Polish J. of Environ. Stud. Vol. 20, No. 2 (2011), 429-434

Original Research

Toxicological Affects of Essential Oils from Eucalyptus *Eucalyptus globules* and Clove *Eugenia caryophyllus* on Albino Rats

Shehata E. M. Shalaby[1]*, Mona Magd El-Din[1], Sherif A. Abo-Donia[2], Monir Mettwally[2], Zakaria A. Attia[1]

[1]Department of Pests and Plant Protection, National Research Centre, El-Tahrer Street, Dokki, Cairo, Egypt
[2]Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

Received: 8 March 2010
Accepted: 8 July 2010

Abstract

The present work was planned to determine the median lethal doses (LD_{50}) and to evaluate the haematological and biochemical changes, and histopathological effects of the essential oils from eucalyptus *Eucalyptus globules* L. and clove *Eugenia caryophyllus* on liver and kidney of albino rats. The LD_{50} was 2,334.4 and 3,597.5 mg/kg b.w. of eucalyptus and clove oils, respectively. Obtained data revealed that $^1/_{10}$ LD_{50} of both tested oils resulted in a significant increase in WBC counts and produced a significant decrease in haemoglobin concentration and platelets count at 5th and 10th doses, as well as RBC counts (-17.1 and -9.4% below normal level) at 10th dose. The activities of serum GOT and GPT enzymes were a significant increase at 5th and 10th doses in treated rats by both tested oils. While two essential oils had mild effect on kidney function, these oils produced a significant increase in creatinine and urea concentration at the 10th dose. Histopathological studies on liver and kidney revealed that both essential oils caused relatively moderate pathological changes in the liver as congestion of the blood vessels in the portal area associated with inflammatory infiltration. Also, two tested oils induced desquamation of the epithelial cells of the renal tubules.

Keywords: toxicological impacts, essential oils, eucalyptus, clove, rats

Introduction

Heavy reliance on the use of conventional insecticides has led to problems of insect resurgences, resistance, negative impact on non-target organisms, health and environmental hazards. These have raised concern among the public for the need to search for safe and environmentally friendly pest control options [1]. There is now a growing interest in the exploration of natural vegetation for possible alternatives [2]. Recently, attention has been given to the isolation and identification from plant sources for various botanical compounds that possess insecticidal properties. Plants attracted the attention of entomologists because most botanical extracts are not toxic to warm-blooded animals and show no or moderate side effects on natural enemies [3]. They are known to provide effective control against insects that have become resistant to other insecticides [4]. Several plants have been found to possess insecticidal activities against a wide range of agricultural pests. The pesticidal and biological activities of plant extracts were extensively studied by several researchers [5-8]. Also, previous study from our laboratory concluded that the eucalyptus and clove essential oils induced high toxicity against the European corn borer *Ostrinia nubilalis* Hb [9].

*e-mail: sh_shalbynrc@yahoo.com

However, there is a dearth of toxicological information on the essential oils. Therefore, the present study was conducted to determine the median lethal doses (LD_{50}) and to evaluate the haematological and biochemical changes, and histopathological effects of the essential oils from eucalyptus *Eucalyptus globules* L. and clove *Eugenia caryophyllus* on liver and kidney of albino rats.

Materials and Methods

Essential Oils

Eucalyptus oil was extracted from *Eucalyptus globules* leaves, while volatile clove oil was extracted from *Eugenia caryophyllus* flowers using a Klevenger apparatus as described by Gunther [10]. The leaves of eucalyptus and flowers of clove were air dried in the shads. Twenty-five grams of dried leaves or flowers were separately mixed with 500 ml of water in a rounded one-liter flask and subjected for hydrodistillation for three hours. The resulting volatile oils were dried over anhydrous sodium sulphate and then stored in dark bottles in a refrigerator until used.

Animal Model

Male albino rats (wistar strain) weighing 150±20 gm were procured from an animal breeding house of the National Research Centre (NRC) in Dokki, Cairo, Egypt. Animals were acclimatized for laboratory conditions and fed on standard diet as per formula of NRC animals breeding house. Water was supplied *ad libitum*.

