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Evaluation Cytotoxicty and Histopathy of the efficacy of *Silybum marianum* seeds on the lung and colon of Albino mice after induced by Urethane

Dr . Rana Naseer abdul-Hameed
North technic university, institute al-door

Abstract:-

Detection of the joint effect of silymarin and urethane in laboratory animals by dosing animals with different concentrations as well as its effects on cytogenetic indicators Comet assay technique for bone marrow, lung and colon cells in albino mice. the aim this study(Studying the effects of urethane cytotoxicity using cytogenetic techniques (comet assay) in albino mice, also indicated histopathy developed cells tissue in lung and colon also can conclusion increased effects of silymarin cytotoxicity ,it able to kill abnormal cells(malignant cell)

Keyword:Urethane,cytogenetic,Silybum marianum

تقييم السمية الخلوية والتغيرات النسيجية لفعالية بذور نبات الكلغان *Silybum marianum* وتأثيرها على الرئة والقولون في الفئران البيضاء بعد تحريضها بمادة اليوريثان

م.د. رنا نصير عبد الحميد
الجامعة التقنية الشمالية، معهد التقني الدور

خلاصة

الكشف عن التأثير المشترك لمستخلص السيليمارين(المستخلص من بذور نبات الكلغان) مع مادة اليوريثان في حيوانات المختبريةعن طريق تجريع الحيوانات بتراكيز مختلفة وكذلك تأثيره على المؤشرات الوراثية الخلوية وهي تقنية فحص المذنب لخلايا النخاع العظمي والرئة والقولون في الفئران البيضاء. هدفت هذه الدراسة (دراسة تأثيرات السمية الخلوية لليوريتان باستخدام تقنيات الوراثة الخلوية (فحص الهالة) في الفئران البيضاء لمعرفة اذا كان فحص الهالة كبير يعني ان هناك سمية خلوية عالي ، كما أشارت إلى وجود اعتلال نسجي في أنسجة الخلايا المتطورة في الرئة والقولونممكن ان نستدل ان المادة لها القابلية على قتل الخلية الخبيثة.

الكلمات المفتاحية: يوريثان، وراثة خلوية، السيليمارين .

INTRODUCTION:-

Description of the star flower family *Silybum marianum* also called (Milk thistle) The original home of the plant is the Mediterranean region, as well as spread in the eastern regions of the Arab world, such as Palestine, Jordan and Iraq. [1]

The stem is simple, slightly branched, tall (1-2 m) , semi-smooth, pale green, as a biennial plant, (Asteraceae). hollow (containing grooves), surrounded by villi, giving a cottony appearance. The leaves are large, embroidered with white veins, sinuses lobed or needle-lobed.to triangular, containing serrated sinuses, spiny lobes, basal leaves narrowing to a seated base, leaves in stem enlarged with spiny ciliated axils, strongly erect [2,3]. The plant contains many active compounds which are known as Flavono- lignans medically and these substances are concentrated in the seeds and then the leaves (figure1).

Complex phenolic compounds such as Al-silymarin, the most used compounds in medicine and for treatment against many diseases .[2,4,5]

figure 1. *Silybum marianum*

More recently, All of these studies showed the effective protective action of silymarin on cells preventing damage to DNA and tissue. Scientists have begun to

investigate silymarin's anti-inflammatory capabilities in additional disorders, increasing the number of possible uses related to this ancient medical practice.

