Study the effect of acrylamide on some physiological and histological properties of rabbits kidney who drenched with Flavonoids that extracted from grape seeds

دراسة تأثير الاكرالامايد على بعض الصفات الفسيولوجية والنسيجية في كلية الأرانب المجرعة بمادة الفلافينويدات المستخلصة من بذور العنب

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Abstract

Acrylamide is a chemical that is produced for use in the manufacture of plastics as well as various other materials. Although acrylamide is used in making some food packing. Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. Forty eight albino rabbits are divided in to three groups, G1 fed on basal diet (control group), G2 drenched with acrylamide 10% W/V at three doses weekly, and G3 drenched with acrylamide 10% and Flavonoids 40% W/V at three doses weekly. After 16 weeks we revealed significant increase ($P< 0.05$, $P<0.02$) in plasma creatinine and uric acids in G2 as compared with G1 and G3, while there are no significant changing($P>0.05$) between G1 and G3. In the other hand Kidney histological changes for G2 demonstrated acute nephrotoxicity and glomerular degeneration as compared with G1 and G3.

الخلاصة:

الاكرالامايد هي عبارة عن ماده كيماوية واسعة الانتشار في المحمصات ومقرمشات البطاطس والصناعات البلاستيكية ومواد التجميل, ومن جهة أخرى الفلافينويدات هي واحدة من 4000 مركب من مركبات المتعددة الفينولات الموجودة بصورة طبيعية في النباتات.

استخدم في هذه التجربة 48 أرنبا من الأرانب البيضاء المختبرية بعمر ست أسابيع وتتراوح أوزانها بين (1.5 إلى 2) كغم وقسمت إلى ثلاثة مجاميع متساوية وبواقع 16 حيوان لكل مجموعة, وغذيت جميع الحيوانات على العلف المركز المجهز على شكل حبوب (120غم/حيوان/يوميا) وشرب الماء حسب حاجة الحيوان واعتبرت المجموعة الأولى مجموعة ضابطه بينما أعطيت المجموعة الثانية الاكرالامايد بتركيز 10% عن طريق الفم وبواقع ثلاثة جرع أسبوعيا ولمدة ستة عشر أسبوعا وجرعت المجموعة الثالثة الاكرالامايد بتركيز 10% والفلافينويدات بتركيز 40% وبواقع ثلاثة جرع أسبوعيا ولمدة ستة عشر أسبوعا وفي نهاية هذه المدة تم قياس نسبة كرياتينين ويوريا الدم لجميع الأرانب وتم عمل مقاطع نسيجية من الكلية وأظهرت النتائج وجود فرق معنوي ملحوظ بزيادة مستوى الكرياتينين ويوريا الدم للمجموعة الثانية عند مقارنتها مع المجموعة الضابطة والمجموعة الثالثة في حين أظهرت الصور النسيجية التهاب تسممي كلوي حاد للمجموعة الثانية عند مقارنتها مع المجموعة الضابطة والمجموعة المعاملة بالفلافينويدات.

Introduction

In recent years there are increasing studies on pollution substances that used in human life and nutrition such as cosmetics which are substances contain from chemicals like acrylamide have hazardous effect on human health(1),however acrylamide (C_3H_5NO) is a small hydrophilic molecules that polymerizes readily in the present of an initiate or because of the double bound between the first and second C-atoms, which makes it a versatile industrials chemicals (2,3).

In 1994, the international agency for research on cancer classified acrylamide as a probable human carcinogen on the basis of its carcinogenicity in rodents (4).

Before 2002 acrylamide exposure was thought to occur mainly through occupational exposure although cigarette smoke, and to a minor extend through the consumption water and the use of cosmetics(5).

However, in 2002, Swedish scientists reported its present in carbohydrates rich foods that industrials at high temperatures, such as French fries and potato chips (6).

