J. Agric. Sci. Mansoura Univ., 29 (10): 5993 - 6004, 2004

SAFETY EVALUATION OF SOME SYNTHETIC AND NATURAL PRESERVATIVES OF COSMETIC PRODUCTS

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ABSTRACT

The present study deals with the safety evaluation of two commonly used synthetic preservatives, i. e., germaben II (methyl-4-hydroxybenzoate) and euxyl K100 (a mixture of benzyl alcohol + methyl chloroisothiazolinone + methyl isothiazolinone) for cosmetic products. The safety of the essential oils of thyme white, thyme red and tea tree was also evaluated as susbstituents for the synthetic preservatives. Therefore, the activities of some enzymes of rat organs, i. e., alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), ribonuclease (RNase) and deoxyribonuclease (DNase) and rat serum contents, i. e., urea, total lipids, total proteins, total cholesterol, uric acid, bilirubin and creatinine were determined for rats administered both synthetic and natural preservatives under study. In addition, histopathological studies were carried out in order to emphasis the results of biochemical determination for liver and kidney functions. In general, the natural preservatives induced little changes in rat enzyme activities and serum contents. On the contrary, the synthetic preservatives caused remarkable alterations in some rat enzyme activities and serum constituents.

Keywords: Thyme oil, Tea tree oil, Germaben II, Euxyl K100, Enzyme activity,Serum constituents, Rats, Histopathological examination.

INTRODUCTION

Cosmetic products are quite important for the beauty of human being. The ingredients of the cosmetic products are proned to deterioration during preparation, handling and storage. Therefore, it is highly required to add a preservative to keep the quality of cosmetic products. It seems that micro-organisms are among the factors responsible for cosmetic spoilage. In this respect, there are several chemicals can act as antimicrobial and antioxidant agents. For instance, BHA and BHT are added to Brut lotion and cream body lotion (Faberge, 1987). Other examples are p- aminobenzoic acid derivatives and digalloyl trioleate in sunscreen preparations, bitlhionol and hexachlorophene in toilet soaps and deodorants and the halogenated salicylanilides for example tetrachlorosalicylanilide (T_4CS) as a bacteriostatic substance (Godwin, 1982). In recent years, plant products are used as natural preservatives and induced encouraging results (Farag *et al.*, 1990).

The essential oils which obtained from many thymus species are used as flavoring in the manufacture of perfumes and cosmetics and for medicinal purposes such as in the preparation of antispasmodics or tonic. In addition, thyme was found to possess inhibitory effect on synthetic media containing bacteria, yeast and fungi (Farag *et al.*, 1989).

Fitzi *et al.* (2002) reported that the topical preparations containing tea tree oil from *Melaleuca alterniolia* possessed antimicrobial and

antipruriginous effects. The antiviral effects of Australian tea tree oil (TTO) against herpes simplex virus were examined by Schnitzler *et al.* (2001). The toxicity of TTO was moderate for RC-37 and approached 50% (TC_{50}) at concentration of 0.006%.

In recent years, tea tree oil has become increasingly popular as an antimicrobial for the treatment of conditions such as tinea pedis and acne. In this respect, Koh *et al.* (2002) investigated the anti-inflammatory properties of tea tree oil on histamine induced wheal and flare. They showed experimentally that tea tree oil reduced histamine which cause skin inflammation.

The present study suggests the use of tea tree oil and thyme oil as a preservative to cosmetic products instead of the synthetic preservatives. Euxly K100 and germaben II as the synthetic preservatives are actually added to shampoo, cream and care products. It seems that the safety limits of synthetic preservatives are not known. Consequently, nutritional experiments were conducted to study their safety limits through evaluation of their effects on the liver and kidney functions. Therefore, the activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, deoxyribo-nuclease, ribonuclease, as well as the serum levels of total proteins, total lipids, total bilirubin, creatinine, urea, uric acid, cholesterol, were determined. Microscopical examinations of liver and kidney tissues of rats were conducted in order to emphasis the results of the biochemical determinations for liver and kidney functions.

