and nervous systems while in the human causes only

neurotoxicity (3). Metabolism of acrylamide occurs by two ways either by conjugation with glutathione or oxidation to glycidamide. Melatonin secretion is not only in blood and also in all types of bodily fluid which including: saliva, cerebrospinal fluid, aqueous humor of the anterior chamber, follicular fluid and also in breast milk. Melatonin receptors are distributed in all tissues and organs and also present so far been confirmed in the brain (including the SCN), pituitary gland, spinal cord, retina, thymus, spleen, liver, heart, kidney, adrenal gland, lungs, testes, ovaries, blood vessel, osteoblasts and lymphocytes (4 and 5). Melatonin is a direct scavenger of radical oxygen and nitrogen species (OH, O<sub>2</sub><sup>-</sup> and NO) (6). The melatonin is a most effective lipophilic antioxidant has been proven to be twice as active as vitamin E and C, which differs from other classic antioxidants, it has amphiphilic properties and proved to be better protected against mitochondrial oxidative stress (7). Stimulator actions of melatonin on the synthesis of another important antioxidant intracellular, glutathione (8) and also its protection of antioxidative enzyme from oxidative damage (9). The present study was aimed to determine the ameliorative effect of Mel, Vit.C and their combination on ACR induced liver and kidney disfunctions by biochemical and histological changes.

## MATERIALS AND METHODS

**Animals and housing:** sixty adult male rats were used in this study. They were kept in animal house under constant environmental condition for 2 weeks to acclimatization before beginning of the experiment. Food and drinking water were provided *ad libitum* throughout the experiment.

**Experimental Design:** rats were divided randomly into two groups as follows:

**1-Control group (n=20):** adult male rats administered distilled water daily by gavage for 45 days. **2-Treatment group (n=40):** adult male rats administered ACR (5mg/kg BW/day) for 45 days by gavage.

At the end of experimental period the animals of each group were divided into the following subgroups: The control group was divided into two equal groups: G1(-ve control) (n=10) administered distal water by gavage for 21 days. G2 (+ve control) (n=10) administered (5 mg/kg BW/day) orally for 21 days by gavage. The ACR group (n=40) divided into four equal subgroups: ACR+distal water (G3): administered distal water. ACR+Mel (G4): administered Mel 5mg/kg BW/day. ACR+Vit.C (G5): administered Vit.C (200 mg/kg BW/day). ACR+Mel+Vit.C (G6): administered both Mel (5mg)+Vit.C (200 mg)/kg BW/day by gavage for 21 days.

**Collection of blood samples:** At the end of treatment period the animals were anesthetized using diethyl ether and sacrificed. The blood samples were collected directly by cardiac puncture into clean dry test tube and serum were separated and stored at -20°C until used for hormonal analysis. The liver and kidney were excised directly and fixed in neutral buffered formalin 10% for histological study according to Luna (10).

**Biochemical Measurements:** Some biochemical measurements were done on the serum after separation by using special enzymatic kits as follow:

**Serum Aspartate aminotransferase (AST) and Serum Alanine aminotransferase (ALT) Estimation(U/I):** Aspartate and alanine aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (11).

**Serum Alkaline Phosphatase (ALP) measurement(U/I):** This estimation was done by using the colorimetric determination of alkaline phosphatase activity (12).

**Total Protein Measurement:** Colorimetric method described by Young (13) and Titez (14). By using the biuret reagent contains sodium potassium tartrate to complex cupric ions and maintain their solubility in alkaline solution

**Urea Measurement :** Urea is hydrolyzed in the presence of water and urease to produce ammonia and nitrogen dioxide (15).

**Serum Creatinine Measurement:** Creatinine is endogenously produced and released to body fluids at a stable rate and its plasma and serum levels are maintained within narrow limits, it can be measured as an indicator of glomerular filtration rate (GFR) (16).

## RESULTS

The results as demonstrated in the table (1) showed that serum ALT concentration increased significantly ( $P < 0.05$ ) in ACR-non treated group compared with control and other treated groups after 21 days of treatment. While no significant differences were observed between ACR+Mel, ACR+Vit.C and ACR-Mel+Vit.C treated groups compared with the control. No significant differences were recorded in serum ALT concentration between ACR+Mel and ACR-Vit.C treated groups compared with control. While serum ALT concentration still significantly ( $P < 0.05$ ) higher in ACR+DW group compared with control and other treated group. Serum AST concentration was significantly ( $P < 0.05$ ) higher in ACR-non treated group compared with control and other treated groups, while no significant differences were recorded in control+Mel and ACR+Mel treated groups

compared with control. The low significant ( $P<0.05$ ) value of serum AST concentration was recorded in ACR+Mel+Vit.C treated group compared with control and all other treated groups. No significant differences were observed in serum ALP concentrations between all treated groups and control at the end of treatment period except in ACR-non treated group in which ALP concentration still significantly ( $P<0.05$ ) higher than those of control and all other treated groups.