Flavonoids or bioflavonoid are ubiquitous group of polyphenolic substances which are present in most plants which concentrating in seeds, fruit skins or peel, park and flowers, so a great numbers of plants medicines contain Flavonoids which have been reported by many others as having antibacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti-thrombotic and vasodilator actions(7,8,9).

In this experiment we investigated to extract Flavonoids from grape seeds and studied its antitoxic effect in kidneys rabbits who drenched with acrylamide orally, so we prepared histological section from kidney to examine pathological changing in slides sections and measured some biochemical's plasma parameters as indicator for kidney function like creatinine and uric acids.

Materials and methods :

1- **Experimental animals :**

Forty eight(6 weeks) rabbits (males and females) were obtained from Karbala market and housed in animals house in special cage 50 *100*50 cmm in Karbala University/ Education College. In the same time all animals leave for two weeks for acclamation before experiment has started for four months in 2010 . However all animals were diet on pellets 120 gm daily and water ad libitum with condition 12 hr dark/12 hr light and temperature 26 – 30 C^0 .

2- Animals design :

All animals were divided in to three groups 16 animals per group in which each group has eight female and eight male, however these groups named G1 fed basal diet and water ad libitum,G2 drenched with acrylamide 10% orally in three times weekly for four months and third group G3 drenched with acrylamide 10% and Flavonoids 40% extraction from grape seeds.

3- Extraction and preparation :

a- **Grape seeds :** Grape (*vitis vinifera*) were collected from market in Karbala city through April to May in 2009, so damaged and disease grapes were expelled out and then following manual separation of the seeds from whole berries, seeds and bagasse. Grape seeds were dried by direct sun light for 7 days and dried grape seeds were ground to fine powder with a grinder, then the extraction was done according to Harbone(1973), which modified by AL-Kawry (2000)(10,11). To 100 gm of crushed seeds powder in 600 ml conical flask, 200 ml of 0.2 N HCL were added and mixed well then covered by aluminum foil and boiled in a water bath at 100^o C for 45 minutes (to complete the hydrolysis process). Gentle mixing should be done every 15 minutes intervals, then the mixture was cooled to 25 – 27^0 C and filtered under vacuum using what man No.4 filter paper. The filtrate was transferred in to separator funnel, the carotinoids, chlorophylls and waxes were separated from filtrate by 100 ml petroleum ether for four times (25 ml for each separation), Flavonoids then extract four times by separating funnel using ethyl acetate (25 ml for each separation).finally Flavonoids compounds were dried under vacuum using rotary evaporator at 40^o C. The dried Flavonoids as a pasta were collected and keep in glass tubes at 20^0 C till used it .

b- Acrylamide preparation and its doses :

Acrylamide solution was prepared by W/V, 10% acrylamide in distilled water, rabbits were drinking orally with acrylamide solution for four months at three frequent doses in a week(12,13).

c- Flavonoids solution and its doses :

Flavonoids were prepared by W/V, 40% in distilled water, animals drenched orally with this solution for four months at three frequent doses weekly.

4- Blood samples :

Animals blood samples were collected after four months by heart puncture by 3ml syringe with 24 gage then put in EDTA tubes and then centrifuged for 10 minutes with 10000 rpm to collect plasma and kept in frozen at 20^0 C until assay .

5- Biochemical assay of plasma samples for creatinine and uric acids :

The kidney function markers including creatinine and uric acids were measured in plasma by colorimetric method :

a- Creatinine in the plasma determined according to the method of Schirmeister *et.al.*(14), where creatinine reacted with picrate in alkaline medium forming a color complex. The amount of the complex formed is directly proportional to the creatinine concentration and could be measured at 500 nm.

b- Uric acids in plasma originated by means of the coupled reactions described by Fawcett and Soctt (15). The blue dye indophenols production reaction absorbs light between 530 and 560 nm proportional to initial urea concentration .

