Egypt J. Chem. Vol. 65, No. SI:13, pp. 1301-1312 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Toxicological effect of camphor oil nanoemulsion on cotton leafworm and its safety evaluation on Swiss albino mice.

Heba S. Rafea [a] , Safwat H. Ali [b] , Tarek A. El-Shiekh [a], Dalia A. Youssef [c], Heba M. Amer [d*] and Abd- Elmoneem M. El- Aasar [b]

a Department of pest physiology, plant protection research institute, Agricultural research center , Dokki, Giza, Egypt..

b Department of Biochemistry, Faculty of Agriculture, Ain Shams University, Egypt.

c Department of Pests and Plant Protection, National Research Centre, 12622, Dokki, Cairo, Egypt.

d* Department of Medicinal and Aromatic Plants Research, National Research Centre, 12622, Dokki, Cairo, Egypt.

Abstract

In the current study camphor oil was investigated at the form of bulk and nanoemulsion to evaluate protective effect against cotton leafworm and their toxicity on and Swiss albino mice. Camphor oil nanoparticles (CamONPs) were prepared and characterized using GC-MS, TEM and FTIR techniques. Results showed that the main component of both bulk and nanoemulsion was eucalyptol. The particle size of CamONPs ranged between 18-36 nm. Fourier Transforms Infrared revealed distinct peaks at wavenumber 3350 and 1640 cm^{-1} where those peaks were disappeared in the bulk form. LC_{50} value of CamONPs on *Spodoperta littoralis* larvae was 1664 ppm, while of bulk emulsion was 20232 ppm. LD_{50} values of CamONPs and its bulk form on mice recorded 1.12 and 1.52g/kg b.w., respectively. Mice treated with LD_{10} of bulk camphor showed increase in white blood cells counts which slightly decreased in mice treated with CamONPs. Red blood corpusles counts, Haemoglobin level and Hematocrit % were significantly changed in CamONPs treatment comparing to bulk form. Both treatments altered the mean values of corpuscular volume, corpuscular hemoglobin, corpuscular hemoglobin concentration and platelet counts comparing to untreated mice. Significant changes were noticed in liver tranceaminases, urea and creatinine. CamONPs revealed significant decrease against tumor markers carcino-embryonic antigen and α-Fetoprotein in comparison to its bulk form and both treatments showed significant changes comparing to control group.

Keywords: Toxicity, Camphor Nanoemulsion, bulk camphor, Spodoptera littoralis.

1. Introduction

Cotton leafworm, *Spodoperta littoralis* (Boisd.) is a lepidopterous insect pest that affects a wide range of field crops. *S. littoralis*, the Egyptian cotton leafworm, is a polyphagous pest of many crop plants (e.g. soybeans, strawberry, lettuce, cotton, eggplant, pepper, tomato, alfalfa) that is widely cultivated in the Middle East, Mediterranean and African regions. *S. littoralis* larvae mostly feed on leaves and stems, causing growth to be stunted and crop yields to be reduced [1].

Several methods and strategies utilizing traditional and chemical synthetic pesticides were and are still followed. However, these insecticides have several health and environmental hazards. In this concern, green insecticides offer unique mechanisms of action as a way to lessen or reduce these hazardous effects. This also may allow effective control of pests which have already evolved resistance to traditional insecticides.

*Corresponding author e-mail: dr.heba_nrc@yahoo.com.; (Heba M. Amer).

Receive Date: 21 May 2022, Revise Date: 29 May 2022, Accept Date: 31 May 2022, First Publish Date: 31 May 2022

DOI: 10.21608/EJCHEM.2022.139037.6140

In crop production and Integrated Pest Management (IPM), biopesticides based on plant extracts are promising alternative strategy techniques. The main advantage of these bioinsecticides is being cost-effective tools to the small holders in developing countries, since they can simply prepare it from the available plant resources [2].

Camphor (*Eucalyptus globules*) is a unique source for one of the most bioactive essential oil (CEO). Generally, CEO is extracted from leaves and contains several bioactive compounds such as phenolics and terpenoids. These secondary metabolites are formed by plants to protect themselves against insects, bacteria, fungi and virus infections using different mode of actions [3 and 4].

Camphor essential oil exhibits wide range of biological activities such as insecticidal, antimicrobial, antiviral, anticancer, anticoccidial, and antinociceptive activities [5]. It has great commercial value to the cosmetic industry and also used to enhance skin penetration enhancer [5]. Furthermore, the CEOs obtained from leaves of *Cinnamomum camphora* (L.) were found to be repellent insecticides against *Sitophilus oryzae* L. [6]. *C. camphora*, rich in camphor, grows natively in Asian countries [5].

On the other hand, phytochemicals as essential oils and secondary metabolites face stability and cost effectiveness problems. Moisture, air, light, and high temperatures sensitivities, which induce rapid evaporation and degradation of some bioactive compounds, are serious concerns in the case of essential oils [7].

To overcome the drawbacks of traditional botanical extracts, Green nanotechnology is an effective technique, which has recently been employed to increase the pesticidal effects of botanical pesticides and improve their pesticidal properties. It has become a vital research field in all areas. Since, modification of size, orientation and physical qualities of the produced nano-particles can increase the performance of any matter [8].

Synthesis of nanoformulations using green processes enhances and controls the releasing properties. Also it increases the stability properties of bioactive components and minimizes the effective dosages due to coating process [7]. The prepared nanoformulations are expected to be more effective compared to their respective bulk substances [9 and 10]. Moreover, pesticide nanoformulations were found to have higher specificity towards the target organisms compared to bulk or commercial formulations, reflecting lower toxicity [11].

Therefore, the current study deals with nano-particles in the form of nanoemulsion prepared from camphor essential oil carrying on Na- alginate to study its insecticidal and biological activity on both cotton leafworm, , as an insect model and mice, as a mammalian model, compared to its bulk form.

2. Materials and methods

2.1. Tested Spodoptera littoralis

The 2nd instar *S. littoralis* larvae were used in the present investigation. A stock culture of the cotton leafworm, *S. Littoralis* was obtained from a laboratory strain maintained in the Agricultural Genetic Engineering Research Institute, Agriculture Research Center, Giza, for several generations without any insecticidal and/or microbial pressure. *S. littoralis* was reared on castor-oil leaves, *Ricinus communis*, under laboratory conditions at 25±2°C and 60±5% R.H. for several generations.