Treatments of Mice and material:

Used the samples Silymarin extract from *Silybum marianum* seeds, and extract by sohslet method) given daily oral gavage at the dosages 500 Mg/Kg b.w in o.2 Ml). Silymarin extract used alcoholic ethanol 96% ,Urethane (Sigma ChemicalCoSt.Louis, MO,USA) (ready to prepare),was administered in a single SC (can use IP) injection, dissolved in water, at the dose of (0.5 g/kg body weight) Urethane crystals ,Its freshly prepared via dissolving in sterile distilled water (adequate volume) of to obtain the desired concentration this extract dose in tikrit university/collegesciences labratory of genetic

1-Toxicity of silymarin and median lethal dose LD50:-

Silymarin having very little acute toxicity and a median lethal the dosage (LD50): The LD50 in male and female mice was 1050 and 970 mg/kg, respectively. In mice, the intravenous median lethal dosage (LD50) of silymarin has been shown to be 500mg/kg. Silymarin is administered via gradual intravenous infusion (over 2-3 hours). Tolerance is significantly higher after oral medication, with values above 10 g/kg. In the case of rapid intoxication, the cause of death appears to be heart failure.

Cytotoxicity Methods :

2-**COMET Assay :** the experiment of this method was conducted according to the method described via Tice. [6]

3-Histological examination

Both lung and colon tissue were remove and taken to be kept for histological analysis. After embedding in paraffin, the tissues were sectioned and kept in 10% neutral buffered formalin. We stained each slice with eosin and haematoxylin.

Statistical Analysis:

Results were shown with the mean arithmetic mean ± standard error after the results were statistically increased using the statistical analysis program of the Statistical Package for Social Sciences (SPSS). The results of this study were statistically examined using the ANOVA test by comparing the statistical averages of the various experimental groups and by utilizing the TUKEY testing in order to identify significant differences.

RESULTS and Discussion

In this study the cytogenetically Alterations in female mice (Adult) as related to treatment SILYMARIN.A series of experiments were designed in order to evaluate the SILYMARIN modulation of cellular and cytogenetical effects by SILYMARIN, Different kinds of examines were developed in order to investigate the SILYMARIN rules of cellular and cytogenetical effects by SILYMARIN, mice were given daily by gavage, Of mice adult females mice groups of either adult mice, every one composed of 10 mice, and there were examined .(Table1)

In vivo comet assay contribute to genotoxic potential identification of agent and assessment of dose response, and understanding substances mechanism of action [7,12,13]. In the here current study DNA migration was evaluated in lung and colon cells of white mice treated with 0.5 g/kg body weight of urethane, damage levels in negative and treated groups, Figure 2.The comet assay results showed a high significant increase lung damaged cell in both treatment groups different in

negative group . Also there was a significant increase in lung and colon total damage in treatment groups, compared to negative control. tables 2,3 explain these results.

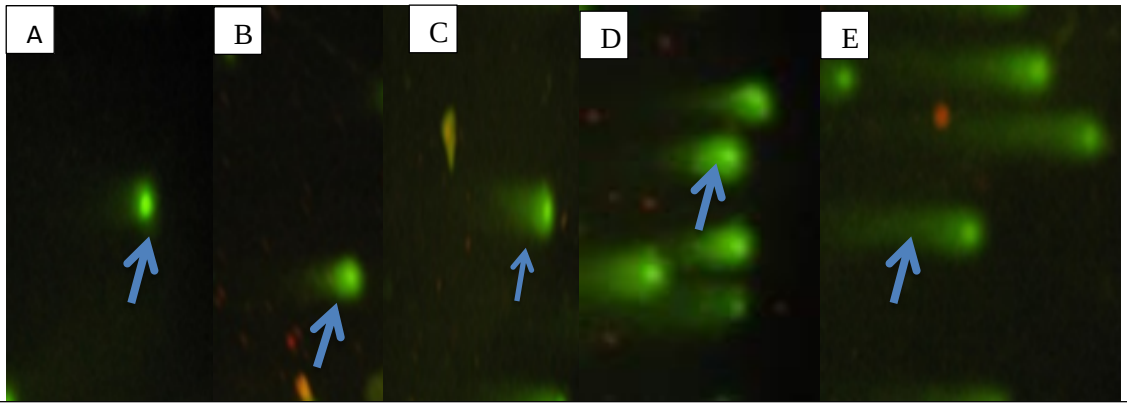


Figure (2) different levels of total damage in colon cells of albino mice(a)cells with undamaged DNA from the negative control group (b, c, d, e) cells with damaged DNA from groups treated with the dose of 5 mg.kg-1(D) Cells co-treated with alcoholic extract at a dose of 470 mg. KG-1, damage of varying degrees (mild, medium, severe) (blue arrow). X100, Cybergreen.