6- Histological section :

Soon after the animals scarified, the abdomen was opened and rabbit kidney was removed and immediately washed with normal saline and fixed with 10% formalin saline solution PH(7.4) and processed by successive dehydration with a sequences of ethanol solution and embedded in paraffin. The serial sections were cut 5µm thick and stained with haematoxylin and eosin (HE) stain using standard process (16). Stained kidney section were examined for structure and architecture changes photomicroscope. The kidney sections were examine for the type of the pathological changes.

Statistical analysis :

Data were expressed as mean $\pm$ SD. Differences between control and other groups were tested for significances using a one - way analysis of variances (ANOVA). P- values of 0.05 or less were considered significant.

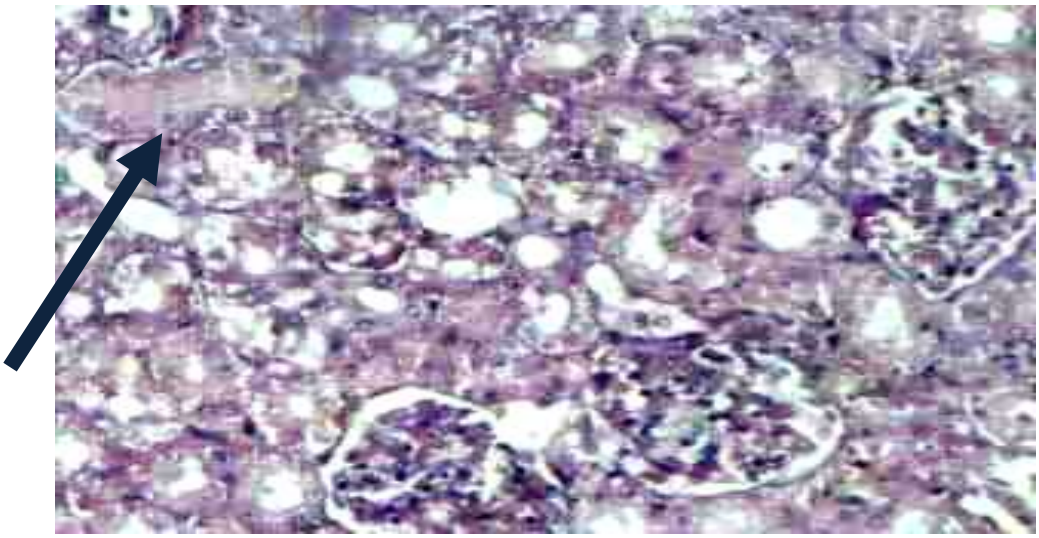
Table (1) summarizes the mean value $\pm$ SD of plasma creatinine and uric acids, so there are no significant changing between negative control group and treated group in plasma creatinine and uric acids value($P \leq 0.05$) and ($P \leq 0.02$) respectively while there are significant changing between positive control and treatment group with Flavonoids in plasma creatinine and uric acid in which ($P \leq 0.05$ and $P \leq 0.02$) respectively, in the other hand there are a significant changing between negative control group and positive control group in plasma creatinine and uric acids

Table 1: mean value of plasma creatinine and uric acid

Groups	Creatinine	Uric acids
Negative control	$3.455 \pm 0.216^{*}$ (B)	$56.38 \pm 0.165^{**}$ (B)
Positive control	4.708 ± 0.518 (A)	79.00 ± 7.22 (A)
Treatment	3.521 ± 0.278 (B)	47.53 ± 4.977 (B)