**Table (1): Effect of Mel, Vit.C alone and their combination on serum liver enzymes of ACR-treated adult male rats (M±SD.): (n=10)**

Parameters Groups	ALT (U/l)	AST (U/l)	ALP (U/l)
G1(Control)	18.38±3.37 <sup>c</sup>	21.98±2.10 <sup>c</sup>	11.44±0.72 <sup>b</sup>
G2(Control+Mel)	19.95±4.88 <sup>c</sup>	22.17±3.04 <sup>c</sup>	12.00±0.47 <sup>b</sup>
G 3(ACR +DW)	31.73±4.82 <sup>a</sup>	39.66±1.21 <sup>a</sup>	18.09±1.38 <sup>a</sup>
G4(ACR+Mel)	20.21±4.91 <sup>bc</sup>	22.16±1.65 <sup>c</sup>	10.84±0.61 <sup>b</sup>
G5(ACR+Vit.C)	21.35±2.56 <sup>bc</sup>	25.25±3.60 <sup>b</sup>	14.14±0.63 <sup>b</sup>
G6(ACR+Mel+Vit.C)	24.03±1.24 <sup>b</sup>	18.48±1.54 <sup>d</sup>	11.54±1.25 <sup>b</sup>
LSD	4.08	3.08	1.16

Values expressed in small letters mean significant differences at ( $P<0.05$ ) levels.

The data in table (2) revealed the serum total protein concentration that no significant difference was observed between control+Mel, ACR+DW, ACR+Mel, ACR+Vit.C and ACR+Mel+Vit.C treated groups compared with control. While serum total protein concentration still significantly ( $P<0.05$ ) lower in all treated groups compared with control. After 21 days of treatment serum urea concentration indicated no significant differences between control+Mel and ACR+Mel treated groups compared with the control. However significant ( $P<0.05$ ) elevation in serum urea concentrations were recorded in ACR+DW, ACR+Vit.C and ACR+Mel+Vit.C treated groups compared with control. While serum urea concentrations still significantly higher in ACR+DW group compared with control and all other treated groups. Finally serum creatinine concentrations revealed that no significant differences were observed between control+Mel and ACR+Mel compared with control. However in ACR-non treated and ACR+Vit.C treated groups serum creatinine concentrations were significantly higher compared with control and other treated groups. The creatinine concentrations still significantly higher compared with control and other treated groups.

**Table (2): Effect of Mel, Vit.C alone and their combination on serum total protein, urea and creatinine concentrations in ACR-treated adult male rats (M±SD): (n=10)**

<b>Parameters Groups</b>	<b>Total protein g/l</b>	<b>Urea mg/dl</b>	<b>Creatinin Mbn kg/l</b>
G1 (Control)	8.39±0.51 <sup>a</sup>	62.39±7.86 <sup>d</sup>	4.23±0.50 <sup>c</sup>
G2(Control+Mel)	7.87±0.59 <sup>ab</sup>	61.95±8.87 <sup>d</sup>	3.59±0.78 <sup>cb</sup>
G 3(ACR+DW)	7.33±0.82 <sup>b</sup>	107.72±11.43 <sup>a</sup>	6.84±0.47 <sup>a</sup>
G4 (ACR+Mel)	7.31±0.67 <sup>b</sup>	65.51± 5.40 <sup>d</sup>	3.58±0.70 <sup>cb</sup>
G5(ACR+Vit.C)	7.36±0.61 <sup>b</sup>	84.32±4.59 <sup>b</sup>	5.47±1.04 <sup>b</sup>
G6(ACR+Mel+Vit.C)	7.72±0.63 <sup>b</sup>	76.01±3.93 <sup>c</sup>	2.72±0.64 <sup>d</sup>
LSD	0.67	8.31	0.85

Values expressed in small letters mean significant differences at (P<0.05) levels.

## DISCUSSION

The results of the present study as illustrated in the table (1) revealed that no significant differences were observed in serum ALT, AST and ALP enzymes in normal Mel. treated group for 21 days compared with control. These results are in accordance with Bhatti *et al.* (17) and Sulaiman (18) who found that no significant differences were observed in adult male and female rats treated with 10 mg/kg BW of Mel compared with control group. Similarly Ebaid *et al.* (19) mentioned that adult male rats treated with Mel 10mg/kg BW/day for 3 weeks revealed no significant differences in plasma ALT, AST and ALP levels compared with control. The results also indicated that the ACR-non treatment group the levels of serum ALT, AST and ALP enzymes still significantly higher at the end of the experiment which may be occurred either due to the toxic effects of ACR. on liver tissue and other organs which not regenerated at the end of experiment or the period of withdrawal (21 days) not enough to regeneration takes place.

