Effects of Camphor on Enzymes, Hormones and Liver Tissues of Male White Mice

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ABSTRACT

The natural product Camphor was structurally confirmed by elemental analysis (C, H) and spectroscopic measurements (U.V, I.R and ^{1}H-NMR). Camphor effects at sublethal doses (25, 50, 100 and 200 mg/kg) was tested on Plasma alanine amino-transferase (ALT), Aspartate amino-transferase (AST), Alkaline phosphatase (ALP) and Creatine Kinase (CK) blood enzymes;blood contents (WBC, RBC, Hg and HC); hormones (Testosterone and Follicle stimulating hormones (F.S.H) in addition to histopathological studies on liver tissues in male white mice.(*Mus musculus var.albus*)

The results indicated that the higher doses (100 and 200mg/kg) of camphor highly elevated the activities of the tested enzymes where as the lower doses caused lower changes of activities, in comparison with control. The tested camphor reduced testosterone and induced FSH levels which may be caused adverse and lesion effects of male mice reproductive system. The results showed also that WBC counts in male mice increased with increasing the tested doses of camphor. Finally, all the tested doses of camphor caused harmful changes in liver tissues of the tested white male mice according to the histopathological studies, in comparison with control.

key words:Camphor, ALT, AST, ALP, WBC, RBC, Hg, Hc, Testosterone, Follicle Stimulating Hormone, Histopathological Studies on Liver, White Male Mice *(Mus musculus var.albus)*.

INTRODUCTION

Camphor is a terpenoid with the chemical formula $C_{10}H_{16}O$. It is a natural product derived from the wood present in the stem and roots of the camphor laurel (*Cinnamomum camphora L.*) trees (Compadre *et al,* 1986).

Camphor can also be produced synthetically from vinyl chloride and cyclopenta-diene passing through the intermediate dehydro norbornyl chloride (Zuccarini, 2009). Camphor is used as an aromatic material and for different purposes such as stimulation of circulatory and respiratory systems and psychological stimulation (Reynolds and Martindale; 1996, Gerald *et al,*2002).

Camphor can be used for modulating sexual activity, contraception, including abortion and reducing milk production in lactating women (Jacobziner and Raybin,1962; Liebelt and Shannon, 1993; Gibson *et al*, 1989; Blackmon and Curry,1957; and Goel *et al.*, 1985). Administration of 100 mg/kg of camphor to mice, which have been under gamma rays, has modulated spermatogenesis in their testes (Goel *et al*., 1985). Camphor derived oxidant substances have been traced in umbilical cord, blood, and fetal tissues (including brain, liver, and kidneys) and it has been shown that camphor can easily pass placental barrier and affect fetal development (Riggs *et al,* 1965).

The oral LD_{50} of camphor was found to be 1.31g /kg body weight in mice (Opdyke, 1978), whereas the sublethal dose (300 mg/Kg body weight) caused increase of hepatic cytochrome P_{450} and cytochrome b_5 levels, aryl hydroxylase activity, glutathione–s–transferase activity and levels of reduced glutathione in the liver of female swiss albino mice (Banerjee et al., 1995). The symptoms of oral camphor poisoning have been reported as blurred vision, nausea, vomiting, colitis, dizziness, delirium, contraction of heart muscles, and difficulty in breathing, seizures and death. In large quantities, it is poisonous when ingested and can cause seizures, confusion, irritability and neuromuscular hyperactivity (Manoguerra *et al*, 2006; Zuccarini, 2009, and Michiels and Mazor ,2010). On the other hand, the intraperitoneal injection of 20 mg/kg of camphor female rats caused an increase in estrogen and progesterone concentrations; in addition camphor could alter both hormonal and structural aspect of uterus that ultimately reflected on fertility of exposed animals (Al-Qudsi and Linjawi, 2012). However, several doses of camphor affected all parts of the rat male reproductive system such as testis, seminal vesicles and vas deference (Jamshidzadeh and Sajedianfard 2006).