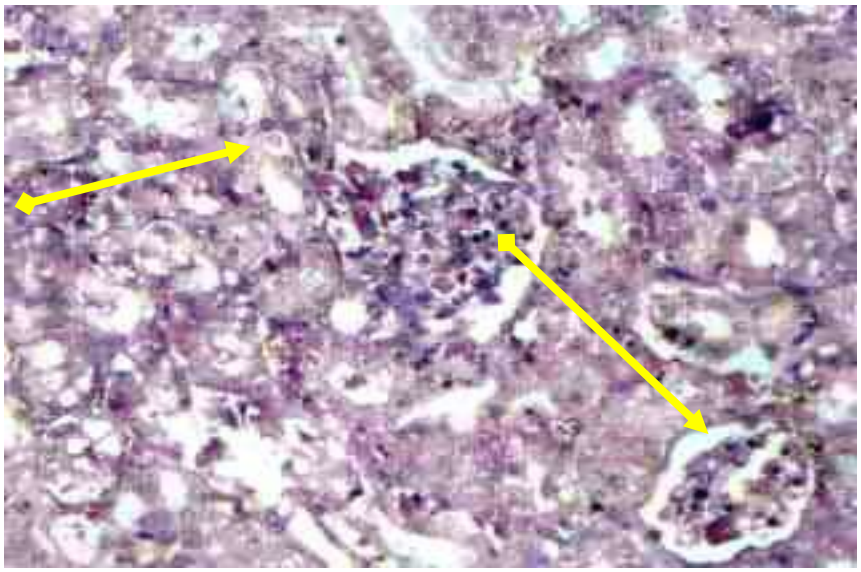
- **Same capital letter mean no significant changing**
- **Different capital letter mean significant changing**
- * **$P \leq 0.05$**
- ** **$P \leq 0.02$**

In the other hand there are several histological changes in slide section in which we can summarized as below

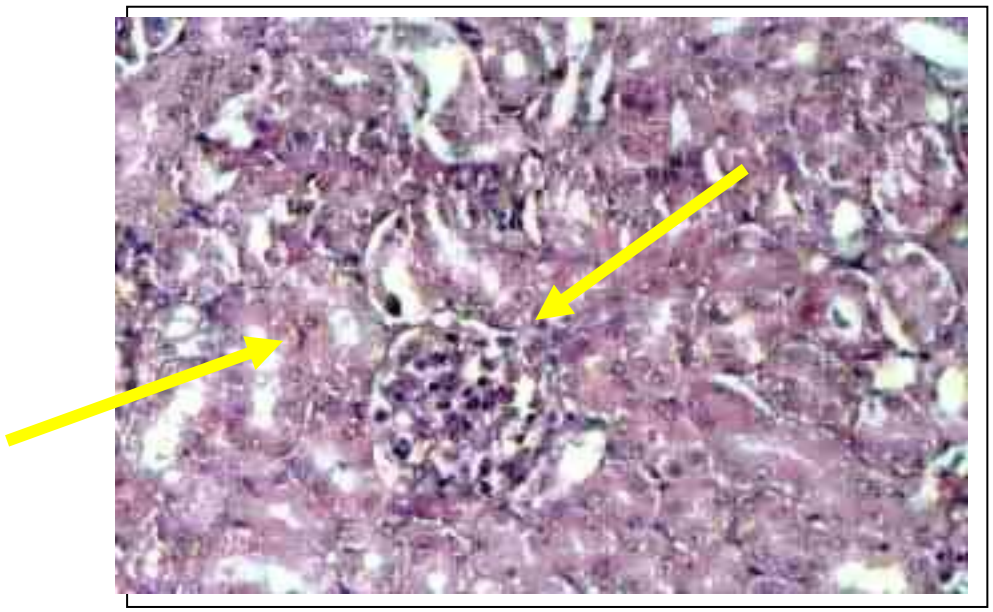


Picture 1(H and E X 400) positive control group of rabbits were drenched with acrylamide 10%

In picture one of positive control group there are chronic pyeloynephritis which develops after recurrent attack of acute pyelonephritis, so the glomeruli are completely fibroses and the result is crowding of hyalinised glomeruli (thick arrow). While in second and third slides of positive control groups the cells that lining tubules are necrotic in which no nuclear in its and the cytoplasm is deeply eosinophilic and the glomeruli in the left side of the slide appear necrotic (yellow arrow)