While in ACR+Mel, ACR+Vit.C and ACR+Mel+Vit.C treated groups the levels of serum ALT, AST and ALP enzymes showed non significant differences compared with control. These results are in line with those of Sulaiman (18) who concluded that the intoxicated of both adult male

and female rats with both chlorpromazine and lipopolysacchride revealed a significant increase in ALT, AST and ALP enzymes compared with control. While pretreatment with 10 mg/kg BW of Mel cause significant reduction in serum ALT and ALP levels and nonsignificant reduction in serum AST level compared to the control group. Similarly fluoride intoxicated adult female rats showed a significant increase in serum ALT, AST and ALP enzyme levels compared with control and the cotreatment with Mel 10 mg/kg BW. for 28 days resulted in a significant elevation of these parameters compared with fluoride intoxicated female rats but still significantly higher than that of control group (20).

The results are also in agreement with Ebaid *et al.* (19) who demonstrated that adult male rats treated with carbon tetrachloride (1 mg/kg) for 15 days cause significant elevation in serum liver enzymes AST, ALT and ALP levels while Mel treatment for 3 weeks resulted in improvement of these parameters compared to CCl<sub>4</sub> intoxicated group but still significantly higher compared with control. The results of ACR-groups treated with Vit.C consistent with Mongi *et al.* (21) who indicated that Wistar rats administrated deltamethrin (1.28 mg/kg) resulted in a significant increase in serum AST, ALT and ALP. While pretreatment with Vit.C (200mg/kg) normalize the above cited parameters. Similarly Hussein *et al.* (22) showed a significant elevation in serum AST and ALT in adult male rats treated with fenvalerate in dose of (2-8mg/kg) for 30 days compared with control. However the administration of Vit.C causes significant decrease in serum AST and ALT activity compared with control. Shawky *et al.* (23) showed a significant increase in serum levels of AST, ALT and ALP enzymes in Lead acetate treated male mice compared with control. However coadministration of Vit.C resulted in ameliorating the above cited parameters compared with the Lead intoxicated group but still significantly higher compared with control. Soliman (24) mentioned a significant elevation in serum AST, ALT and ALP activity in adult male rats treated with ACR compared with control. The coadministration of Vit.C cause significant reduction of these parameters compared to the group of control. The present results also coincidence with Elzoghby *et al.* (25) who found that administration of Vit.C to malathion intoxicated male rats resulted in significant reduction of serum AST, ALT and ALP toward the normal values compared with the control group. In agreement with these results Ahmadizadeh *et al.* (26) reported that adult male rats exposed to different doses (200, 400 and 600 mg/kg) styrene cause significant elevation in serum AST, ALT and ALP levels in a dose dependent manner. While pretreatment with Vit.C (200mg/kg BW) I/P cause a significant reduction in these parameters compared with styrene group but still significantly higher than those of controls. Vit.C is water soluble (hydrophilic) and considered one of important antioxidant trapping the free radicals in extracellular fluid and protecting the biomembranes from

peroxidation damage. On other hand the co-treatment of Mel and Vit.C causes an improvement in serum AST, ALT and ALP levels in male rats treated for 21 days compared with ACR-untreated group, but still significantly higher than that of control these results are inconsistent with Soliman (23) who indicated that the ACR-intoxicated adult male rats showed a significant elevation in serum ALT levels these parameters begin to improve after treatment with Mel, Vit.C and both Mel and Vit.C but still significantly higher than that of control. The present results as illustrated in table (2) indicated that no significant differences were observed in serum total protein, urea and creatinine concentrations between Mel-treated group compared with control. Moreover no significant difference was recorded in serum total protein between Mel treated male rats and control (27 and 17). Our results are also parallel to those recorded by Bharti & Srivastava (20) and Ebaid *et al.* (19) who found that no significant differences were recorded in plasma total protein, creatinine and urea between Mel treated female rats compared with control.