MATERIALS AND METHODS

Sources of preservatives

Euxyl K100 (a mixture of benzyl alcohol, methyl chloroisothiazolinone and methyl isothiazolionone), and germaben II (methyl-4-hydroxybenzoate) were purchased from Diomed S.A. Company (Geneva 29/ Switzerland,) and International Specialty products (ISP, Guilfrord, Surrey, England), respectively. The essential oils of tea tree, thyme red and thyme white were obtained from Dainel LTD Company (Billinghan, Cleveland, England).

Nutritional experiments

Albino male rats (128) with an average weight of 80-100 g were obtained from a private market at Helwan district, Cairo, Egypt. The animals were housed individually in cages and fed on a basal diet and water was available *ad-libitum* for one week as an adaptation period.

After the adaptation period, the rats were randomly divided into six main groups according to the synthetic and natural preservative types. The first group (8 rats) was fed on the basal diet for whole experiment (one week) and considered as a control. The other five groups comprised three subgroups each of 8 rats. The first subgroup of the second group was continued to be fed on a basal diet and intrapretonial injected by 0.05 ml of germaben II, whilst, the 2nd and 3rd subgroups were injected with 0.10 ml and 0.15 of ml germaben II, respectively.

The same design was adopted for 3rd, 4th and 5th rat groups using euxyl K100 (0.05, 0.10 and 0.15 ml), thyme white oil (0.05, 0.10 and 0.15 ml), thyme red oil (0.05, 0.10 and 0.15 ml), and tea tree oil (0.05, 0.10 and 0.15 ml), respectively.

At the end of the experiment (one week) blood samples were taken, centrifuged at 300 rpm for 20 min. and the sera were kept in a deep freezer (-7°C) until analysis. The rats were, then killed by decapitation and the rat organs (liver, heart and kidney) were excised and washed with ice-cold isotonic saline solution (0.15 M KCL). Organs were stored at - 20°C until analysis.

Preparation of samples for analysis

The rat liver and kidney organs were individually homogenized according to the method of Zheng *et al.* (1993). The homogenates were filtered through 5 layers of muslin at 4°C and centrifuged at 5000 rpm for 15 min. to isolate the nuclei. After separation of the nuclei, 1% Triton X100 (3 ml) was added to the obtaine pellets and directly used for the determination of enzyme activities.

Liver function tests

The activities of serum alanine aminotransferase (ALT, EC 2.6.1.2), serum aspartate aminotransferase (AST, EC 2.6.1.1) and serum alkaline phosphatase (ALP, EC 3.1.3.1.) were determined according to Bergmeyer and Harder (1986) Nilkinson (1976) and Belfield and Goldberg (1971), respectively. The activities of ribonuclease (RNase, EC 3.1.27.5) and deoxyrabonuclease (DNase, EC 3.1.22.1) were measured in the homogenates of liver, heart and kidney organs according to Bergmeyer (1974).

Total proteins (Lowry *et al.*, 1977), total lipids (Frings and Dunn 1970) and total cholesterol (Roeschlau *et al.*, 1974) were determined in serum of rats at the end of the experimental period.

Kidney function tests

Urea (Fawcett and Soctt, 1960), uric acid (Barham and Trinder, 1972), total bilirubin (Walter and Gerade, 1970) and creatinine (Larsen, 1972) were determined in rat serum at the end of the experimental period.

Histopathological study

At the end of the experimental period the rats were killed using diethyl ether. The liver and kidney organs were removed, stored in 10% neutral formalin and embedded in paraffin wax. The organs were sectioned at the thickness of 5.6 microns and stained with haematoxylin and eoxin according to Culling (1965). Tissue sections were then examined using ordinary microscope for histological evaluation.

Statistical analysis

The present data were subjected to analysis of variance and the least significant difference (I.S.D.) test was calculated to allow comparison

between the average values of the studied factors (Snedecor and Cochran, 1972).

RESULTS AND DISCUSSION

Germaben II is a versatile preservative and used as an ingredient of some cosmetic products such as hand and body lotion, moisturising, face, nail, shave and sunscreen creams and body shampoo and hair moisturizer (Gowin, 1982). Also, it induces bactericidal activity (ISP, Guilford, Surrey, England). Euxyl K100 is also used as a part of the following cosmetic products: conditioning shampoo body glaze sunscreen, creams, lotions, bath additives (Diomed S.A. Company, Geneva 29/ Switzerland). Thyme white oil, thyme red oil and tea tree oil are used as natural preservatives and exhibit an antimicrobial activities (Farag *et al.*, 1989, 1990 and 1998 and Kima *et al.*, 1994).