2.2. Tested camphor essential oil

Essential oil of camphor was extracted from 100 g of the fresh leavesare collected from Orman botanical garden was subjected to hydro distillation for 3h.The obtained essential oil was dried over anhydrous sodium sulphate and kept in deep freezer till used for GC-Mass analyses, the essential oil of sample was performed.

2.3. Preparation of Loaded Nano-emulsion

Alginate nanocapsulesof camphor essential oil wasperformed using the method used by [12], (a the modified version of the procedures reported by [13]), alginate nanocapsuleswere made by emulsifying oil in water (o/w) and then crosslinking with calcium chloride. Sodium alginate solution (3 % w/v) was prepared usingdistilled water at 50˚C for 45 min. Tested camphor essential oil was diluted by distilled water using Tween 80 as an emulsifier with mechanical stirring for 10 min. Briefly, sodium alginate o/w emulsion was made by drop wise dispersion of diluted oil into alginate solutionin the ratio of1:2 (w/w) oil to alginate) under continuous mechanical stirring at room temperature.The formed emulsion was then sonicated for 30 min using an ultrasonic cleaning set (model WUC-DO3H 290 W, 60 Hz) and then for 2 min with a high-energy ultrasonication probe (model VCX 750, 750 W, 20 kHz). An adequate proportion of CaCl2 (2:10, w/w CaCl2 to alginate, respectively) was then added into the resultant emulsion and stirred for another 30 min and sonicated as described previous. Nano-capsules were obtained finally, as adispersion in aqueous solution.

2.4. Characterization of nano-formulations

Different methods of characterization were used to measure the morphological shape, size, uniformity content, and chemical interactions of the produced nano-formulations and/or nano-particles to ensure that the prepared camphor oil formulation was converted into nano-sized particles as follow:

2.4.1. Transmission Electron Microscopy (TEM)

Transmission Electron Microscope (Jeol, JEM-2100, USA) was used to examine the morphological shape and size of the prepared nano-formulation. The nano-capsule suspensions was diluted with distilled water and placed onto a carbon-coated copper grid and stained with a1%phosphotungstic acid then examined by magnification (20000X) and photographed [14].

2.4.2. Fourier Transforms Infrared (FTIR)

The chemical interactions between camphor oil nano-emulsion and Na-Alg or the cross linking agent CaCl2 were investigated using FTIR. This was accomplished by extracting the following samples from the camphor oil that had been tested: pure bulk oil and loaded Nano-emulsion. The FTIR measurements were performed using FTIR 6600, JASCO according to the method described by Jerobin *et al*. [15].

2.5. Analysis of chemical constituents of camphor oil by GC-MS:

Gas chromatography/mass spectrometry (GC/MS) Gas liquid chromatographic analysis of essential oil constituents: The identification of the components of essential oil constituents was carried out using gas liquid chromatography on a Hewlett Packard Model 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped under the following conditions:

(a) Separation was done on an INNO wax polyethylene glycol, Model No. 19095 N123, 240°C maximum, capillary column 30 m x 250 µm x 0.25 µm, nominal flow 1 mL/min, with average velocity 36.445 cm/sec and pressure 7.6522 psi. Column temperature was 350 °C with temperature programming as follows: initial temperature 50 °C maximum, with 10°C rising for each minute, and then hold at 240°C for 10 min.

(b) Injection temperature 280°C, back inlet, with split ratio 8:1, split flow 120 ml/min., and gas saver 20 ml/min.

(c) Carrier gas was helium with flow rate 1 ml/min.

(d) Flame ionization detector temperature 280°C.

(e) Hydrogen flow rate 30 ml/min.

(f) Air flow rate 300 ml/min.

2.6. Toxicity tests:

2.6.1. Bioassay tests on S. littoralis:

Bioassays of camphor essential oil emulsion and its loaded nanoform on 2nd instar larvae of *S. littoralis* were carried out. Larvae were fed on a semi-synthetic diet as described in Shorey and Hale [16]. The diet was prepared using 500 g kidney beans, 30 g agar, 65g yeast, 3g sorbic acid, 5 g benzoic acid, 10ml formalin, and 10 g ascorbic acid. The kidney beans and agar were individually autoclaved in 600ml of distilled water and were then ground with the other components, except ascorbic acid, which incorporated with the prepared media after it had reached to warmed temperature.

Series of concentrations of both camphor oil bulk emulsion (1:2, oil:water) and its loaded nano form were used to calculate the LC_{50} values. All concentrations were prepared according to the active ingredient content in each emulsion per the semi-synthetic diet. Concentrations of 519, 1037, 2075, 2603, 4150 and 6516 ppm of camphor loaded nanoemulsion were used while concentrations of 13650, 18200 and 22750 ppm were used in case of camphor oil bulk emulsion. All concentrations of both two emulsions were incorporated into 100 g of the semi-synthetic diet immediately before gelling in order to avoid decomposition. Media treated with distilled water and a 100 µl of Tween 80 was used as control.

The selected larvae were tested using four replicates per concentration, for both camphor bulk oil emulsion and its loaded nano form, with ten larvae in each replicate. Each replicate was housed in a glass tube and was fed 1 g of the treated diet. The larvae were incubated at 25 ± 2 °C and 65-70% RH. Larval mortality was recorded daily for 5 days after treatment and was compared with the control larvae. The mortality percentage was corrected using Abbott's formula [17].

The logarithmic relationship between the oil concentrations and larvae mortality were plotted and the LC_{50} values were calculated using Ld-p line program according toFinney [18]. Concentrations mortality regression lines were plotted in form of log/probity relation and the LC_{50} values were calculated using Ld-p line program according to [18]. The equation ofSun [19] used to determine LC_{50} index as follow:

$$\text{Toxicity Index for } LC_{50} = \frac{\text{LC50 of the most effective compound}}{\text{LC50 of the least effective compound}} \times 100$$

2.6.2. Toxicity tests on mice

Acute oral toxicity:

The acute oral toxicity study of both nano and bulk camphor formulations were performed according to OECD NO (401) while LD_{50} values were determined after 24 h. using the formula of Weil [20].

Experemintal design:

Fifteen mice were allocated into three groups (5 mice/each) where Groupe 1; served as control group and intubated with distilled water, group 2; mice were orally treated with one fifth (1/5) of LD_{50} bulk camphor oil and group 3; mice were orally treated with one fifth (1/5) of LD_{50} of nanoemulsion camphor oil. All mice groups were treated for 7 days, then blood samples were collected from eye retro plexes (using capillary tubes) in centrifuge tubes contain EDTA for haematological analysis and plasma was used to measure liver, kidney functions and tumormarkars.