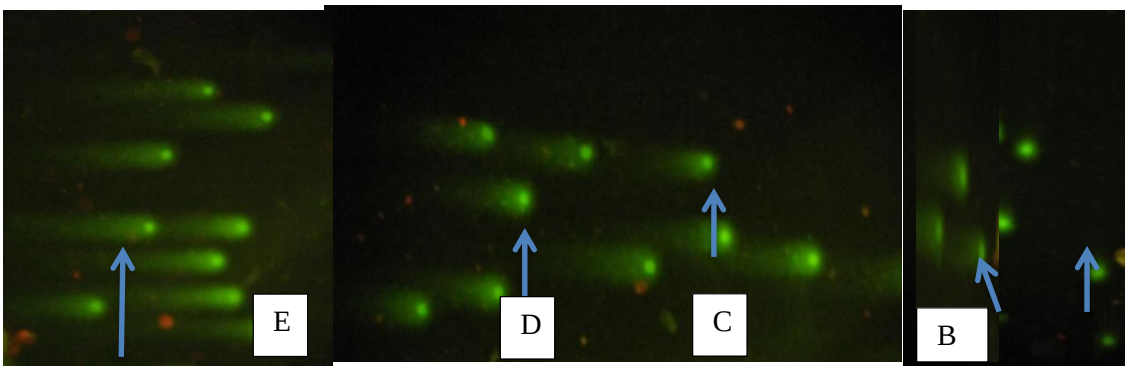


Figure (3) different levels of total damage in the lung of albino mice after treatment with alcoholic and aqueous extract at a dose of (a) cells with undamaged DNA from the negative control group, (b) cells co-treated with alcoholic extract at a dose of 630 mg. KG-1 cells appear similar to control cells, cells with undamaged DNA (C), -treated cells with alcoholic extract at a dose of 630 mg. (D) Kg-1 cells were co-treated with the alcoholic extract at a dose of 420 mg. kg-1 (E) (mild, moderate, severe) (blue arrow), X100, Cybergreen.

Table (1) Mean values of differences for cells with damaged DNA and total damage in lung cells of albino mice in groups treated with urethane and silymarin alcoholic e

xtract.

Treatment dose Mg.Kg-1.Bwt	Med total damage score MD± SE	Average total damage score MD+/-SD	Average cells with damaged DNA MD± SD	Average cells with damaged DNA MD+/-SD
Distal Water	_	2.33 +/- 0.78	-	0.71±2.00
Urethane 5	3.75 ±101.4 **	-	2.79*±088.2	-
Urethane + alcoholic ext.silymarin 470	32.08± *2.75	-	21.12±2.79*	-
420	*2.75± 34.05		25.10± *2.79	-

***Significant at 0.01 , **Significant level 0.05 paired sample T-TEST**

(Table2) Values of Mean Differences for mice

cell and have Damage in DNA ,with AVERAGE Total damage in colon cells in groups also treat with different doses the urethane .

Treatment dose mg.kg-1.b.wt	Med total damage score MD± S.E	Average total damage score MD± S.E	Average cells with damaged DNA MD± S.E	Average cells with damaged DNA MD± S.E
D.W	-	**1.08±3.03**	-	**0.06±2.00**
Urethane 5	**2.77*±80.14**	-	**2.15±68.02**	-
Urethane + alcoholic ext.silymarin 470	**2.77*±31.29**		**2.15±28.28**	
420	**2.77*±30.33**		**2.15*±44.22**	-

***Significant at 0.01 , **Significant level 0.05 paired sample T-TEST**

Histological examination-

Studying histology and classifying lesions and overexpression (see Figure 4). The histological characteristics of this model effectively show that adenomas are the