Camphor compound was identified for elemental analysis (C,H) and structurally confirmed by (U.V, I.R and ^{1}H-NMR).

The biochemical and histopathological studies of structurally confirmed pure camphor in male mice were conducted. The effect of camphor on Alanine aminotransferase (ALT), Aspartate amino-transferase (AST), Alkaline phosphatase (ALP) and Creatine Kinase (CK) blood enzymes. In addition to the effect on the charactersity of blood contents (WBC, RBC, Hg and HC). The effect of camphor on Testosterone

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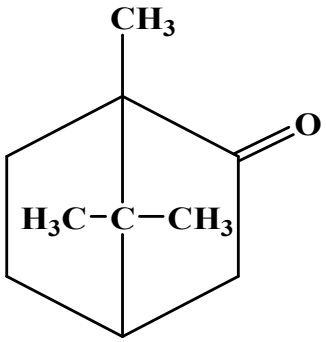
and Follicle stimulating hormones (F.S.H) was also studied. Histopathological investigation of the effect of camphor on liver organ was also conducted.

MATERIALS AND METHODS

I- Tested Compound

Camphor, M.A.R, B.D.H, laboratory Reagent, was structurally confirmed by elemental analysis (C,H) and spectroscopic measurements (U.V, I.R, and ^{1}H-NMR)

Camphor : 1,7, 7- Trimethyl bicycloheptane-2-one



II -Tested Mice and Experimental Design:

Albino mice males (*Mus musculus* var. *albus*, laboratory strain) were maintained for two weeks accommodation period before conducting the experiment. Four sublethal doses 25, 50, 100 and 200 mg/kg body weight were orally given daily in fourteen dosages consecutively to mice by gauvage tube. Four replicates were taken for each tested dose. Untreated mice males were concurrently carried out.

III- Blood and Tissue Samples:

On the day fifteen, blood samples were collected (in heparin containing tubes) from the retro orbital venous or retro orbital sinus puncture and centrifuged at 4000 rpm for 10 minutes. The obtained clear supernatant serum plasma was taken for the biochemical test. All the tested mice were sacrificed at the end of experiment to isolate liver organs for the histopathology studies.

1- Enzymes Measurements

Plasma alanine aminotransferase, ALT (sGPT) and Aspartate amino-transferase, AST (sGOT), were measured in vivo based on the methods of IFCC, 1986). Alkaline phosphatase(ALP) was determined using kits of biodiagnostics. Creatine Kinase(CK) was estimated according to IFCC, (1989).

2- Hormone assays:

Testosterone and Follicle stimulating hormone were assayed by Automated Enzyme Immunoassay system (AIA-360) called Immulite/ immolate 1000 system according to the methods described by Baird (1976), Abraham (1977) and Santner *et al*. (1981).

3- Haematological determination:

The haematological determinations were conducted according to Dacie and Lewis (1995) to determine WBC'S, RBC'S , Hg and HC.

4- Histopathological studies

Histopathological studies were carried out on liver organ in both treatments and control. The isolated liver was cleaned of extraneous material and fixed in neutral buffer (10% formalin), then dehydrated in ascending grades of alcohol, cleaned in xylene and embeded in paraffin, cut into 4 micron thickness paraffin sections and stained with hematoxylin – eosin by standard techniques. (Banchroft *et al* ,1996). The sections were examined and photographed using digital camera connected to computer. These techniques were performed at Pathology Department, Faculty of Medicine, Cairo University.

IV- Statistical Analysis.

The data were statistically analyzed according to Steel and Torrie (1980) and SAS (2007) to get L.S.D the values were recorded as mean ± S.D.

RESULTS AND DISCUSSION

A. Identification and Confirmation Studies :

The identification and confirmation of camphor were carried out for elements analysis (C,H) and U.V., I. R and ^{1}H-NMR spectrometry at Micro-analytical center, Faculty of science, University of Cairo, Giza, A.R.E.