Determination of the Median Lethal Dose (LD_{50})

According to the method of Finney [11] for determination of the median lethal dose (LD_{50}), exploratory trials were performed in five groups each of two rats, eucalyptus and clove oils were administered orally at doses of 500, 1000, 1,500, 2,000, and 2,500 mg/b.w. correspondingly in five groups to find the smallest toxic dose to start with. Dose 1,000 and 1,500 mg/b.w of eucalyptus and clove, respectively, which was the first dose to cause signs of toxicity multiplied by constant factor (1.5) for each succeeding groups of rats. Nine groups of rats were used (10 of each), 1st, 2nd, 3rd, and 4th groups were given 1000, 1,500, 2,250, and 3,375 mg/kg b.w. of eucalyptus oil, while the 5th, 6th, 7th, and 8th groups were given 1,500, 2,250, 3,375, and 5,062.5 mg/kg b.w. of clove oil in four groups. The 9th group was kept as a control. Mortality of rats was recorded after 24 hr. The toxicity index of each oil was determined according to Sun [12].

Subchronic Toxicity

For studying the effect of repeated administration of tested oils on male albino rats, thirty animals were divided into 3 groups (5 rats of each). The 1st and 2nd groups were treated by $^1/_{10}$ LD_{50} of eucalyptus and clove essential oils and the 3rd group was served as a control. Tested oils were dissolved in corn oil and administered orally by convenient stomach tube day after two days for one month. After 15 and 30 days of administration blood samples were collected in two tubes, the first containing heparin (7.5 I.U. /ml) according to Schalm [13] for haematological investigation.

Haematological Investigation

Erythrocytic count (RBCs), leukocytic count (WBCs), platelets count and haemoglobin concentration were determined according to the methods served by Schalm [13].

Biochemical Changes

To study the effect of tested essential oils on serum constituents of rats, serum was collected after centrifugation at 3,000 rpm for fifteen minutes and kept at -20ºC until used. Chemical analysis was carried out on serum, to assess the state of the liver and kidney. This included: serum glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) activities, creatinine, and urea concentration. Analysis was performed according to the set description of Bayer Corporation, SERA PAK [14], with the aid of AMES QUIK-LAB chemistry analyzer, Miles Inc., Germany.

Histopathological Studies

At the 10th dose, all the sacrificed rats were necropsied. Specimens were collected from liver and kidney organs and fixed in 10% neutral buffer formalin. Paraffin sections (6-8 microns) were prepared and stained with Harris haematoxylin and eosin [15] for microscopic examination.

Statistical Analysis

Statistical significance was assessed by Duncan and Tukey test at $P<0.05$ and $P<0.01$ [16].

Results and Discussion

Determination of the Median Lethal Dose (LD_{50})

Obtained data (Table 1) concerning the median lethal dose (LD_{50}) of active principle of eucalyptus and clove essential oils revealed that the doses of the LD_{50} were 2,334.4 and 3,597.5 mg/kg b.w., respectively, for rats under environmental conditions.

Obtained data revealed that eucalyptus essential oil is moderately hazardous (LD_{50} 2,334.4 mg/kg b.w), while clove essential oil is only slightly hazardous (LD_{50} 3,597.5 mg/kg b.w.) according to the WHO classification [17].

Table 1. Toxicity levels of Eucalyptus and Clove essential oils against albino rats.

Compound	LD_{50} (mg/kg b.w)	LD_{95} (mg/kg b.w)	Slope	Toxicity index		Folds	
				LD_{50}	LD_{95}	LD_{50}	LD_{95}
Eucalyptus oil	2334.4	7632.13	3.2	100	100	1.54	2.36
Clove oil	3597.5	18037.1	2.3	64.89	42.3	1.0	1.0

Table 2. Effect of Eucalyptus and Clove essential oils on haematological parameters on albino rats.

Parameters / Treatments		WBC (10^3/ml)		RBC (10^6/ml)		Hemoglobin (mg/ml)		Platelets (10^3/ml)	
		5th dose	10th dose	5th dose	10th dose	5th dose	10th dose	5th dose	10th dose
Eucalyptus oil		10.5aA (+29.6)	13.25aA (+42.5)	4.28aA (-6.1)	3.63bB (-17.1)	11.2cC (-9.7)	9.8cC (-17.6)	276.0cC (-30.1)	207.0cC (-32.4)
Clove oil		8.25bB (+1.85)	12.65bB (+36.0)	4.28aA (-6.1)	3.97bAB (-9.4)	11.8bB (-4.8)	10.3bB (-13.4)	310.0bB (-21.5)	256.0bB (-16.3)
Control		8.1bB	9.3 cC	4.56aA	4.38 aA	12.4aA	11.9aA	395.0aA	306.0aA
LSD	5%	0.46	0.11	0.35	0.35	0.26	0.13	13.1	4.7
	1%	0.76	0.19	0.57	0.57	0.43	0.22	21.7	7.8

*(According to Duncan test) Letters means the significant differences between treatments and control. Each figure between brackets represents the percentage of content as check.