2.7. Determination of hematological parametrs

Red Blood Corpuscles (RBCs), White Blood Cells (WBCs) and haemoglobin were determined in fresh blood samples by counter, SYSMEX; KX-21N.

2.8. Biochemical analysis:

2.8.1. Liver transaminases (ALT & AST)

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were performed as colorimetric procedure reported by [21] using DiaSyskits; Diagnostic system

2.8.2. Kidney functions (urea & creatinine)

The biochemical parameters urea and creatinine were determined according to [22 and 23], respectively, using kits of LINEAR (LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2a planta. 08390 Montgat (Barcelona) SPAIN) in case of urea determination. However, in case of creatinine, kits of Biomed Diagnostic purchased from EgyChem for Lab technology was used.

2.8.3. Tumor markers (AFP &CEA)

Tumor markers alpha –fetoprotein (AFP) and carcino embryonic antigen (CEA) were detected as described by [24] and [25], respectively, using kits of ParkinElmer (ParkinElmer health sciences, Inc. US10 Division).

2.9. Statistical Analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) using Duncan multiple test in SAS. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Characterization of camphor loaded nanoemulsion

3.1.1. Electron microscopy examination

Morphological shapes and size of the prepared camphor lodednanoemulsion, nano sample was examined by TEM Figure (1). Camphor loaded nanoemulsion show spherical shape with smooth surface as shown by the TEM Figure (1). The particle sizes were ranged from 18 nm (image, a) 36 nm (image, b).

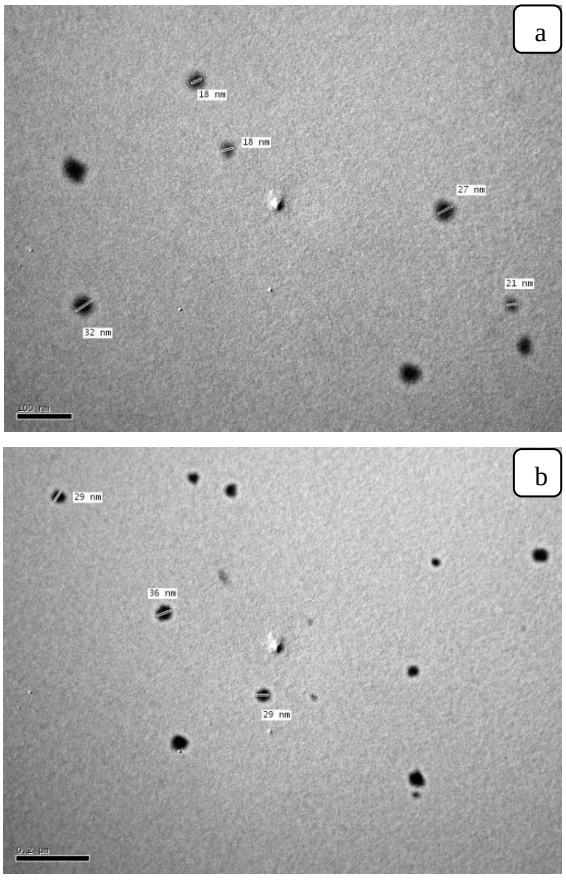

Fig.(1): TEM (a&b) of camphor loaded nanoemulsion particles with size 18-36nm

3.1.2. GC -Ms analysis

The chemical composition of camphor oil nanoemulsion and its bulk form was estimated and

compared by GC/MS as shown in Table (1) and illustrated in Fig. (2). Using GC-MS, a total of seven compounds, which represent 100% of total content of bulk camphor (*Eucalyptus globulus*) oil were identified (a), whereas total of nine compounds representing 99.99 % of nanoemulsion volatile composition were detected (b). The predominant compounds in oil of both bulk and nanoemulsion were Eucalyptol (1,8-Cineole) representing 64.58 & 73.77 %, respectively followed by p-Cymene (17.53 & 8.19 %, respectively), γ-Terpinene (7.28 & 3.94%, respectively), Carvacrol(4.95 & 1.93%, respectively) and α-Pinene (3.18 & 1.29%, respectively).

These results are similar to those obtained by [26] camphor (*Eucalyptus globulus*) EO. They showed that the major chemical compounds of CEO were 1,8-Cineole (87.78%), β-cymene (7.77%), D-limonene (2.29%), α-pinene (1.04%) and α-terpineol (0.10%). Several researchers analyzed and identified the chemical compositions of EO obtained from Eucalyptus genus by GC/MS. Maciel *et al*. [27] analyzed the chemical profiles of three essential oils obtained from three Eucalyptus species. They found that 1,8-Cineole dominated the profiles of the tested EOs (83.89%), in addition (+) limonene, o-cymene and α-pinene represented 8.16, 2.93 and 4.15%, respectively. Moreover, Ghaffa *et al*. [28] studied the chemical profiles of EOs obtained from seven Eucalyptus plant species cultivated in Pakistan. 1,8-Cineole was the predominant compound in the tested *E. Globules* EOs representing lower value (56.5%), while limonene represented higher value (28.0%). α-pinene (4.2%), α-terpinol (4.0%) and globulol (2.4%) were also detected. Our results are in parallel with those early reported by Mossa *et al*. [26]. They attributed the variations in chemical constituents to the geographical, climatic and environmental conditions, which affect the chemical profiles of the EOs of *E. globules* cultivated in different locations.

Table 1: Volatile constituentsidentifed from bulk and nanoemulsion of *camphor (E. glopulus)* essential oil using GC-MS.

No	Component Name	RT	Camphor crude oil	Alginate loaded Camphor
1	α-Pinene	6.416	3.18	1.29
2	β-Myrcene	7.789	0.86	n.d.
3	α-Phellandrene	8.173	1.62	n.d.
4	p-Cymene	8.774	17.53	8.19
5	D-Limonene	8.831	n.d	8.39
6	Eucalyptol	9.014	64.58	73.77
7	γ-Terpinene	9.649	7.28	3.94
8	Carvacrol	16.31	4.95	1.93
9	Dibutyl phthalate	31.404	n.d.	0.76
10	Oleyl amide	36.817	n.d.	0.89
11	Erucylamide	40.142	n.d.	0.83
Total Identified			100	99.99

*n.d.: not detected.