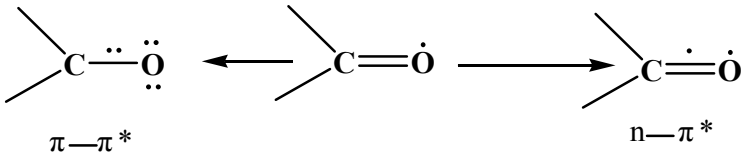
b. Confirmation of Camphor:

The elucidation of spectra (U.V, I.R and ^{1}H-NMR) measurements were conducted according to Dyke et al (1978) ; Silverstein and Bassler (1967)

a. Identification of Camphor:

Camphor was determined for melting points and elements microanalysis (C,H):

Compound	Molecular Formula	M.W	m.p	Element analysis			
				Calculated		Found	
				%C	%H	%C	%H
Camphor 1,7, 7- Trimethyl bicycloheptane- 2- one	$C_{10}H_{16}O$	152.0	179-180	78.9	10.6	77.8	9.46



Which appeared in the short wave length at 216 nm $(\pi \rightarrow \pi^*)$ and longer wave length at 267nm $(n \rightarrow \pi^*)$. Camphor is capable of existing in the enol form at 224 nm for $(\pi \rightarrow \pi^*)$

* U. V measurements indicated that the bicycloheptanone has C=O chromphore undergoes three transitions:

*** I.R measurements**

C – H stretching sharp band (2960 – 2882.09 cm^{-1}), OH (in enol form) stretching wide and (3465.46 cm^{-1}) , C=O stretching sharp band (1747.3 cm^{-1}) and = CH (in enol-form) stretching band (1744.3 – 1452.4 cm^{-1}) region.

*** ^{1}H-NMR Measurements**

The hydrogen nuclei at ortho of C=O group are magnetically deshielded at 1.293 δ (ppm); protons of methylene at C_5 and C_6 in addition to proton of methine at C_4 in a paramagnetic shift at 1.829 – 1.768 δ (ppm). The protons of methyl groups at C_1, C_7 and C_7 were dismagnetically shielded as triplet resonate spectrum at (2.052 δ) ppm.

B-Biochemical Measurements:

1-Enzymes Measurements.

The effects of camphor sublethal doses (25, 50, 100 and 200 mg/kg body weight) on Alanine aminotransferase (ALT)and Aspartate amino-transferase(AST), Alkaline phosphatase (ALP) and Creatine Kinase(CK) , enzymes are recorded in Table (1) mean ± sd and elevation (+E) or reduction (R) percents.

Camphor highly significant elevated the activity of ALT enzymes in the blood of white male mice (*Mus musculus* var. *albus*) at all the tested doses. The percentages of elevation ranged from + 40.6 to +322.0 % at 25 to 200 mg/kg body weight range.

Camphor caused also significant elevation of the activity of AST enzyme in the blood of white male mice but to less extent than ALT, the range of elevation percents were +10.2 to +44.4% elevations at the same range of doses (25–200mg/kg). So, according to Dacie and Lewis (1995) that ALT is primarily present in liver but AST is present in many tissues including the heart, skeleton, muscles, kidney and brain in addition to liver. The highest level is found in association with extensive hepatic necrosis, toxin induced liver injury, whereas lesser elevations are encountered in diffuse and local chronic liver diseases.

The activity of alkaline phosphatase enzyme (ALP) was also highly elevated with significant differences by the tested doses of camphor, 25,50, 100 and 200mg/kg in the blood of white mice with range of elevation percents between +30.5 – 150.3% for the lowest and highest tested doses, respectively. The highest levels of ALP may be associated with bone diseases (Young,2000), which indicated that camphor may be caused some bone diseases at the tested sublethal doses against the males of mice (*Mus musculus* var. *albus*).

Creatine kinase (CK) enzyme activity was found to be reduced by 22.7% reduction by 25mg/kg treatment but afterward it was elevated by +29.5, +89.2 and +108.3% elevation in the blood of male mice at 50, 100 and 200 mg/kg body weight respectively. Thus, an elevation of CK level caused by camphor treatments may be associated to mycocardial infraction or diseases of skeletal muscles (Young,2000). With these findings it can concluded that camphor at the tested doses (25, 50, 100 and 200 mg/kg) may harmfully affected several organs in relation to ALT, AST, ALP and CK of white mice male (*Mus musculus* var. *albus*).