In the present investigation, two sets of nutritional experiments were conducted to demonstrate the effects of the aforementioned synthetic and natural preservatives on the rat serum constituents, liver and kidney functions and histological examinations of liver and kidney tissues of rats.

Table (1) shows the influence of thyme white, thyme red and tea tree essentials as natural preservatives and gramaben II and euxyl K100 as synthetic preservatives on the RNase activity of rat liver, heart and kidneys. In control rats, the RNase activity in different rat organs followed the decreasing order: liver (36.24 IU)> heart (29.31 IU) > Kidneys (26.71 IU). The application of thyme white oil to rats at various levels caused gradual and slight increase in the RNase activity (the maximum increased was 102% over the control rats 100%). The same results on RNase activity were obtained for various rat organs. Also, similar findings for RNase activity at various levels (0.05ml, 0.10ml, and 0.15ml) for different rat organs for thyme red and tea tree essential oils were recorded. In other words, the essential oils used in the present study did not cause any breakdown on rat microsomes.

The administration of the synthetic preservatives caused gradual increase in RNase activity at various levels on different rat organs. In fact, the high dose (0.15) induced remarkable increase in the RNase activity in comparison with low and moderate doses of the germaben II and euxyl K 100. Therefore, the high dose of these synthetic compounds can alter the chromosomal structure of RNA which is responsible for the synthesis of enzyme structural proteins and ultimately induce mutant effect.

Table (2) shows the effect of thyme white, thyme red and tea tree essential oils as natural compounds and germaben II and euxyl K 100 as synthetic compounds on the DNase activity of rat liver, heart and kidneys.

Table (1): Effect of natural and synthetic preservatives on nuclear RNase activity (I.U.) of rat organs.

Preservative	Dose	Liver		Heart		Kidneys	
		Activity	%	Activity	%	Activity	%
Control		36.23 ± 2.97	100	29.31 ± 2.01	100	26.71 ± 2.61	100
Thyme white oil	0.05	35.42 ± 2.81	97.73	29.98 ± 2.72	102.28	26.98 ± 2.64	101.01
	0.10	36.71 ± 3.01	101.32	30.02 ± 2.41	102.42	27.72 ± 2.31	103.78
	0.15	37.01 ± 2.96	102.15	31.20 ± 2.61	106.45	27.61 ± 2.81	103.37
Thyme red oil	0.05	37.21 ± 3.04	102.67	29.84 ± 2.82	101.81	27.31 ± 2.05	102.25
	0.10	38.41 ± 3.71	106.02	29.71 ± 2.51	101.36	26.99 ± 2.67	101.05
	0.15	38.52 ± 2.65	106.32	30.24 ± 3.11	103.17	26.76 ± 2.51	100.18
Tea tree oil	0.05	36.99 ± 3.41	102.09	28.67 ± 2.45	97.82	27.30 ± 2.66	102.21
	0.10	37.31 ± 2.67	102.98	29.84 ± 2.61	101.81	27.67 ± 2.67	103.59
	0.15	37.39 ± 2.77	103.20	29.96 ± 2.54	102.22	27.98 ± 2.43	104.75
Germaben II	0.05	38.49 ± 2.96	106.23	31.71 ± 2.61	108.18	28.67 ± 2.38	107.34
	0.10	39.87 ± 2.81	110.04	31.89 ± 2.73	108.80	29.37 ± 2.76	109.95
	0.15	41.21 ± 2.54	113.74	32.61 ± 2.68	111.26	30.45 ± 2.53	114.00
Euxyl K100	0.05	39.29 ± 2.65	108.44	31.79 ± 2.91	108.46	28.67 ± 2.01	107.34
	0.10	41.04 ± 3.21	113.27	32.04 ± 2.54	109.31	28.99 ± 2.61	108.54
	0.15	41.99 ± 3.42	115.89	32.92 ± 2.50	112.32	30.21 ± 2.45	113.10

- Each value represents the mean ± SE of 8 rats.
- I.U. indicates international unites.