Moreover, Ali *et al.* [29] found that volatiles composition of the Algerian *Origanum glandulosum* Desf oil nanoemulsion was different from that of the hydro-distilled oil. In addition, Donsi *et al*. [30] found that both high pressure and high shear homogenizations decomposed the active components of volatile oils particularly terpinenes, p-cymene, carvacrol, carveol and other compounds. Noteworthy, they found a significant decrease in carvacrol concentrations as the intensity of high shear homogenization increased to high pressure homogenization. This may interpret the decreased amount of α-pinene, p-cymene and γ-terpinene found in nanoemulsion compared to bulk oil in the current study which may be as a result of steering and/or ultrasonication during nanoemulsion formation. The same authors observed significant quantitative difference in montoterpenes level between *O. Glandulosum* essential oil obtained by hydro-distillation and their prepared nanocapsules. Furthermore, they attributed the absence of major sesqueterpenes in the encapsulated oil extract to the homogenization process in processing technique.

3.1.3. FTIR analysis of camphor nano-emulsion and camphor bulk emulsion

FTIR data are presented in Fig. (2) and Table (2) for camphor (*E. globulus*) bulk oil (spectrum a) and its loaded nanoemulsion (spectrum b). The FTIR spectra represent the signal peaks of the main compounds in EO. 1,8-cineol, the main responsible for the insecticidal properties of camphor (*E. globulus*) EO, were observed. Signal peaks of this bicyclocamphane compound are shown as follow: a strong absorption at 984.15 and 984.13 cm^{-1} for

camphor bulk oil and its loaded nanoemulsion (Fig. 2 a&b, respectively) allocated to symmetrical bending out of the CH_2 plane. Other peaks were observed for both camphor bulk oil and its loaded nanoemulsion at 1214 and 1079 cm^{-1} are attributed to asymmetric and symmetric stretches of C–O–C group, respectively. Other characteristic peaks for both camphor bulk oil and its loaded nanoemulsion are discerned at 1374 cm^{-1}, corresponding to CH_3 deformation.

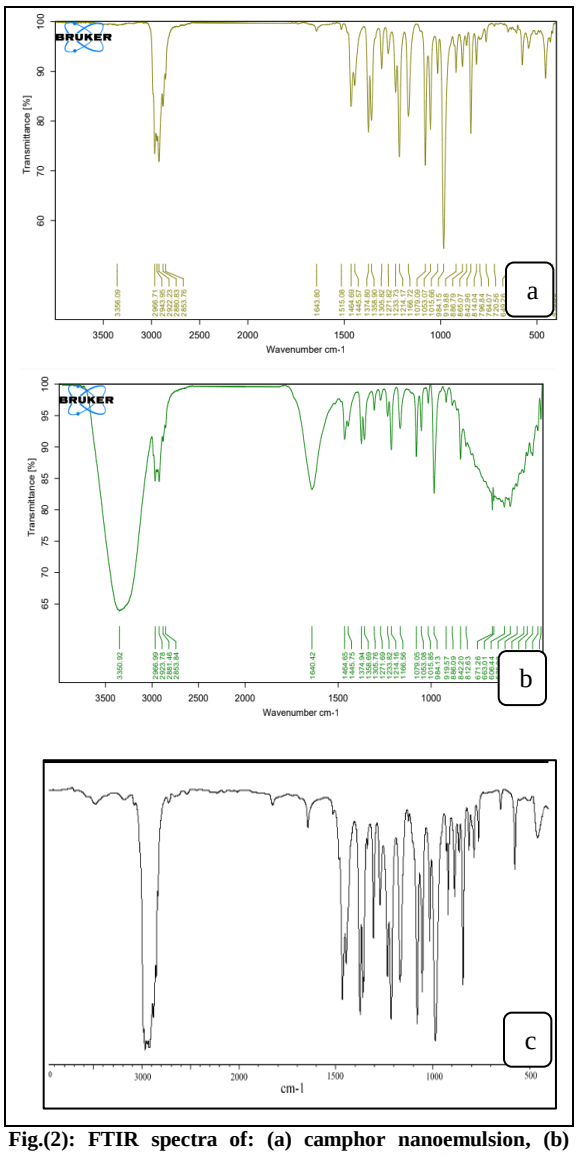

Fig.(2): FTIR spectra of: (a) camphor nanoemulsion, (b) camphor bulk emulsion and (c) 1,8 cineole from spectral database for organic compounds.

The obtained results are the same as reported by Garcia *et al.* [31], who used FT-IR spectroscopy technique to analyze the essential oil of eucalyptus. 1,8-cineol was found to be the major compound in eucalyptus oil. Generally, signal bands in the EO of *Eucalyptus globulus* are attributed to the groups and bonds of all compounds, such as methylene and methyl groups and double bonds, as documented by Garcia *et al.* [31]. Peaks at 2943 cm^{-1} in bulk oil spectrum, and 2966 cm^{-1} in both bulk oil and loaded nanoemulsion spectra can be attributed to CH_3 symmetric and asymmetric stretches.

Table 2: Summarizing of major chemical groups FTIR spectra of Camphor (*E.globulus*) bulk oiland its loaded nanoemulsion

Chemical group	Camphor bulk oil (wave No.cm^{-1})	Camphor nanoemulsion (wave No.cm^{-1})
CH2 symmetrical bending	984.15	984.13
C–O–C symmetric stretches	1079.09	1079.05
C–O–C asymmetric stretches	1214.17	1214.16
CH3 deformation	1374.8	1374.94
C–H symmetrical bending	1445.57	1445.75
C–H asymmetrical bending	1464.69	1464.65
C=O group	-	1640.42
C=C stretches vibrations	1643.80	-
CH2 asymmetric stretch	2922.23	2923.78
CH3 asymmetric stretch	2966.71	2966.99
CH3 symmetric stretch	2943.95	-
- OH group	-	3350.92

Other peaks at 2922 and 2923 cm^{-1} were observed in bulk oil and its loaded nanoemulsion respectively corresponds to CH_2 asymmetric stretch. In addition, an absorption was observed at 1640 cm^{-1} in loaded nanoemulsion spectrum corresponding to carbonyl group (C=O) which may be a result of C=O found in Na-alginate used in loading nano camphor emulsion. Also an absorption at 1643 cm^{-1} in bulk oil spectrum, can be allocated to stretches vibrations in C=C bonds. Peaks at 1464 and 1445 cm^{-1} detected in both bulk oil and its loaded nanoemulsion spectra are associated with symmetrical bending in the plane of C–H bonds. These results were similar to those obtained by *Garcia* et al. [31], who observed peaks at 2957, 2870, 2923, 1642 and 1456 cm^{-1} in FT-IR spectrum of *Eucalyptus globules* essential oil.