2-Effect on Hormones:

The effects of Camphor sublethal doses (25, 50, 100 and 200mg/kg) on testosterone and follicle stimulating hormone (F.S.H) hormones in serum blood of white male mice
(*Mus musculus* var. *albus*) are showed in Table (2).

Camphor caused fluctuated reduction in the levels of testosterone at the tested doses as compared with control with highly significant differences. The reduction percents of testosterone were 73.4, 47.1, 49.5 and 76.8% at the doses 25,50,100 and 200 mg/kg body weight, respectively.

On the other hand, the level of F.S.H was induced by camphor treatments is non-significant differences and the percentages of elevation of F.S.H levels were +32.0, +59.1, +22.6 and +7.0% relatively in descending order with increasing the tested doses, respectively. These results indicated that the administration of the sublethal doses of camphor reduced testosterone secretion and induced F.S.H level in male mice, which may be causing adverse and lesion influences of mice male reproductive system such as testis, seminal and spermatocytes.

Table 1. Effect of Camphor on Some Blood Enzymes in Serum Blood of White Male Mice *(Mus musculus var.albus)*

	Doses mg/kg									
	0	25		50		100		200		
Enzymes	Mean ± S.D	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	$LSD_{0.05}$
ALT μ / L	45.47 ± 2.53	63.9 ± 1.27	+40.57	66.51 ± 3.32	+46.27	141.8 ± 1.44	+211.91	191.9 ± 4.60	+321.99	1.8047
AST μ / L	66.37 ± 2.85	73.16 ± 0.62	+10.22	74.79 ± 2.71	+12.67	81.59 ± 1.45	+22.91	95.8 ± 1.44	+44.42	1.0765
ALP μ / L	257.02 ± 11.57	335.35 ± 3.88	+30.47	356.65 ±4.80	+38.76	600.47 ± 6.47	+133.62	643.2 ± 4.13	+150.26	2.8116
Creatine Kinase μ / L	2039.27 ± 183.9	1575.97 ±327	22.71	2641.22 ±281.4	+29.51	3858 ± 18.42	+89.18	4248.65 ±274	+108.34	5269.4

Shown as Mean ± S.D ; % Reduction (%R) or % Elevation (+ value) (% E) :
The reduction or Elevation percent was determined according to Kaukinen (1979).
As follows: % Reduction or % Elevation = 100 – (Treatment / Control × 100)
Shown as % Reduction (%R) or % Elevation (+ value) (% E).

Table 2. Effect of Camphor on Some Hormones in Serum Blood of White Male Mice *(Mus musculus var.albus)* shown as Mean ± S.D ; % Reduction (%R) or % Elevation (+ value) (% E)

Hormones	Doses mg/kg									
	0	25		50		100		200		
	Mean ± S.D	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	$LSD_{0.05}$
Testosterone ng/ml	5.48 ± 0.64	1.46 ± 0.11	73.4	2.90 ± 0.40	47.1	2.77 ± 0.35	49.5	1.27 ± 0.35	76.8	0.5585
F.S.H MIU/ml	0.767 ± 0.11	1.02 ± 0.30	+33	1.22 ± 0.057	+59.1	0.94 ± 0.04	+22.6	0.82 ± 0.55	+7	0.4845

Table 3. Effect of Camphor on Blood Components of White Male Mice *(Mus musculus var.albus)* shown as Mean ± S.D ; % Reduction (%R) or % Elevation (+ value) (% E)