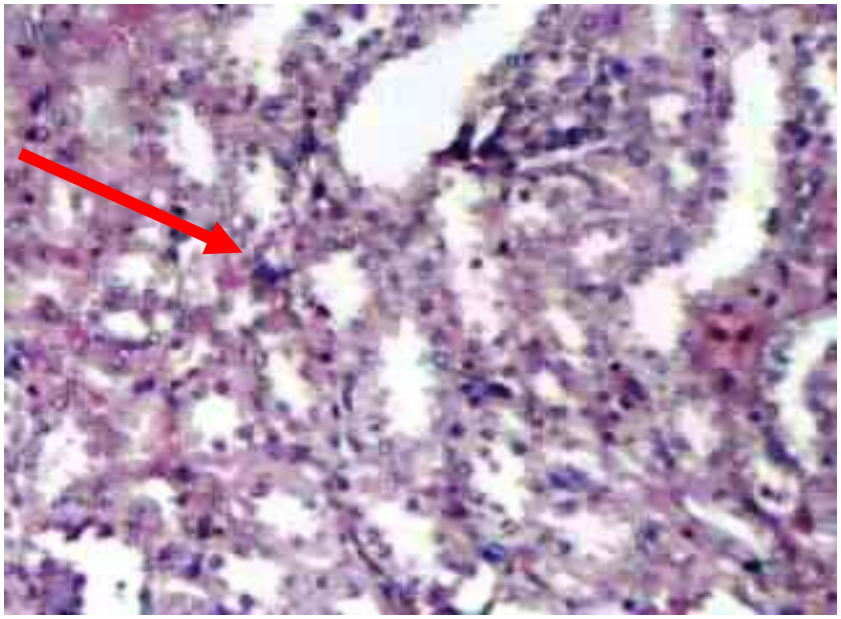


Picture 2(H and E X 400) positive control group of rabbits were drenched with acrylamide 10%



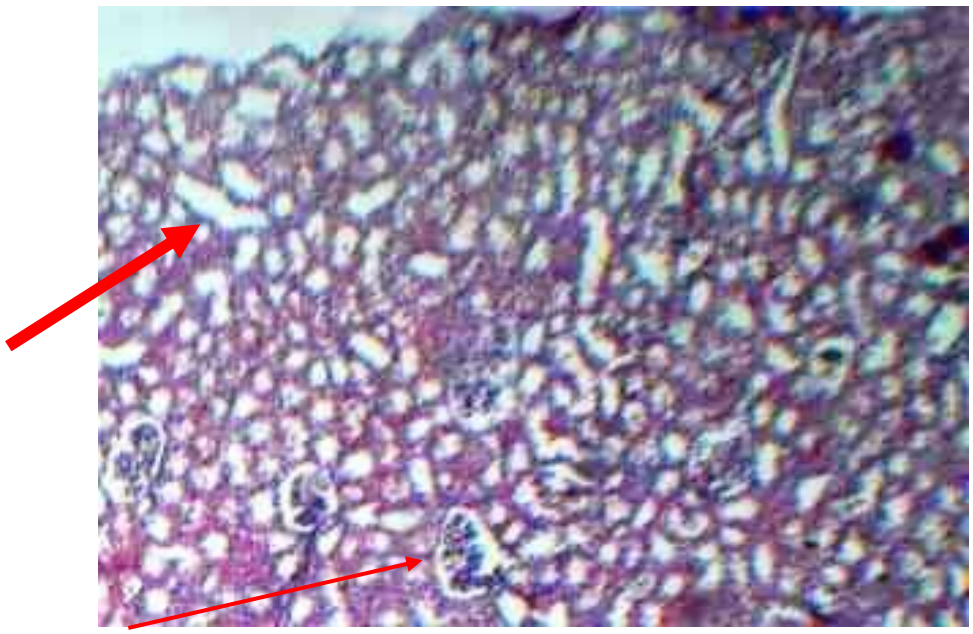
Picture 3(H and E X 400) positive control group of rabbits were drenched with acrylamide 10%