Also FTIR show that the functional groups were more appeared in camphor nanoemulsion (spectrum a) than that of its bulk form (spectrum b), where hydroxyl functional group (-OH) was strongly appeared in camphor nanoform (a) as shown at wave number 3350 cm^{-1}, but it was absent in its bulk form, this may be due to OH group found in Na alginate used in loading camphor nanoemulsion. The intensity of peaks regarding frequency has a high coincidence with the FTIR spectrum of Eucalyptol (1,8-cineol) as shown in Fig (2c). This coincidence was obtained from the database denominated Spectral Database for organic compounds, SDBS No. 2002

3.2. Insecticidal efficiency of camphor oil bulk emulsion and its nano form against 2nd instar larvae of S. littoralis

Data present in Table (3) show the toxicity efficiency of camphor oil bulk emulsion and its nano form to *S. littoralis* as evaluated by toxicity parameters (LC_{50}, LC_{90} and toxicity index). Based on LC_{50} and LC_{90} values, it is obvious that camphor oil nanoemulsion had remarkable toxic effects (LC_{50} = 1664 & LC_{90} = 9866 ppm) comparing to its oil bulk emulsion (LC_{50}= 20232 & LC_{90}= 26221 ppm). The toxicity index indicates that camphor oil nanoemulsion was about 12 times as toxic as its oil bulk emulsion

The remarkable toxic effect of camphor loaded nanoemulsion comparing to its bulk form resulted in the present study was similar to that obtained by Amal *et al* [32] who found that camphor nanoemulsion had considerable toxic effect comparing to camphor bulk emulsion on 2nd instar larvae of *S. littoralis* with about 19 times between nano and bulk form. In addition, Osman *et al*. [33] indicated that the LC_{50} value for camphor extract was 13.3 x 103 ppm when it was applied on the 4th instars larvae of *S. littoralis*. Moreover, the response of larval mortality caused by camphor oil in the current investigation is similar to the findings of Amal *et al*. [34] who reported that the LC_{50} value of bulk camphor oil was 20000 ppm against 2nd instar larvae of *S. littoralis*.

The results obtained are in agreement with those of Dimetry *et al*. [35]. They tested the toxicity effects of 4 EOs (thyme, peppermint, sage and camphor) in bulk and their nanoemulsions against the 4th instars larvae of *Agrotisipsilon*, they noticed higher toxicity of nanoemulstions compared to bulk essential oils. Also, El-Shewy [36] show that the effectiveness of Jojoba oil nanoemulsion was more than the crude oil against the 4th instars larvae of the black cutworm (*Agrotis ipsilon*). The results of our study and the other studies show clearly that the nanoemulsion of essential oils is active as pesticide compared to bulk oils against different insects.

Table 3: LC_{50} and LC_{90} values of both camphor oil nanoemulsion and bulk form against 2nd larval instar of *S. littoralis.*

Camphor form	LC_{50} (PPM)	95% fiducial limit (ppm)		LC_{90} (PPM)	95% fiducial limit (ppm)		Slope	Toxicity index
		L.C.L.	U.C.L		L.C.L.	U.C.L		
Bulk	20232	19280	21345	26221	24285	29736	11.38 ±0.51	8.22
Nano	1664	1339	2017	9866	6923	17125	1.66 ± 0.21	100

3.3. Toxicity of camphor oil bulk and nano emulsion on mice:

Data obtained in Table 4 show the LD_{50} of bulk camphor oil emulsion and its nano form. Results reveal that the doses of the LD_{50} were1.52 and 1.12 g/kg b.w., respectively. These values indicate that nanoemulsion was more toxic than its bulk form. The same trend was observed when the sub lethal dose (1/5 LD_{50}) values were 0.304 and 0.224 g/kg b.w for bulk camphor oil emulsion and its nano form, respectively.

Table (4): LD_{50} and 1/5 LD_{50} values of the prepared nanoemulsion and bulk camphor form after 24 hr of oral administration to Swiss albino mice.

Compound Tested	Compound form	LD_{50} (g/kg)	1/5 LD_{50} (g/kg)
Camphor	**Bulk**	1.52	0.304
	Nano	1.12	0.224

These results are similar to that obtained by Shalaby *et al*. [37] who revealed that eucalyptus essential oil is moderately hazardous (LD_{50} 2334.4 mg/kg b.w), calculated according to WHO [38].The marked influence (effectiveness) occurred in hematological and biochemical parameters of mice treated with nano camphor emulsion more than bulk form in present study may be attributed to nanoparticles which appear reduced size molecules leads to increase in surface area exposed to the reaction as a result of transformation of camphor oil to its nanoemulsion.

3.3.1. Effect of bulk and nano camphor emulsions on some haematological parameters of albino mice

Data presented in Table (5) show the effect of camphor oil in bulk and nanoemulsion on the hematological analyses in the mice after 7 and 21 days. The data show that the levels of white blood cells (WBCs) significantly ($P<0.05$) increased in the mice treated with bulk emulsion of camphor oil compared to that for the control group. While, the levels of WBCs in mice treated with nanoemulsion of camphor oil showed No significant ($P<0.05$) changes compared to the control group. Also data reveal that the levels of red blood cells (RBCs), hemoglobin (Hb) and hematocrit (Hct) of the mice treated with nanoemulsion of camphor oil significantly ($P<0.05$) decreased compared to those for the control group, while these parameters recorded non-significant

(P<0.05) changes when the mice were treated with bulk camphor oil. RBCs measurements such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLTs) counts for the mice treated with camphor oil in bulk or nanoemulsions showed no significant (P<0.05) changes compared to those of the control group.

Table (5): Complete Blood Picture of mice treated with camphor oil in bulk and nanoemulsions after 7 and 21 days compared to that of untreated control.