Blood Components	Doses mg/kg									
	0	25		50		100		200		
	Mean ± S.D	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	Mean ± S.D	% Elevation & Reduction	$LSD_{0.05}$
W.B.C 10^3Cell/µL	2.92 ± 0.26	7.15 ± 0.24	+144.90	3.12 ± 0.25	+6.85	7.70 ± 0.18	+163.7	5.325 ± 0.05	+82.5	0.0961
R.B.C 10^6Cell/µL	7.28 ± 0.29	8.06 ± 0.22	+10.7	5.80 ± 0.08	20.6	7.98 ± 0.15	+9.60	8.05 ± 0.09	+10.6	0.1598
Hgb g/dL	10.95 ± 0.31	13.15 ± 0.36	+20.1	8.37 ± 0.12	23.6	11.2 ± 0.23	+2.8	12.65 ± 0.43	+15.52	0.1374
HCT %	35.82 ± 1.22	41.52 ± 0.57	+15.91	27.25 ± 0.20	23.93	34.70 ± 0.08	3.1	36.67 ± 0.20	+2.5	0.2009

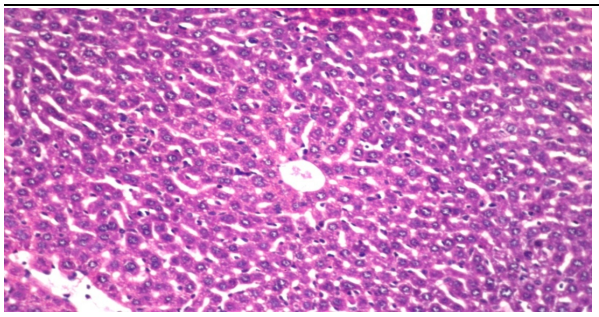


Fig. 1. liver of mice in control :Showing normal histologicalstructure of central vein and surrounding hepatocytes in hepatic Parenchyma H&E,× 40

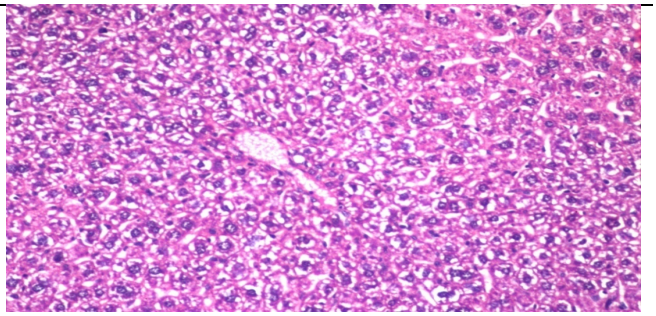


Fig. 2. liver of mice treated by camphor dose 25 mg/kg Showing intracytoplosmic vacuolar degeneration in the hepatocytes. H&E,× 40

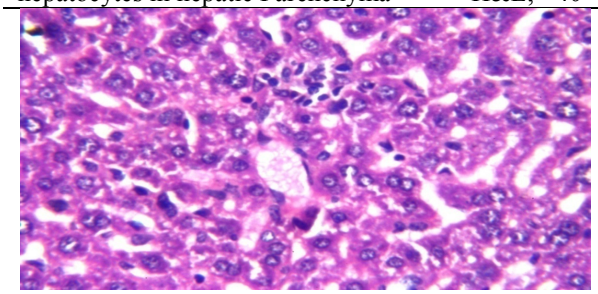


Fig. 3. liver of mice treated by camphor dose 25 mg/kg Showing diffuse kupffer cells proliferation in between the hepatocytes. H&E,× 40

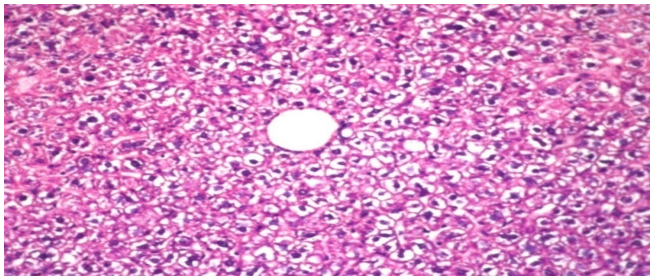


Fig. 4.liver of mice treated by camphor dose 50 mg/kg Showing intracytoplasmic vacuolar degeneration. H&E,× 40