Table (2): Effect of natural and synthetic preservatives on nuclear DNase activity (I.U.) of rat organs.

Preservative	Dose	Liver		Heart		Kidneys	
		Activity	%	Activity	%	Activity	%
Control		62.14 ± 5.11	100	53.62 ± 4.11	100	58.27 ± 4.89	100
Thyme white oil	0.05	61.18 ± 4.81	98.45	52.76 ± 4.91	98.39	59.32 ± 4.68	101.80
	0.10	62.94 ± 5.01	101.28	54.82 ± 4.61	102.24	59.84 ± 4.69	102.69
	0.15	63.72 ± 4.99	102.54	54.71 ± 3.99	102.03	60.41 ± 5.02	103.67
Thyme red oil	0.05	62.44 ± 5.13	100.48	53.67 ± 4.82	100.10	58.99 ± 5.01	101.24
	0.10	62.99 ± 4.67	102.61	54.21 ± 3.64	101.10	59.14 ± 4.96	101.49
	0.15	63.76 ± 4.98	101.36	55.14 ± 4.81	102.83	60.11 ± 5.81	103.16
Tea tree oil	0.05	61.68 ± 5.04	99.26	52.67 ± 4.61	98.23	60.84 ± 4.99	104.41
	0.10	63.87 ± 5.14	102.78	54.11 ± 4.32	100.91	60.91 ± 5.24	104.53
	0.15	64.53 ± 4.88	103.85	53.89 ± 3.86	100.50	61.08 ± 5.31	104.82
Germaben II	0.05	64.72 ± 5.61	104.15	55.76 ± 4.61	103.99	62.21 ± 4.64	106.76
	0.10	67.81 ± 4.89	109.12	56.11 ± 4.81	104.64	63.67 ± 4.86	109.27
	0.15	68.03 ±5.84	109.47	58.34 ± 4.31	108.80	63.82 ± 5.14	109.53
Euxyl K100	0.05	64.67 ± 4.96	104.07	55.76 ± 4.64	103.72	61.24 ± 5.03	105.09
	0.10	68.12 ± 4.81	109.62	56.81 ± 4.89	105.95	62.36 ± 4.82	107.02
	0.15	69.54 ± 5.41	111.91	57.46 ±4.14	107.16	62.52 ± 5.31	107.29

- Each value represents the mean ± SE of 8 rats.
- I.U. indicates international unites.

In control rats, the activity of DNase was in the decreasing order depending upon the rat organ: liver (62.14 IU)> kidney (58.27 IU)> heart (53.62 IU). The administration of the essential oils as natural preservatives at various levels (0.05 ml, 0.10 ml and 0.15 ml) on different rat organs caused gradual and non-significant increase in DNase activity. The highest percentage increase in DNase activity was 104.82% over the control (100%) when tea tree was administered at 0.15 ml. Hence, one would except that the application of cosmetic products containing these oils do not induce any changes on the DNA chromosomal structure.

The synthetic substances caused gradual increase in DNase activity, this increase was dependent upon the applied dose in all rat organs. In this context, the moderate and high doses from these substances show remarkable increase in DNase activity. This increase might affect the cellular processes comprising the synthesis of ribonucleic acids (RNA). Generally speaking, the administration of essential oils of thyme red, thyme white caused little increase in the enzyme activities compared with the synthetic substances.

Table (3) shows the influence of natural and synthetic preservatives at 0.05 ml concentration on ALT, AST and ALP activities. The administration of the essential oils of thyme white and thyme red to rat induced slight increase in the activities of the aforementioned enzymes at the end of the experiment (one week). Whilst, the administration of tea tree oil caused further increase in the enzyme activities compared with thyme oil. The administration of synthetic preservatives especially euxyl K 100 exhibited remarkable increases in enzyme acyivity. These findings clearly indicate that the synthetic materials used in the present study caused detrimental effect on liver function.

Table (3): Influence of natural and synthetic preservatives on rat serum alanine (ALT), aspartate (AST) aminotransferases and alkaline phosphatase (ALP) activities.