Haematological Changes

Obtained data (Table 2) revealed that the $^1/_{10}$ LD_{50} of eucalyptus oil caused a significant increase in WBC count (29.6% above normal level) at 5 doses of treated rats as compared with $^1/_{10}$ LD_{50} clove treated rats and normal animals, while at the 10th dose both tested oils caused a significant increase in WBC count (+42.5 and +36.0%). The high increase of leucocytes may be due to the inflammatory response induced as a defensive mechanism. Also, both compounds may affect the leucocytic count by the stressogenic effect of these insecticides on the reticuloendothelial system [18].

Although the tested essential oils did not produce any significant effect on RBC count at the 5th dose, there was a significant decrease at the 10th dose (17.1 and 9.4% below the normal level) that caused a significant decrease ($P<0.01$) in haemoglobin concentration and platelet count at all the doses investigated. The present findings revealed reduction in numbers of both RBCs and platelets probably due to a suppressive and toxic effect on bone marrow and subsequently on haematopoiesis. Since platelets are synthesized in bone marrow, so the double suppressing effect on RBCs and platelets would be explained [19]. On the other hand, the significant decrease in haemoglobin percent and lowering of erythrocytes was noted, indicating anemia during the toxicity study [20].

Biochemical Changes

The result on liver function showed that the eucalyptus and clove essential oils produce a significant increase on the activity of GOT at all doses, but revealed significant ($P<0.05$) increase in GPT activity at the 5th dose (14.2 and 11.9% above the normal level, respectively) and 10th dose (+62.6 and 27.2%, respectively). GOT and GPT activities were activated in liver of treated animals. The disruption of transaminases from the normal values denotes lesions of tissues and cellular function because they are involved in the detoxification process, metabolism, and biosynthesis of energetic macromolecules for different essential functions [21]. Also, these results are in accordance with those obtained by Arise et al. [22], who reported that repeated administration of varying concentrations of the aqueous extract of *Eucalyptus globules* leaves caused a significant increase in acid and alkaline phosphatase activities in the liver and serum of albino rats. GOT and GPT were significantly increased, implying negative impact on liver functions. According to Witthawaskul et al. [23] the two enzymes mainly associated with hepatocellular damage in liver are AST and ALT. Also, AST is present in a wide variety of tissues, including heart, skeletal muscles, kidney, brain, and liver [24].

Therefore, the significant increase of GOT in rats treated with eucalyptus and clove essential oils sub-chronically

Table 3. Effect of Eucalyptus and Clove essential oils on liver and kidney function indices of albino rats.

Parameters / Treatments		GOT (U/l)		GPT (U/l)		Creatinine (mg/dl)		Urea (mg/dl)	
		5th dose	10th dose	5th dose	10th dose	5th dose	10th dose	5th dose	10th dose
Eucalyptus oil		30.5aA (+38.0)	38.7aA (+58.0)	28.9aA (+14.2)	41.8aA (+62.6)	0.8 aA (+33.3)	1.0 aA (+42.9)	25.7aA (+13.7)	30.8aA (+24.7)
Clove oil		27.0bA (+22.2)	30.4bB (+24.1)	28.3aA (+11.9)	32.7bB (+27.2)	0.7 aA (+16.7)	0.9abA (+28.6)	24.4aA (+8.0)	26.8bAB (+8.5)
Control		22.1cB	24.5cC	25.3bA	25.7cC	0.6 aA	0.7 bA	22.6aA	24.7bB
LSD	5%	2.62	2.62	2.62	3.93	0.26	0.26	3.46	3.46
	1%	4.34	4.34	4.34	6.51	0.43	0.43	5.74	5.74

*(According to Duncan test) Letters means the significant differences between treatments and control. Each figure between brackets represents the percentage of content as check.

effect, may be due to the release of enzymes from the cells of the affected organs, or to change in cell membrane permeability [25]. In the same trend, obtained data by El-Mahrouky et al. [26] indicated that a house sparrow treated with ¼ LD_{50} of camphor leaf extract induced a gradually significant increase in plasma GOT and GPT activities at intervals 3, 6, 12, 24, and 48 hours post-treatment.

Also, obtained results revealed that the tested oils have a mild effect on kidney function (creatinine and urea concentration), whereas the administration of eucalyptus oil caused a significant increase ($P<0.05$) in creatinine and urea concentrations after 30 days (+42.9 and +24.7%, respectively), but clove oil did not produce any significant changes in both parameters at all doses. These changes may be due to epithelial necrosis to the renal tubules with nuclear and chromatin changes in the epithelium of cortical tubules [27]. The occurrence of generalized convulsions in humans was observed after camphor ingestion [28].