In the other hand we can diagnoses infiltration of macrophage, polymer leukocytes and fibroblasts that organize the dead tissues (picture 4 red arrow)

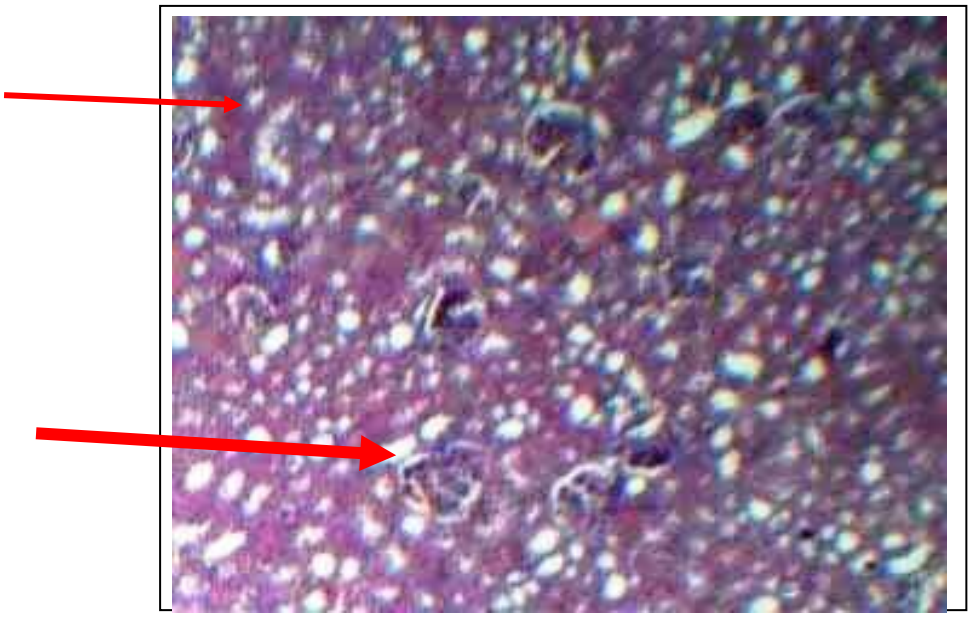


Picture 4(H and E X 400) positive control group of rabbits were drenched with acrylamide 10%

While in picture 5 and 6 we prepared slide section for treatment group in its we can recognize a marked recover of tubule cells from degeneration and retardation of necroses as compared with above slides (thick and thin arrow)