In ACR-non treated group which administered distilled water for 21 days showed a significant increase in serum urea and creatinine compared with control while serum total protein showed no significant differences compared with control. A significant restoration of above parameters to normal values were recorded in ACR+Mel treated group compared with ACR-non treated and control groups. These results were matched with Bharti & Srivastava (20) who demonstrated that Mel treatment of fluoride intoxicated female rats lead to significant reduction of serum urea and creatinine levels compared with the control. In agreement with the results of the present study Ismail (28) showed a significant increase in serum urea and creatinine in adult male rats intoxicated with the malathion compared with the control. While no significant difference in serum total protein was observed. The administration of Vit.C to malathion intoxicated group resulted in restoration of above parameter to near normal value. However Ismail & Ismail (29) mentioned the mercury intoxicated adult rats showed a significant increase in serum urea concentration and non significant changes in serum creatinine concentration compared with the control. Administration of Vit.C with mercury kept the urea level within its normal range compared with the control. In the same line Elzoghby *et al.* (25) demonstrated a significant increase in serum BUN and creatinine and a significant decrease in serum total protein concentrations in adult male rats intoxicated with malathion compared with control. However coadministration with Vit.C lead to restoration of these parameters toward normal values compared with control. A similar results were recorded by Ahmadizadeh *et al.* (26) in styrene indicated toxicity in adult male rats treated with Vit.C, the present results also observed that ACR group treated with combination of Mel and Vit.C cause improvement in serum urea and creatinine concentrations compared with control and ACR-non treated groups. The results consistent with those

obtained by Soliman (23) who found a significant increase in serum urea and creatinin concentrations compared with control. But administration of Mel, Vit.C alone and their combination lead to restoration of all these parameters toward the normal values but still significantly lower than those of the control. The data are in accordance with Quiroz (30) and Ebaid *et al.* (19) who reported that a significant increase in serum urea and significant decrease in serum total protein levels in adult male rats treated with carbontetrachloride while administration of Mel, Vit.C alone and their combination resulted in restoration of all above cited to their normal levels. The administration of Vit.C protect the liver from the effect of free radicals resulted from oxidative stress. Vit.C also cause improvement of total protein concentration due to reduction in apoptotic properties to white blood cells due to its antioxidant properties (31).

### التأثير الوقائي للميلاتونين وفيتامين ج لوحدهما و كلاهما على وظائف الكبد والكلية في ذكور الجرذان

#### البالغة المسممة بالاكريلاميد

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#### الخلاصة

تم تصميم هذه الدراسة لتحديد التأثير التحسيني للميلاتونين وفيتامينج وحدهما وكلاهما معاً على وظائف الكبد والكلية في جرذان الذكور البالغة المسممة بالاكريلاميد (جرعت بالاكريلاميد لمدة 45 يوم) ثم تم تقسيم جرذان الذكور البالغة (60 جرذ) بشكل عشوائي إلى مجموعتين رئيسيتين. مجموعة السيطرة (عدد=20) قسمت إلى مجموعتين فرعية: المجموعة الأولى G1 : عشرة حيوانات من مجموعة السيطرة جرعة بماء مقطر والمجموعة الثانية G2: عشرة حيوانات اعطيت ميلاتونين (5 ملغم / كجم من وزن الجسم) لمدة 21 يوماً. المجموعة الرئيسية الثانية: المجموعة التي جرعت بالاكريلاميد ACR (عدد = 40) قسمت كما يأتي إلى الاكريلاميد + ماء مقطر عن طريق الفم ومجموعة الاكريلاميد + ميلاتونين (5ملغم/ كغم من وزن الجسم / يوم)، ومجموعة الأكريلاميد+ فيتامين ج (200 ملغم/ كغم من وزن الجسم / يوم)، ومجموعة الأكريلاميد + ميلاتونين (5ملغم)+ فيتامين ج (200 ملغم)/ كغم من وزن الجسم / يوم ولمدة 21 يوماً. وبينت النتيجة عدم وجود فروق معنوية في مستويات الانزيمات AST وALT وALP في مصل الدم بين مجموعة السيطرة التي عولجت بالميلاتونين ومجموعة السيطرة. وتم تسجيل انخفاض معنوي في مستويات AST وALT في مصل الدم، ومستويات ALP في جميع المجموعات المعالجة مقارنة مع مجموعة الأكريلاميد + ماء مقطر والمجاميع المعالجة بالمذكور أعلاه لا تزال أعلى بكثير بالمقارنة مع مجموعة السيطرة. لم تسجل أي اختلافات معنوية في تراكيز البروتين الكلي واليوريا والكرياتينين في مصل الدم بين مجموعة السيطرة + ميلاتونين مقارنة مع مجموعة السيطرة. وتم تسجيل تحسن كبير في تراكيز البروتين الكلي واليوريا والكرياتينين في المصل لجميع المجموعات المعالجة مقارنة مع مجموعة الأكريلاميد + ماء مقطر.



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