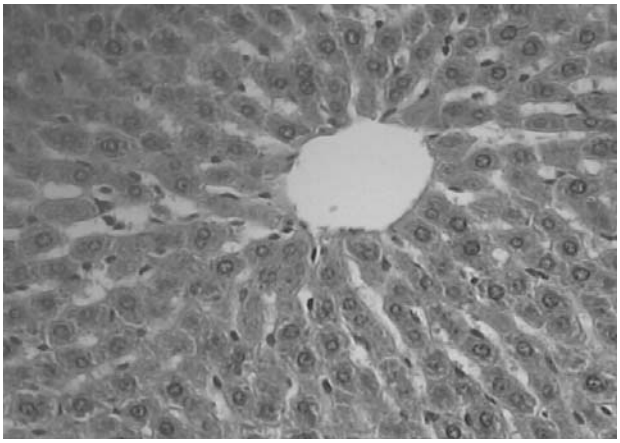

Fig. 1. Section of control liver showing the normal structure of the hepatic lobules (H & E stain -X 150).

Histopathological Changes

Histopathological effects of eucalyptus and clove essential oils on liver and kidney of treated albino rats were observed.

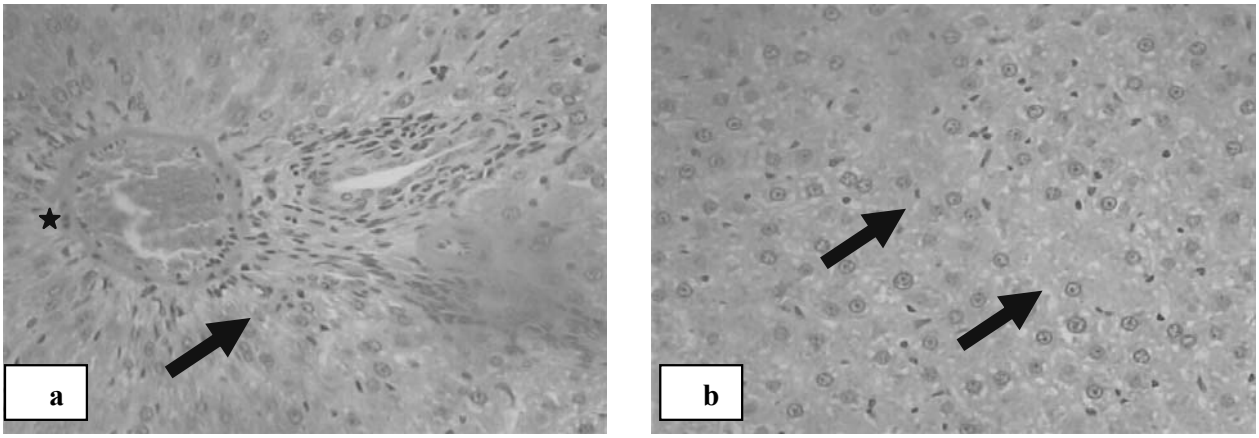

Fig. 2ab. Section of liver of rats treated by 1/10 LD_{50} eucalyptus essential oil showing congestion of the blood vessels (asterisk) in the portal area associated with inflammatory infiltration (arrow) (Fig. 2-b) and showing small vacuoles in the hepatocytes (arrow). (H & E stain –X 150).

I-Liver

Microscopic examination of liver sections of control rats showed the hepatic lobules, the structural units of the liver; each is formed by cords of hepatocytes and blood sinusoids in between (Fig. 1).

Administration of $^{1}/_{10}$ LD_{50} of eucalyptus essential oils after two days for one month caused congestion of the blood vessels in the portal area associated with inflammatory infiltration. Also, small vacuoles were shown in hepatocytes (Figs. 2 a, b). Our results agree with those obtained by Abd El-Gawad et al. [29], who reported that $^{1}/_{4}$ LD_{50} of camphor leaf ethanol extract caused dilation of the central and portal veins, with lymphocytic infiltration at liver of house sparrow and palm dove. Arise et al. [22] found that aqueous extract of *E. globules* leaves may have deleterious effects on liver membrane structure and functional integrity.