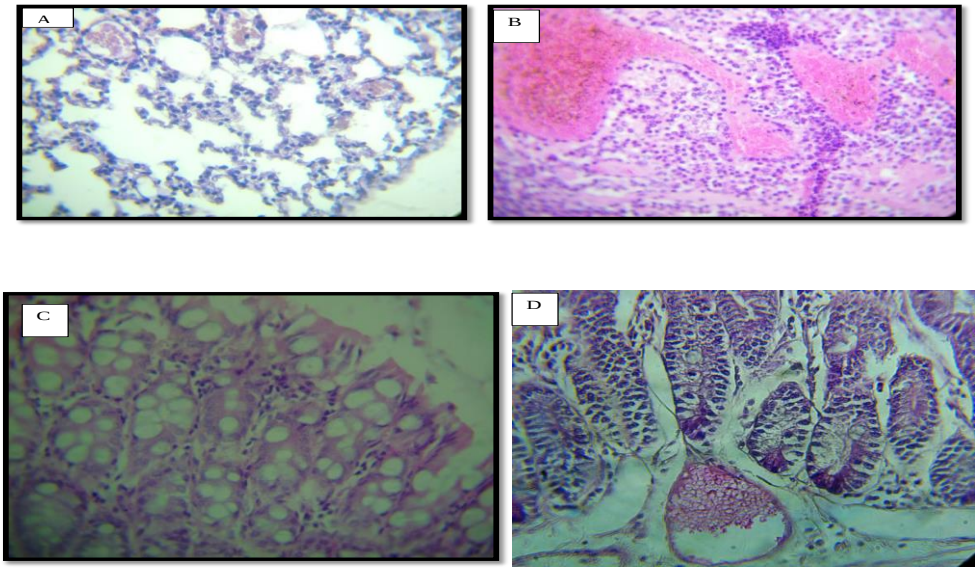
lesions found in abnormal tissue, while a category was determined to check the cancer diagnosis [11]. The criterion for classifying a disease as hyperplasia was a sign of alveolar proliferation of cells without the obliteration of over four intra-alveolar spaces; the nodule was designed to spread with the obliteration of more than four intra-alveolar spaces. From both lungs, every lesion was specified. Histopathological investigation of the lesions resulted in a grade of nodules and hyperplasia.

Comparing lung tissue to controls(figure A) Significant alterations and tissues damage were observed (figure B) these alterations include oedema, damage of tissue and a significant lymphocyte infiltration ,the degree of damage and lymphocyte infiltration were decreased after silymarin treatment (figure C) While compared to control animals (figureA) a histological analysis of the colon showd(figure B) These alteration include lymphocyte infiltration, vacuolated cytoplasm and o edema ,most of morphologic changes were avoided by dosing silymarin and became healing .(Seefigure4)

This study like previous studies (8).while the administration of silymarin did not change the control group of the parameters evaluated in this study. When Discussion the current study, the histological parameters chosen are standard for the assessment of lung and colon cell damage. Focal cell necrosis was the indication that a considerable an important number of lung cells have died (8-9).

The Histopathological alteration in both lung and colon of mice treatment with urethane ,results suggest additional ,different pathogenic systems might be activated in the lung Parenchyma and the colon cells.It is widely recognized that the Urethane consists of a significant endogenous anti-oxidant that scavenges a wide range of free radicals, including the highly toxic(˙OH) (9).the Electron-rich

stricture of the Urethane enabling it species at high rates of reactive scavenge directly (10).



(Figure4) A characteristic photomicrograph(H&E stain, 100x, respectively) show in A normal lung tissue, B abnormal lung nodules and hyperplasia, C normal colon tissue, D abnormal colon hyperplasia Inducer by smoke (5 cigarette twice a day) and urethane (5 Mg/Kg b.Wt), while in E abnormal colon (tumor) overgrowth of the cell, Lymphocytic infiltrate with blood vessel congestion, and elevated A lymphocyte infiltration in (B, D, E, and F)

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