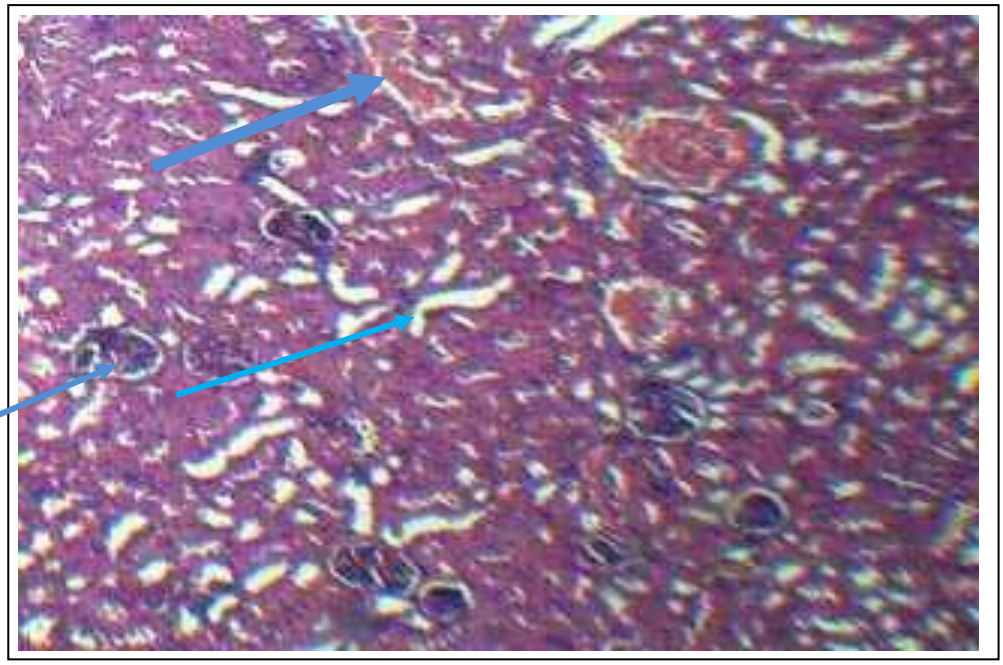


Picture 5(H and E X 100) section in rabbits treated with acrylamide 10% and flavonoids 40%

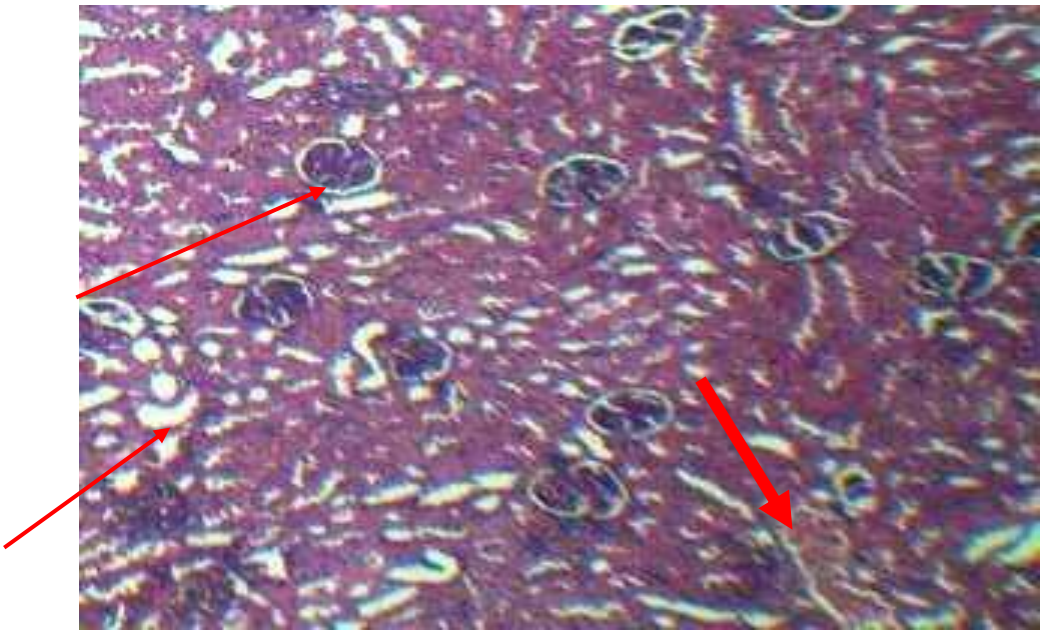


Picture 6(H and E X 100) section in rabbits treated with acrylamide 10% and flavonoids 40%

Also we recognized in slides of treatment group that have lesion of hyaline casts in the lumen of renal tubules (thick arrow), but in the other side of the slides we can recognize normal renal tubules and glomeruli (thin arrow in picture 7 and 8)



Picture 7(H and E X 100) section in rabbits treated with acrylamide 10% and flavonoids 40%



Picture 8(H and E X 100) section in rabbits treated with acrylamide 10% and flavonoids 40%