Parameter	*After 7 days*			*After 21 days*		
	Nano camphor oil	**Bulk camphor oil**	**Control**	**Nano camphor oil**	**Bulk camphor oil**	**Control**
WBCs ($x10^3$/µl)	9.38±0.97[a]	5.52±0.96[b]	5.93±0.64 [b]	10.50±1.04 [a]	6.48±1.03 [b]	6.57±0.66[b]
RBCs ($x10^6$/µl)	8.84±0.31[a]	7.16±0.62 [b]	9.23±0.38 [a]	10.39±0.33 [a]	8.09±0.71 [b]	10.63±0.49 [a]
Hemoglobin (g/dl)	15.78±0.23[a]	12.76±0.80[b]	16.67±0.44 [a]	16.52±0.31 [a]	13.66±0.91 [b]	17.43±0.62 [a]
Hematocrit (%)	53.20±1.24[a]	45.00±2.77[b]	57.33±1.77 [a]	59.14±1.52 [a]	48.14±3.23 [b]	60.80±2.60 [a]
MCV (fl)	60.40±0.87[a]	63.40±2.18 [a]	62.00±1.53 [a]	56.90±0.52 [a]	60.04±2.02 [a]	57.20±0.50 [a]
MCH (pg)	18.00±0.32[a]	17.80±0.49 [a]	18.00±0.58 [a]	15.90±0.31 [a]	17.04±0.55 [a]	16.40±0.47 [a]
MCHC (g/dl)	29.60±0.40[a]	28.60±0.24 [a]	29.00±0.00 [a]	10.50±1.04 [a]	6.48±1.03 [b]	6.57±0.66[b]
Platelet count ($x10^3$/µl)	1375.00±86.15[a]	1476.60±120.24 [a]	1486.67±46.72 [a]	10.39±0.33 [a]	8.09±0.71 [b]	10.63±0.49 [a]

Data were analyzed in five replicates and presented as means ± SE, different letters in the same raw for each period refer to significant differences at (P<0.05).

It can be noticed that the mice treated with bulk emulsion of camphor oil after 7 and 21 days recorded non-significant (P<0.05) changes in the levels of all hematological parameters except WBCs which significantly (P<0.05) increased compared to those of the control group. On the other hand, the mice treated with nanoemulsion of camphor oil recorded significant (P<0.05) decrease in the levels of RBCs, Hb and Hct, and non-significant (P<0.05) changes in the levels of other hematological parameters compared to those of the control group. It is evident that camphor oil in nano formulation reduced the counts of RBCs, and consequently reduced the hemoglobin level and hematocrit percentages of the mice administered on this oil for 7 and 21 days.

Similar results were obtained by Shalaby *et al.* [37] who stated that 1/10 LD_{50} of eucalyptus oil significantly increased WBCs, while all the doses investigated caused significant decreases in haemoglobin concentration and platelet count of treated rats as compared with normal mice. They attributed the increase of leucocytes to the inflammatory response induced as a defensive mechanism. Also, both forms of camphor oil may affect the leucocytic count by the stressogenic effect of these insecticides on the reticuloendothelial system [39].

The toxic and suppressive effects of eucalyptus oil on bone marrow and subsequently on haematopoiesis probably explain the present findings of both RBCs and platelets reductions. Since platelets are synthesized in bone marrow, so the suppressing effects on RBC and platelets counts would be explained [40]. While, the significant reductions in haemoglobin level and erythrocytes indicate anaemia occurrence during the toxicity study [41].

3.3.2. Effect of bulk and nano camphor emulsions on liver transaminases, kidney functions and tumor markers of albino mice

In order to evaluate the safety of tested formulations, urea and creatinine as kidney function parameters, plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as liver function parameters, beside some tumor markers such as carcino-embryonic antigen (CEA) and α-Fetoprotein (AFP) were determined in plasma of mice treated with camphor oil in bulk and nanoemulsions after 7 and 21 days. Results presented in Tables (6) indicate significant (P<0.05) increase in the levels of measured parameters in the mice treated with camphor oil in bulk and nano forms after 7 and 21 days compared to those in the control group. It is pointed out that these significant increases in the levels of various parameters in the mice treated with camphor oil in bulk and nanoemulsions did not exceeded the normal ranges of these parameters which demonstrated the safety of these formulations.

Comparatively, the mice treated with bulk emulsion of camphor oil recorded (P<0.05) significant increases in the levels of serum urea, ALT, CEA and AFP, and non-significant (P<0.05)

changes in the levels of serum creatinine and AST compared to corresponding parameters in the mice treated with nano emulsion of camphor oil for 7 and 21 days. Also the data illustrate that the safety of camphor oil in nano emulsion was comparable to that of camphor oil in bulk emulsion, and consequently the camphor oil in nanoemulsion can be used as safe natural pesticide compared to toxic synthetic pesticides.

Results of liver functions show that both two forms of camphor oil (bulk and nano) reveal a significant increase on activities of ALT and AST at both periods (7&21 days). The obtained results are in agreement with those of Shalaby *et al.* [37]**,** who found that the eucalyptus essential oil produce a significant increases in the activities of ALT and AST at investigated doses. The liver of treated mice showed elevated ALT and AST activities. The disruptions in transaminase values denote lesions of tissues and cell functions because they are involved in various vital functions including the metabolism, detoxification process, and biosynthesis of energetic molecules [42]. Moreover, the conducted results agreed with those of Arise *et al*. [43]**,** who found that the repeated administration of aqueous extracts prepared from the leaves of *Eucalyptus globules* significantly increased the activities of acid and alkaline phosphatase in the liver and serum of albino rats.

The significant increase in ALT and AST activities imply a negative impact on liver functions, since they are mainly associated with hepatocellular damages [44]. Also, AST could be considered as a biomarker due to its presence in a multiple tissues including brain, heart, liver, skeletal, kidney and muscles [45]. So, the increased activities of transaminase enzymes could be due to their release from the damaged cells, or which have changed thier membrane permeability in the injured organs [46]. The same trend was observed by EL-Mahrouky *et al*. [47] in house sparrows treated with 1/4 LD_{50} of camphor leaf extract. They found that plasma AST and ALT activities gradually increased at intervals 3, 6, 12, 24, and 48 hours post-treatment.

On the other hand, the current results on kidney functions are in accordance with Shalaby *et al.* [37] who stated that eucalyptus oil has a mild effect on kidney functions (urea and creatinine concentrations), whereas the administration of eucalyptus oil for 30 days significantly increased urea and creatinine concentrations. The changes occurred in kidney function tested in our study may be due to epithelial necrosis to the renal tubules with nuclear and chromatin changes in the epithelium of cortical tubules [48]. The occurrence of generalized convulsions in humans was observed after camphor ingestion [49].

Table (6): Effect of camphor oil in bulk and nanoemulsions on the renal profile (urea and creatinine), liver profile (AST and ALT enzymes), and tumor markers (CEA and AFP) in plasma mice after 7 and 21 days treatments.