Preservative	ALT activity		AST activity		ALP activity	
	U/ml	%	U/ml	%	U/ml	%
Control	13.92^{a} ± 0.09	100	14.56^{a}± 0.51	100	64.49^{a}±0.54	100
Thyme red oil	15.43^{b} ± 0.55	110.84	16.20^{b}± 0.44	111.26	64.97^{a}±0.53	100.79
Thyme white oil	15.74^{b} ± 0.38	113.07	17.87^{b}±0.57	122.73	65.57^{b}±0.35	101.67
Tea tree oil	16.62^{c} ± 0.54	119.40	17.98^{b}± 0.55	124.00	65.99^{c}± 1.33	103.88
Germaben II	17.29^{c} ± 0.31	124.21	18.87^{c}± 0.14	130.14	66.02^{c}± 0.01	102.37
Euxyl K100	22.33^{d} ± 0.10	160.42	23.28^{d}± 0.25	159.89	72.45^{d}±0.04	112.34
Value of LSD at p< 0.05	0.67	-	0.79	-	1.14	-

- The data are expressed as mean values ± standard error.
- Numbers in a column followed by the same letter are not significantly different.

Rat serum constituents

Table (4) shows the influence of synthetic (germaben II and euxyl K 100) and natural (thyme red oil, thyme white oil and tea tree oil) preservatives on serum total lipids, proteins and cholesterol. The data show that there were no significant increases in the levels of serum total lipids due to the application of all natural or synthetic preservatives compared with control. Also, the results indicated that there were non-significant increases in the

total cholesterol and total protein levels in rats administered thyme red oil compared with the control experiment. (Farag *et al.*, 1991). On the other hand, thyme white oil, tea tree oil, germaben II and euxyl K 100 induced significant increases in the levels of both total proteins and cholesterol. The levels of these substances in all rat groups were arranged in the following decreasing order according to the type of preservative: no preservative > thyme red oil > thyme white oil > tea tree oil > germaben II > euxyl K 100.

Table (4): Influence of natural and synthetic preservatives on levels of serum total lipids, total cholesterol and total proteins of rats.

Preservative	Total lipids (mg/dl)	Total proteins (mg/dl)	Total cholesterol (mg/dl)
Control	$290.00^{a} \pm 0.00$	$7.96^{a} \pm 0.04$	$162.79^{a} \pm 0.59$
Thyme red oil	$291.66^{a} \pm 0.58$	$8.02^{a} \pm 0.11$	$162.94^{a} \pm 0.05$
Thyme white oil	$291.33^{a} \pm 1.15$	$8.17^{b} \pm 0.06$	$164.00^{b} \pm 0.02$
Tea tree oil	$291.33^{a} \pm 0.58$	$9.13^{c} \pm 0.02$	$165.33^{c} \pm 0.58$
Germaben II	$291.00^{a} \pm 0.00$	$10.11^{d} \pm 0.11$	$166.07^{d} \pm 0.06$
Euxyl K100	$291.00^{a} \pm 1.00$	$11.03^{e} \pm 0.06$	$168.07^{e} \pm 0.07$
Valus of LSD at $p < 0.05$	1.26	0.13	0.61

- The data are expressed as mean values ± standard error.
- Numbers in a column followed by the same letter are not significantly different.

Table (5) shows the effect of natural and synthetic preservatives on serum total bilirubin, creatinine, urea and uric acid levels in rats. The data show that the thyme red oil, tea tree oil and germaben II induced slight increase in serum total bilirubin levels compared with the control. On the contrary, euxyl K 100 as a synthetic preservative induced an obvious increase in serum total bilirubin level. The data for essential oils illustrated that there were slight increases in serum creatinine levels. In contrast, germaben II and euxyl K 100 as synthetic preservatives to cosmetic products induced significant increases in serum creatinine levels at the end of the experiment.

Table (5): Influence of natural and synthetic preservatives on levels of serum creatinine, total bilirubin, urea and uric acid of rats.