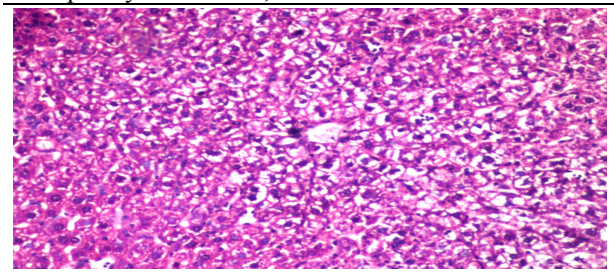


Fig. 5.liver of mice treated by camphor dose 100 mg/kg Showing vacuolor degeneration in hepatocytes. H&E,× 40

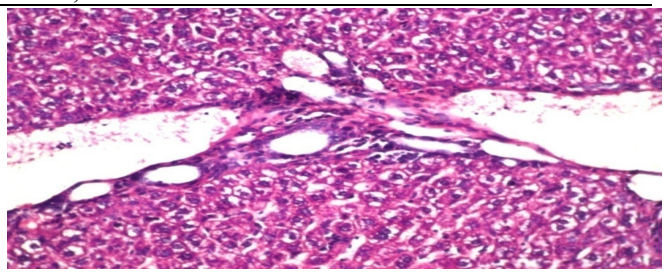


Fig. 6. liver of mice treated by camphor dose 100 mg/kg Showing sever dilation of portal veins with multiple newly formed bile duct and periductal few inflammatory cells infiltration in portal area. H&E,× 40

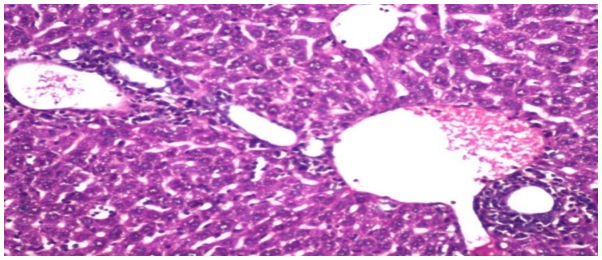


Fig. 7. Liver of mice treated by camphor dose 200 mg/kg Showing dilatation of portal vein with periductal inflammatory cells infiltration surrounding the bile duct in portal area. H&E,× 40

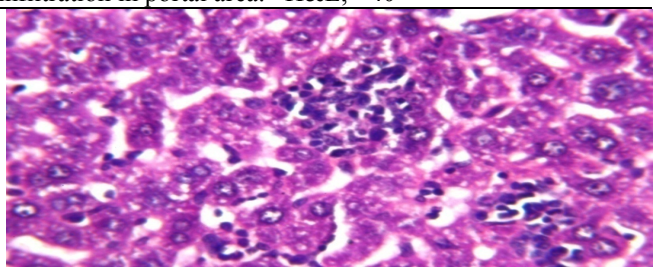


Fig. 8. Liver of mice treated by camphor dose 200 mg/kg Showing focal necrosis with inflammatory cells infiltration in hepatic Parenchyma in and diffuse kupffer cells proliferation in between the hepatocytes. H&E,× 40

3- Haematological Effects:

Camphor highly significant elevated the counts of white blood cells (WBC) in the blood of white male mice (*Mus musculus* var. *albus*) at the tested doses 25,50,100 and 200 mg/kg body weight. So, WBC counts were fluctually elevated with +6.85 – +163.7% range for the tested doses, in comparison with control (Table 3). On the other hand Red blood cells (RBC), haemoglobin (Hg) and haematocrite in blood of white male mice were significantly affected by the tested doses of camphor with fluctuated range between weak elevation or reduction (Table 3).

4- Histopathological Effects on liver:

There was no histopathological alteration and the normal histological structure of the central veins and surrounding hepatocytes in parenchyma were recorded in group of white male mice as control (Fig1). It was detected intracytoplasmic vaculor degeneration in the hepatocytes in mice treated with 25 or 50 mg/kg of camphor (Fig 2 and 4).