Treatments	Urea (mg/dl)	Creatinine (mg/dl)	AST (U/L)	ALT (U/L)	CEA (ng/ml)	AFP (ng/ml)
After 7 days						
Nano	12.82±0.57^{b}	0.56±0.02^{a}	14.66±0.42^{a}	19.56±0.38^{b}	0.59±0.02^{b}	0.40±0.02^{b}
Bulk	14.98±0.14^{a}	0.62±0.01^{a}	14.20±0.30^{a}	24.04±0.55^{a}	0.71±0.01^{a}	0.53±0.02^{a}
Control	8.47±.43^{c}	0.45±0.02^{b}	11.73±0.39^{b}	15.20±0.38^{c}	0.50±0.01^{c}	0.25±0.03^{c}
After 21 days						
Nano	13.18±0.68^{b}	0.622±0.04^{a}	15.94±0.12^{a}	21.54±0.91^{b}	0.67±0.01^{b}	0.51±0.02^{b}
Bulk	15.98±0.16^{a}	0.702±0.01^{a}	15.98±0.38^{a}	27.78±0.61^{a}	0.766±0.01^{a}	0.662±0.02^{a}
Control	8.7±0.50^{c}	0.5±0.02^{b}	12.77±0.38^{b}	15.97±0.18^{c}	0.53±0.01^{c}	0.26±0.02^{c}

Data were analyzed in five replicates and presented as means ± SE, different letters in the same column for each period refer to significant differences at ($P<0.05$).

4. Conclusions

Therefore, according to observed findings in current work, it could be concluded that camphor nanoemulsion treatment has a powerful to reduce density population of *S. littoralis* and its safety was comparable to that of camphor oil bulk emulsion, and consequently the camphor oil in nanoemulsion can be used as safe natural pesticide compared to toxic synthetic pesticides, suggesting that camphor nanoemulsion may contribute in integrated pest management (IPM).

5. Conflicts of interest

There aren't any conflicts to report.

6. Formatting of funding sources

The author acknowledges that financial support was a personal effort

7. References

[1] Pineda S, Schneuder M I, Smagghe G and Martinez A M, Lethal and sublethal effects of Methoxyfenozide and Spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae), Journal of Economic Entomology., 2007, 100, 773–780.

[2] Amoabeng B W, Gurr G M, Gitau C W, Nicol H I, Munyakazi L and Stevenson P C, Tri-Trophic insecticidal effects of African plants against cabbage pests, Plos one, 2013, 8, 10, 1371-1382.

[3] Mareggiani G R, Russo S E and Rocca M A, *Eucalyptus globules* (Mirtaceae) essential oil: Efficacy against *Aphis gossypii* (Hemiptera: Aphididae), an agricultural pest, Review Latinoamer Quim, 2008, 36, 16-21.

[4] Elaissi A, Rouis Z, Abid Ben Salem N, Mabrouk S and Youssef S, Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities, BMC Complement. Alternate Medicine, 2012, 12, 10, 1186-1472.

[5] Chen W Y, Vermaak I and Viljoen A, Camphor—a fumigant during the Black Death and a coveted fragrant wood in ancient Egypt and Babylon— a review, Molecules, 2013, 18, 5434–5454.

[6] Liu C H, Mishra A K, Tan R X, Tang C, Yang H and Shen Y F, Repellent and insecticidal activities of essential oils from Artemisia princeps and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean, Bioresource Technology, 2006, 97, 1969-1973.

[7] Ghormade V, Mukund V D and Kishore M P, Perspectives for nano-biotechnology enabled protection and nutrition of plants, Biotechnology Advanced, 2011, 29, 792-803.

[8] Mubayi A, Chatterji S, Rai P M and Watal G, Evidence based green synthesis of nanoparticles, Advanced Materials and Methods, 2012, 3, 6, 519-525.

[9] Anjali C H, Khan S S, Margulis-Goshen K, Magdassi S, Mukherjee A and Chandrasekaran N, Formulation of water-dispersible nanopermethrin for larvicidal applications, Ecotoxicology Environment Safety, 2010, 73, 1932-1936.

[10] Anjali C H, Sharma Y, Mukherjee A and Chandrasekaran N, Neem oil (*Azadirachta indica*) nanoemulsion – a patent larvicidal agent against *Culex quinquefasciatus*, Pests Management Science, 2012, 68, 158-163.

[11] Frederiksen H K, Kristensen H G and Pedersen M, Solid lipid microparticle formulations of the pyrethroid gamma-cyhalothrin—incompatibility of the lipid and the pyrethroid and biological properties of the formulations, Journal of Control Release., 2003, 86, 243-252.

[12] Youssef D A and Abdelmegeed S M, Polymer-based encapsulation of peppermint oil *(Mentha piperita)* nanoemulsion and its effects on life and some physiological activities of honeybees Apismellifera (Hymenoptera: Apidae), Egyptian Pharmaceutical Journal, 2021, 20, 4, 313-322.

[13] Lertsutthiwong P, Noomun K, Jongaroonngamsang N, Rojsitthisak P U and Nimmannit U, Preparation of alginate nanocapsules containing turmeric oil, Carbohydrate Polymers, 2008, 74, 209-214.

[14] Rivera M C, Pinheiro A C, Bourbon A I, Cerqueira M A and Vicente A A, Hollow chitosan/alginate nanocapsules for bioactive compound delivery, International Journal of Biological Macromolecules, 2015, 79, 95-102.

[15] Jerobin J, Sureshkumar R S, Anjali C H, Mukherjee A and Chandrasekaran N, Biodegradable polymer based encapsulation of neem oil nanoemulsion for controlled release of Aza-A, Carbohydrate Polymers, 2012, 90, 1750-1756.

[16] Shorey H H and Hale R, Mass rearing of the larvae of nine Noctuide species on a simple artificial medium, Journal of Economic Entomology, 1965, 58, 522-524.

[17] Abbott W S, A method of computing the effectiveness of an insecticide, Journal of Economic Entomology, 1925, 18, 265-267.

[18] Finney D J, Probit Analysis. Cambridge: Cambridge University Press, 1971, 333pp.

[19] Sun Y P, Toxicity indexes an improved method of comparing the relative toxicity of insecticides, Journal of Economic Entomology, 1950, 43, 45-53.

[20] Weil C, Tables for Convenient Calculation of Median Effective Dose (LD_{50} or ED_{50}) and Instruction in Their Use, Biometrics, 1952, 8, 249-263.

