Measurement of malondialdehyde and glutathione concentration in plasma of male rats that drenched with acrylamide and omega 3

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Abstract

Acrylamide has toxic effects on most ofliving body systems ,so in this article we studied the effect of acrylamide on malondialdehyde (MAD) and glutathione (GSH) of male rats that drenched omega3 fatty acids. the results showed that there isasignificant increase $p<0.003$ in plasma MDA in fourth group as compared with other groups while this value decreased in second group as compared with the others and this study showed that there isasignificant increased $p<0.0001$ in plasma GSH in fourth group as compared with other groups while this value decreased in second group as compared with the others.

قياس تركيز المالونديالديهايد الكلي و الكلوتاثايون في مصل الدملذكور الجرذان المجرعة بالاكرالامايد و الاوميغا 3

مفتاح البحث:الاكرالامايد، كليوتاثايون، المالونديالديهايد.

الخلاصة

يعد الاكرالامايد مادة ذات تاثير سمي في معظم اجهزة الجسم ،درست تاثير هذه المادة على المالونديالديهايد والكلوتاثايون لذكور الجرذان المجرعة للاوميغا3 .لقد وجدتزيادة معنوية في تركيز المالونديالديهايد تحت مستوى احتمالية$p<0.003$ في المجموعة4 مقارنة مع بقية المجاميع بينما انخفضت هذه القيمة في المجموعة2 مقارنة مع بقية المجاميع ،بينما كانت هناك زيادة معنوية في تركيز الكليوتاثايون تحت مستوى احتمالية$p<0.0001$ في المجموعة4 مقارنة مع بقية المجاميع وانخفضت هذه القيمةفيالمجموعة2 مقارنة مع بقية المجاميع.

Introduction

Acrylamide (ACR) (CH_2=$CHCONH_2$) is a vinyl monomer which exists in the form of a white crystalline powder (1). It can readily polymerize to form a gel which is used in wide variety industrial processes including the preparation of paper and paints ,so most of the acrylamide is used as chemical intermediate or as grouting agents in the construction of drinking water reservoirs and wells (2).So it is found in certain food (starchy food) that is cooked at high temperature, based on the data available in June 2002, food was estimated to make a significant contribution to total exposure of general public to acrylamide (3), it's can be absorbed into body by passage through unbroken skin, by inhalation of air containing dust or droplets of solutions of the material (4),this compound has toxic effect on our life such as its effect on nerves system . Many studies showed the neurotoxicity of ACR because these are the only toxic effects that

have been showed both in humans from occupational exposure and from studies in laboratory animals (5).the other important effect of ACR is on reproductive system as it documentedin many laboratory studies (6).In contrast ,it was reported mutations were induced in the cell transgene in mouse fibroblasts in high concentrations of ACR (7).

The other material is omega3 fatty acids which are essential unsaturated fatty acids with a double bond (C=C) starting after the third carbon atom from the end of the carbon chain(8), although omega3 fatty acids have been known as essential to normal growth and health since 1930s ,awareness of their health benefits has dramatically increased since the 1990s (9).Some researches suggest that these fatty acids may reduce the risk of ischemic and thrombotic stroke (10), as well as several studies reported anti-cancer effects of omega3 fatty acids particularly in breast, colon, and prostate cancer (11), these fatty acids have mild antihypertensive effects ,because when patients consumed n-3 from oily fish on a regular bases, their systolic blood pressure was lowered by about (3.5-5.5) mmHg(12).

There are many sources of omega3 fatty acids such as fish oil(herring, sardines,..) ,krill oil, green-lipped mussels (13) ,also there are plant sources such as flax seeds, perils , chia seed ,…(14).

From the above we concluded that both acrylamide and omega3 are present in diet, the first one occurs in ways out of our desire, but the second can be chosen by ourselves.

The aim from this study adverse the effect of acrylamide which present in our food in trace amount with theeffect of essential nutrition like omega3 on some parameters like glutathione andmalondialdehyde.

Glutathionereductase is a flavoprotein that is required for conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH), which is essential for maintenance of reduced (GSH) in vivo (15),also it is very important in inflammatory processes in living cells (16),while malondialdehyde was an organic compound with formula $CH_2(CHO)_2$ it occurs naturally and is a marker for oxidative stress(17),as well as can be found in tissue sections of joints from patients with osteoarthritis (18).

Materials and methods.

Fourteen adult laboratory male rats (100-150g), aged about 70 days, provided by Al-Nahrain infertility center, they were housed in plastic cages in the animal house of university of Karbala/Education College. The animals placed on a 12-hour light/dark cycle, with food and water freely available . Rats were given an acclimation period up to one week before the beginning of experiment.

The solutions were prepared every week by mixing acrylamide with saline w/v.10% solutions were stored at 4c° for no longer than 1 week prior to use. Drugs were administrate by oral gavage in a total volume of 0.2 ml, four times a week for 12d consecutive week (19, 20, and 21).