Preservative	Creatinine (mg/dl)	Total bilirubin (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control	$0.90^{a} \pm 0.01$	$1.00^{a} \pm 0.01$	$29.15^{a} \pm 0.01$	$4.65^{a} \pm 0.04$
Thyme red oil	$0.94^{a} \pm 0.02$	$1.04^{b} \pm 0.01$	$29.73^{a} \pm 0.15$	$4.98^{a} \pm 0.02$
Thyme white oil	$0.90^{a} \pm 0.01$	$1.06^{c} \pm 0.00$	$30.52^{b} \pm 0.02$	$5.36^{b} \pm 0.01$
Tea tree oil	$0.95^{a} \pm 0.01$	$1.08^{d} \pm 0.01$	$31.48^{c} \pm 0.59$	$5.41^{c} \pm 0.03$
Germaben II	$1.16^{b} \pm 0.07$	$1.06^{c} \pm 0.01$	$39.27^{d} \pm 0.64$	$6.00^{d} \pm 0.07$
Euxyl K100	$1.38^{c} \pm 0.01$	$1.41^{a} \pm 0.01$	$36.05^{e} \pm 0.05$	$7.00^{e} \pm 0.02$
Values of LSD at $p < 0.05$	0.05	0.01	0.64	0.04

- The data are expressed as mean values ± standard error.
- Numbers in a column followed by the same letter are not significantly different

On the other hand, the essential oil of thyme red caused non-significant changes in the levels of urea and uric acid in rat serum. Conversely, the

administration of thyme white oil and tea tree oil induced slight significant increases in the levels of urea and uric acid in rat blood at the end of experimental period. On the other hand, germaben II and exuy1 K100, the synthetic preservatives, remarkably elevated the levels of serum urea and uric acid. The data of this series of experiments suggests that germaben II and exuyl K 100 as preservatives ought to be substituted with thyme red essential oil.

Microscopical examination

Microscopical examination of liver tissues of control rats revealed the normal histological structure of hepatic lobules from central veins and hepatic cords. On the other hand, liver tissues of rats administered exuyl K 100 showed Kupffer cell activation, congestion of central veins and hepatic sinusoids and individual necrosis of hepatocytes (Fig.1).

Microscopical examination of liver tissues of rats given germaben II revealed kupffer cell activation, congestion of central veins, hepatic sinusoids as well as hepatoportal blood vessels. Multiple focal areas of hepatic necrosis associated with mononuclear cells infiltration were also observed.

Examination of liver tissues of rats administered thyme red or thyme white showed no histological changes except activation of kupffer cells. The administration of tea tree oil to rats showed no histological changes except mild hydropic degeneration of hepatocytes.

Examination of kidney tissues from rats fed on normal diet revealed no histopathological alterations. In contrast, marked histopathological changes were observed in kidney tissues of rats given euxy1 k 100 (Fig.2). These changes were described as coagulative necrosis of tubular renal epithelium and dark phyknotic nuclei, and vacuolar degeneration of the endothelial lining the glamerular tufls as well as accumulation of eosinophilic, proteinaceous material in the Bowmen's space.

The microscopical examination of kidney tissues of rats administered germben II showed necrosis of tubular renal epithelium associated with the appearance of dark pyknotic nuclei. On the other hand, no histopathological changes were observed in the kidney tissues of rats administered thyme red essential oil. Meanwhile, kidney of rats administered thyme white essential oil or tea tree essential oil showed no changes except congestion of renal blood vessels as well as the glomerular tufts.

The results of the histopathological studies indicated that the administration of synthetic preservatives (germaben II and euxyl K 100) induced harmful effects on the kidney tissues of rats. Conversely, the natural preservatives (the essential oils of thyme red, thyme white and tea tree) had little effect on liver, kidney tissues of rats. These results agreed quite well with the data of biochemical determinations relevant to liver and kidney function tests. Therefore, the use of synthetic preservatives (germaben II and euxyl K 100) as a constituent of cosmetic products has to substituted by the natural preservatives for the benefit of human health.