[21] Reitman S and Frankel S, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, American Journal of Clinical Pathology, 1957, 28, 56-63.

[22] Burtis, C A, Ashwooad E R and Tietz, N W, Textbook of clinical chemistry 3rd edition (W. B. Saunders), 1999.

[23] Henry RJ, Clinical chemistry: Principles and technics (20 ED), Harper and Row, 1974.

[24] Abelev G I, Alfa- fetoprotein as a marker of embryo-specific differentiation in narmal and human tissues, Transplant Review, 1974, 20, 3-37.

[25] Schwartz M K, Tumor markers in diagnosis and screening, ln: Ting S W, Chen J S, Shwartz M K eds. Human tumor markers, Amsterdam, Elsevier Science, 1987, 3-16.
[26] Mossa F, Jimenez-Krassel F, Scheetz D, Weber-Nielsen M, Evans A C and Ireland J, Anti-Müllerian hormone (AMH) and fertility management in agricultural species, Reproduction, 2017, 154, 1–11.
[27] Maciel N M, Collevatti R G, Colli G R, Schwartz E F, Late Miocene diversification and phylogenetic relationships of the huge toads in the *Rhinella marina* (Linnaeus, 1758) species group (Anura: Bufonidae), Molecular Phylogenetics and Evolution, 2010, 57, 787–797.
[28] Ghaffa A, Ghosh S, Li F, Dong X, Zhang D, Wu M, et al., Effect of biochar aging on surface characteristics and adsorption behavior of dialkyl phthalates. Environmental Pollution, 2015, 206, 502-509.
[29] Ali S, Asaria M, Stranges S, COVID-19 and inequality could spread COVID-19. The Lancet public Health, 2020, 5, 5, e240.
[30] Donsi, et al., Nanoencapsulation of essential oils to enhance their antimicrobial activity in food. LWT-Food Science Technology, 2011, 44, 9, 1908-1914.
[31] Garcia M, Esquivel E U, Hernandez M, Ibarra E and Herrera A, The *Tricoderma atroviride* cryptochrome/photolyase genes regulate the expression of blr1-indepenent genes both in red and blue light, Fungal Biology, 2016, (in press).
[32] Amal E, Marouf F H, Eman A, Shehata G E and Abd-Allah, Efficacy of Camphor Oil and Its Nano Emulsion on The Cotton Leafworm, *Spodoptera littoralis*, Egyption Academic Journal of Biological Science, 2021, 13, 2, 103-108.
[33] Osman H H, Fetoh B A and Mohammed A M, The potency ofChloropyrifos and Camphor extract on *Spodoptera littoralis* (BOISD.). Egyptian Academic Journal of Biological Sciences, (A. Entomology), 2012, 5, 2, 131-139.
[34] Amal S, Sobhi E l, kousy S M and El-Sheikh T A, Some Toxicological and Physiological Aspects Induced by Camphor oil, *Cinnamomum camphora* on the Cotton Leafworm, *Spodoptera littoralis* (Boisduval). (Lepidoptera: Noctuidae), Egyption Academic Journal of Biological Science, 2020, 12, 2, 63-73.
[35] Dimetry N Z, Amin A H, Bayoumi A E, Abdel-Raheem M A and Youssef A D, Comparative toxicity of neem and peppermint oils Nano formulations against *Agrotisipsilon* (Hufn.) larvae (Lepidoptera: Noctuidae), Journal of Botanical Research, 2019, 1, 1, 13-19.
[36] El-Shewy A M, Efficacy of Jojoba oil compared to its Nano particles on biological and physiological aspects of *Agrotisipsilon* and its histological effect on albino rats, Middle East Journal of Applied Science, 2018, 8, 4, 1404-1412.
[37] Shalaby S E M, El-Din M M, Abo-Donia S A, Mettwally M and Attia Z A, Toxicological effects of essential oils from eucalyptus *Eucalyptus globules* and clove *Eugenia caryophyllus* on Albino rats, Polish Journal of Environmental Studies, 2011, 20, 2, 429-434.
[38] WHO, the WHO recommendation classification of pesticides by hazard and guideline to classification. World Health Organization, Geneva, 2005.
[39] Gromysz, M. (1993) Substrate specificity of mouse-liver microsomal enzymes in S-fenvalerate metabolism ACS Symposium series No. 42, synthetic S-envalerate. American Chemical Society, Washington, D C.
[40] Jamel Al-Layl K M S, Toxicological and histopathological effects of the Cyanobacterium *Oscillatoria rubescens* on blood and liver of the white albino rats. Arabian Univercity Journal of Agriculture Science, 2004, 12, 2, 821.
[41] Choudhari C V and Deshmukh P B, Acute and subchronic toxicity study of *Semecarpus anacardium* on haemoblobin percent and RBC count of male albino rat, Journal of Herbicides Medical Toxicology, 2007, 1, 1, 43.
[42] Tordior W F and Van HeemStra-Lequin E A, Field studies monitoring exposure and effects in the deffelopment of pesticides, Elsevier, Amsterdam, Oxford, New York, 1980, 207.
[43] Arise R O, Malomo S O, Adebayo J O and Igunnu A, Effects of aqueous extract of *Eucalyptus globules* on lipid peroxidation and selected enzymes of rat liver. Journal of Medical Plant Research, 2009, 3, 2, 77.
[44] Wittawaskull P, Panthong A, Kanganapothi D, Taesothikul T, Lertprasertsuke N, Acute and subacute toxicities of saponin mixture isolated from *Schefflera leucantha* Viguier, Journal of Ethnopharmathiotic, 2003, 89, 115.
[45] Mukinda J T and Syce J A, Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents, Journal of Ethnopharmathiotic, 2007, 112, 138.
[46] Obici S, Otobone F J, Silvasela V R, Ishida K, Silva J C, Nakamura C V, Cortez D and Audi E A, Preliminary toxicity study of dichloromethane extract of *Kielmeyer coriacea* stems in mice and rats. Journal of Ethnopharmathiotic, 2008, 115, 131.
[47] EL-Mahrouky F, Sanad A S and Khider F, Effect of methomyl and camphor leaves ethanol extract on some transaminases enzymes and total protein in birds, Journal of Agriculture Science, 2001, 26, 10, 6437.

[48] Janssen W, Forensic Histopathology. Spring-Verlag, Berlin, NY, 1984, pp. 314-315.
[49] Ruha A M, Grame K A and Fifld A, Late seizure following ingestion of *Vicks vaporub*, Academic Emerging Medical, 2003, 10, 691.