Animals design

Rats were randomly distributed into four groups/(10/group),the first group without treatment as control(G1),G2(treatment with acrylamide 10% and omega3 according to body weight) G3(treatment with omega3 only according to body weight) and G4 (treatment with acrylamide 10% only).

Sample preparation for malondialdehyde (MAD) and glutathione(GSH).

Blood samples were collected after 12 weeks by heart puncture in anesthetized animals (22). Blood collected in EDTA tubes then centrifuged for 10 minutes with 10000 rpm to separate plasma and kept frozen at 20 C until assay.MDA levels of the plasma were measured according to this procedure : 0.5 plasma was shaking with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube.1 ml of 0.6% 2-thiobarbituric acid (TBA) was added to the mixture,shaken ,and warmed for 30 min in a boiling water bath followed by rapid cooling. Then it was shaken with a 4ml of n-butyl-alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 535 nm by spectrophotometer against butanol (23,24).

Thiobarbuticacid+malondialdehyde→MDA-TBA adduct+2 H_2O

$Absorbance_{535}=1.53*10^5 M^{-1}Cm^{-1}$.

While GSH level was measured by commercial kit (ZeptoMetrix Corporation,872 Main Street, Buffalo, New York 14202, Office Phone:716-882-0920,Fax:716-882-0959, www,Zeptometrix.com).which the principle of thisprocedure:Glutathionreductase catalyzes the NADPH-dependent reduction of glutathione disulfide(GSSG) to glutathione(GSH).

$NADPH+H+GSSG\rightarrow 2GSH + NADP^+$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340nm (A_{340}), thus providing a spectrophotometric means of detection which is directly proportional to the GR activity in the sample. The reaction is thus measured by the decrease in absorbance at 340 nm using the extinction coefficient $6220M^{-1}cm^{-1}$ for NADPH. One unit of NADPH causes the oxidation of 1.0 µmole NADPH at 25°C at pH 7.0.

Statistical analysis

Data were expressed as mean ± SD. Differences between control and other groups were tested for statistical significance using one-way analysis of variances (ANOVA). P-values of 0.05 or less were considered significant, statistical analysis was performed using SPSS for windows version (SPSS, Inc., Chicago, Illinois).

Result

Table (1) summarizes the mean value ±SD of plasma MDA in nmol, there is a significant increase p≤0.003 in plasma MDA in grouptreated with acrylamide(G4) only as compared with G1, G3 and G2 ,whilethere are no significant changes between omega3 and acrylamide groups as compared with control in p≤0.003 and there is no significant increase in plasma MDA in group treated with omega3 fatty acids only as compared with the other groups.

Table (1) Effect of acrylamide and omega3 on plasma MDA in male rats.

Group	Malondialdehyde(MDA) nmol
Control G1	0.000003 ± 0.00037 a
Acrylamide and omega3 G2	0.000006 ± 5.43 a
Omega 3 only G3	0.000007±5.79 a
Acrylamide only G4	0.0027 ± 0.003 b

-Same small letter means no significant changing.

-Different small letter means significant changing.

-p≤0.003.

Table (2) The plasma GSH in nmol, there is a significant increase p≤0.0001 in plasma GSH in grouptreated with acrylamide only (G4) as compared with G1, G3 and G2 ,while there are no significant changes between omega3 and acrylamide groups as compared with control in p<0.0001 and there is no significant increase in plasma GSH group treated with omega3 fatty acids only as compared with the other groups.

Table (2) **Effect of acrylamide and omega3 on plasma GSH in male rats**.

Group	Glutathione reductase (GSH)
Control G1	15.51 ± 0.88 a
Acrylamide and omega3 G2	15.27 ± 0.70 a
Omega3 only G3	16.02 ± 0.57 a
Acrylamide only G4	21.11 ± 1.84 b

-Same small letter means no significant changing.

-Different small letter means significant changing.

-p≤ 0.003.

Discussion

From table (1) we found that there is a significant increase in plasma MDA in group treated with acrylamide only compared with G1,G2 and G3 ,this result was agreed with (25) he suggested that this is an indicator of lipid peroxidation , also (26)suggested that ACR lead to glycolytic enzymes inhibition so this inhibitory due to the lipid peroxidation, while the value was

decreased in G2 that drenched ACR and omega3 so, this is a good evidence that this material act as an antioxidant , this result agreed with (27) which suggested that omega3 has antioxidant effects by inhibiting lipid peroxidation.

From table (2) we found thatthere is a significant increase in plasma GSH in group treated with acrylamide only compared with G1,G2 and G3 plasma GSH was significant increased in treated group with acrylamide only compared with G1,G2 and G3, this result was agreed with (28) ,which suggested that ACR is capable of interacting with GSH and formed glutathione S-conjugates, so its level increase. While the level of GSH was decreased in G2 that its with ACR and omega3, this is an indicatorthat explain the positive effect of fatty acids on the activity of GSH, so this chemical compound is healthy.

Conclusions

1-we found that acrylamide is a very dangerous in afew amount on human specially on MDA and GSH.

2-omega3 effected on the poisonous of acrylamide and lower it .

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