The dose 25 mg/kg of camphor caused diffuse kupffer cells proliferation in between the hepatocytes in white male mice (Fig 3). The histopatholgical studies of white male mice treated with 100mg/kg of camphor on liver showed vacuolor degeneration in the hepatocytes (Fig 5), associated with severe dilatation in the portal veins as well as multiple newly formed bile ductless with periductal inflammatory cells infilitration in the portal area(Fig 6); the treatment 200mg/kg of camphor in liver showed dilatation in the portal vein associated with periductal inflammatory cells infiltration Surrounding the bile ducts (Fig7), in addition to focal necrosis with inflammatory cells infiltration were detected in the hepatic parenchyma and there was diffuse kupffer cells proliferation in between the hepatocyte (Fig8) .

It could be concluded that camphor at the sublethal tested doses (25,50,100 and 200mg/kg), highly elevated the activities of the tested enzymes ALT, ALP, CK and AST of blood of male mice in descending order respectively specially at the higher doses (100 and 200 mg/kg) in addition to lower changes in the activities at the lower tested doses (25 and 50mg/kg). These results may be powerfully affected several organs in relation to of those tested enzymes of white mice males (*Mus musculus* var. *albus*). The reduction of testosterone secretion and induction of F.S.H levels by the sub lethal doses of camphor may be caused adverse and lesion effects of male mice reproductive system such as testis, seminal and spermatocytes. White blood cells count in male mice increased with increasing the tested doses. All the tested doses of camphor caused harmful changes in liver tissues of the tested white male mice according to the histopathological studies in comparison with control.

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الملخص العربي

أثار الكافور علي الإنزيمات والهرمونات وأنسجة الكبد علي ذكور الفئران البيضاء

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تم استخدام مركب الكافور كمنتج طبيعي وتم تحليل عناصر مركب الكافور مثل الكربون و الهيدروجين و التأكد من التركيب الكيماوي بواسطة القياسات الطيفية مثل طيف الاشعة فوق البنفسجية (U.V)،طيف الاشعة تحت الحمراء(I.R) وطيف الرنين المغناطيسي للبروتونات (^{1}H-N.M.R) وتهدف الدراسة الحالية إلى معرفة آثار الكافور على الانزيمات مثل انزيم الانين تراتس امينيز(ALT)، اسبرتات ترانس امينيز(AST)، انزيم الفوسفاتيز القلوي(ALP) وانزيم الكرياتين كينيز(CK) والهرمونات الجنسية مثل هرمون التستوستيرون والF.S.H ومكونات الدم مثل الهيموجلوبين، الهيماتوكريت، عدد كرات الدم البيضاء والحمراء بالاضافه إلى الدراسات التشريحية علي انسجه الكبد في الفئران البيضاء الذكور (*Mus musculus var .albus*).

وتم استخدام ٤ جرعات من الكافور هي ٥٠،٢٥، ١٠٠ و٢٠٠ مغ/كغ لمدة ١٤ يوم و في اليوم الخامس عشر تم ذبح الفئران و اخذ عينات الدم و الكبد لأجراء الفحوصات المختلفة وأشارت النتائج إلى ان الجرعات العالية (١٠٠ و٢٠٠ مغ/كغ) من الكافور احدثت ارتفاع في أنشطه الانزيمات المختبرة حيث تسببت الجرعات المنخفضة في انخفاض نشاط الانزيمات، بالمقارنة مع المجموعة الظابطة. و قد ادت الجرعات المختبرة الي خفض الهرمونات الجنسية لذكور الفئران التي يمكن ان تسبب اثار ضاره و مختلفة لخصوبة الفئران الذكور. وأظهرت النتائج أيضا ان كرات الدم البيضاء زادت مع زيادة الجرعات المختبرة من الكافور. وأخيرا، فان جميع جرعات الكافور المختبرة تسببت في تغيرات ضاره في انسجه الكبد للفئران البيضاء الذكور المختبرة وفقا للدراسات التشريحية، بالمقارنة مع المجموعة الظابطة.