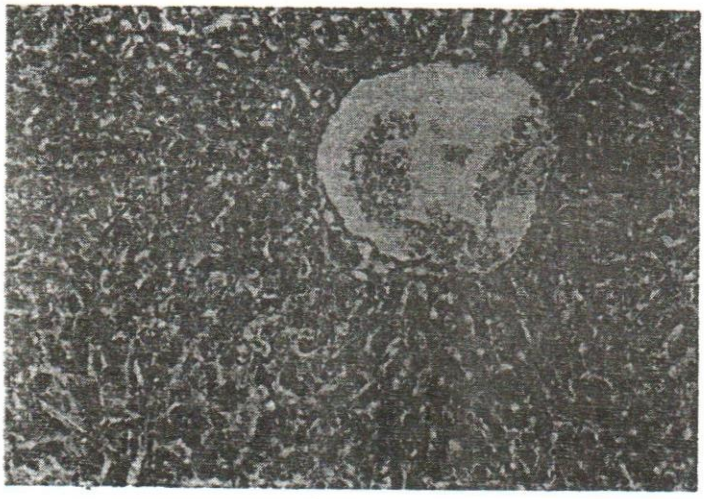

Figure (1). Microscopical examination of liver tissues of rats administered Euxyl K 100 (0.05 ml) showing individual necrosis of hepatocytes (arrows) (H & E. X 200).

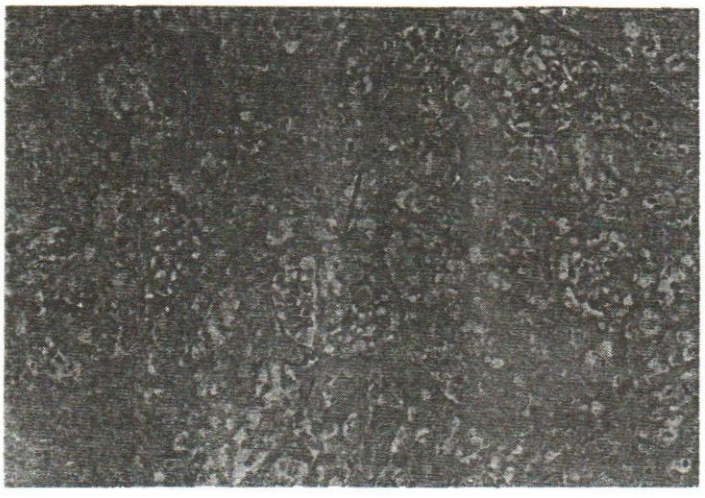

Figure (2). Microscopical examination of kidney tissues of rats administered Euxyl K100 (0.05 ml) showing coagulative necrosis of renal tubules, vacuolar degeneration of glomerular tufts as well as accumulation of proteinaceous pink material in Bowmen's space (H & E. X 200).

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تقييم مأمونية بعض المواد الحافظة الطبيعية والمخلقة المضافة إلى مستحضرات التجميل

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تهدف هذه الدراسة إلى تقييم مأمونية بعض المواد الحافظة المخلقة (جيرمابين٢ وايوكسيل ك١٠٠) وبعض المواد الحافظة الطبيعية (الزيت الطيار لكل من الزعتر الأبيض والزعتر الأحمر وأوراق الشاي) المضافة إلى مستحضرات التجميل . تم معاملة فئران التجارب بتركيزات مختلفة مقدارها ٠,٥ ، ١, ، ١,٥ مل/١٠٠ جم من وزن الحيوان وذلك بالحقن في الغشاء البروتوني. وبعد أسبوع تم تقدير نشاط بعض الإنزيمات في الأنسجة المختلفة (الكبد – القلب – الكلى) والسيرم. وكذلك قياس مستوى بعض المكونات في السيرم مثل الدهون والبروتينات الكلية والكلوسترول واليوريا وحمض اليوريك والبليروبين الكلي . كما أجريت دراسات هستوباثولوجية لأنسجة الكلى والكبد لدراسة مدى تأثرها بهذه المركبات الطبيعية والمخلقة وبتركيز (٠,٥ مل).

ودلت النتائج أن المواد الحافظة الطبيعية أحدثت تغيرات قليلة في نشاط الإنزيمات تحت الدراسة ومحتويات السيرم . وفي المقابل وجد أن المركبات المخلقة أدت إلى تغيرات ملحوظة في نشاط بعض الإنزيمات ومكونات السيرم. ولذلك توصي الدراسة باستخدام المواد الحافظة الطبيعية بدلا من المواد المخلقة في مستحضرات التجميل وذلك لمأمونيتها على صحة الإنسان .