Discussion

In this study there are a significant reduction in blood creatinine and uric acids in group that drinking with Flavonoids and acrylamide when compared that drenched acrylamide only, however there are several opinion may explain this result, first of all acrylamide is listed by the World Health Organize (WHO) as probable human carcinogen (17). It is classified as genotoxic carcinogen on the basis of cells culture and animals data. Acrylamide has been shown to induce cell mutations in cultured animals cells and also in animals treated in animals treated in vivo. Thus it is assumed that exposure also to low doses of acrylamide increases the risk for mutation and cancer(18,19). In the other hand signs of acrylamide toxicity in animals exposed for longer periods of time(months to year) are caused gastrointestinal bleeding, respiratory distress syndrome, hepatotoxicity and peripheral neuropathy (20,21,22), while in my study I record acute nephrotoxicity which supported by histological section in slide (1,2,3), so the elevation of creatinine and uric acids that refers to acute destruction in glomerular infiltration rat.

In the other hand there are another cause may explain the acrylamide toxicity due to metabolic pathway, so conjugation to GSH catalyzed by glutathione-S-transferase(GSH) and excretion as mercapturic acid is a major pathway for metabolism and detoxification of acrylamide (23,24). The marcapturic acid [N-acetyl-s-(2-carbomoylethyl)-cysteine] is excreted in the human and animals urine (25). Conditions that associated with decrease GSH and hence acrylamide toxicity at lower exposure include: a- malnutrition associated withcosumption of diets low in the sulfur amino acids cystine and methionine, which are needed for synthesis of GSH (26,3), and b- Oxidative stress which may result in oxidation of GSH to GSSG (26,27,28). The resulting depletion in GSH lead to reduce protection of cell membrane to oxidative stress and this hypothesis which supported our study and give an explanation for renal cells degeneration which recorded in slide (1,2,3,4).

In the other way to give an appropriate explanation for result of treatment group with acrylamide and Flavonoids in a synchronism way that make me to depend on Flavonoids because Flavonoids are uniquely suited as a support or single therapy in the treatment of inflammatory due to their combination of antioxidants and anti-inflammatory properties of Flavonoids have been well established(29,30). The reason for use Flavonoids/proanthocyanidin based antioxidants in the management of inflammatory disorders include:

1- The radical scavenging capacity of Flavonoids, and particularly proanthocyanidins is several times higher than that of vitamin antioxidants for a wide range of radicals(31). Flavonoids are chemically a diverse range of compounds containing different ring structures with different side chains and polymerization states. This diversity greatly contributed to their radicals scavenging ability and other effect on enzyme systems the body(32). The potency as antioxidants and the chemicals diversity of multi compounds Flavonoids extracts, like ENZO professional, strongly recommend Flavonoids in therapeutic efforts to lower oxidative stress levels(33).
2- Flavonoids have been shown to inhibit the production of ROS, the activity of the ROS generating enzymes MPO and NADPH oxidase, and several signal transducing enzymes involved in the cell activation in neutrophils (30).
3- Flavonoids have been shown to reduce biomarkers of oxidative cell damage in cell culture systems, animal models and human studies (34,35,36)
4- Flavonoids inhibit lipid peroxidation this can be by direct radical scavenging or by chelation properties of transition metals(33).
5- Flavonoids have direct anti-inflammatory properties that have been documented in many studies. The anti inflammatory properties of flavonoids include cyclooxygenase (COX2) inhibition, lowering prostaglandin E2 production, inhibition of several signal transducing molecules and enzymes, such as inhibition of NFkB activation, inhibition of nitric oxide synthesis induction and subsequent NO^{-} production and inhibition of pro inflammatory cytokines, such as interleukin-1β and interleukin-2 (30,37,38,39).

Conclusion :

Acrylamide is hazards effect for human health and Flavonoids have antioxidant effect which present in a wide range of fruits and herbal plants, so I recommended to increase use fruits in diet as a precaution against any pollution and toxicity of chemicals.

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