Examination of liver sections of rats received an oral dose equal to $^{1}/_{10}$ LD_{50} of clove essential oil showed that the structure of the hepatocytes appeared more or less like normal. The specimens also revealed congestion of the blood vessels in the portal area and associated with inflammatory infiltration (Fig. 3).

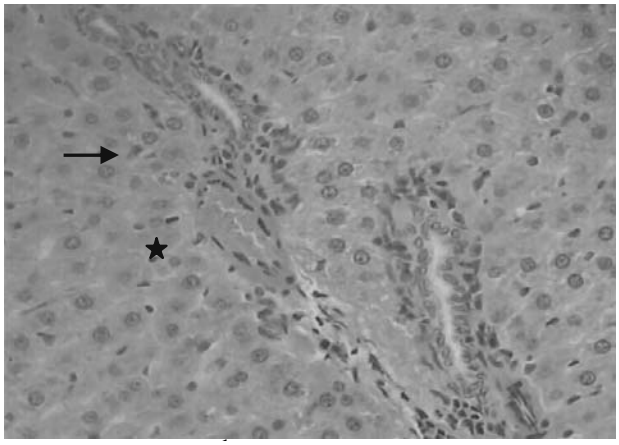

Fig. 3. Section of liver of rats treated by $^{1}/_{10}$ LD_{50} clove essential oil showing congestion of the blood vessels (asterisk) in the portal area associated with inflammatory infiltration (arrow) (H & E stain – X 150).

2-Kidney

Examination of kidney sections of control animals showed renal corpuscle and renal tubules, proximal convoluted tubules and distal convoluted tubules (Fig. 4).

Administration of oral doses equivalent to $^{1}/_{10}$ LD_{50} of eucalyptus essential oil showed lobulated renal corpuscles and desquamation of epithelial cells of the renal tubules. Hemorrhage in the interstium was seen (Fig. 5). Examination of kidney of treated rats by $^{1}/_{10}$ LD_{50} of clove essential oil showed lobulated renal corpuscles and the desquamation of the epithelial cells of the renal tubules (Fig. 6). In the same trend, data obtained by AbdEl-Gawad et al. [29] revealed that congestion with lympho-

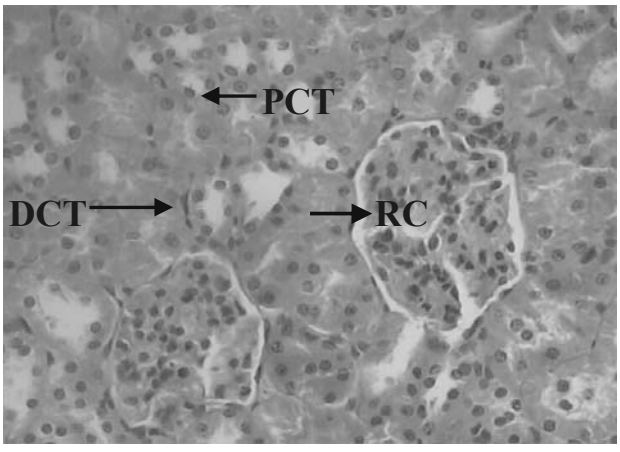

Fig. 4. Section of kidney of control rat showing renal corpuscle (RC) and renal tubules, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) (H & E stain –X 150).

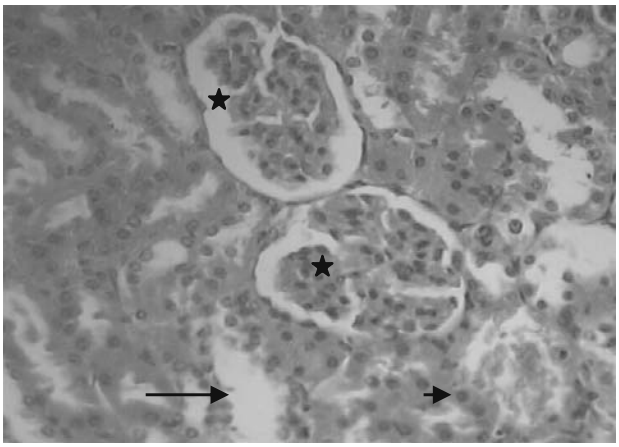

Fig. 5. Section of kidney of rats treated by $^{1}/_{10}$ LD_{50} eucalyptus essential oil showing renal corpuscle and renal tubules, proximal convoluted tubules and distal convoluted tubules. Notice the lobulated renal corpuscles (asterisk) and the desquamation of the epithelial cells of the renal tubules (arrow). Hemorrhage in the interstium also can been seen (arrowhead) (H & E stain – X 150).

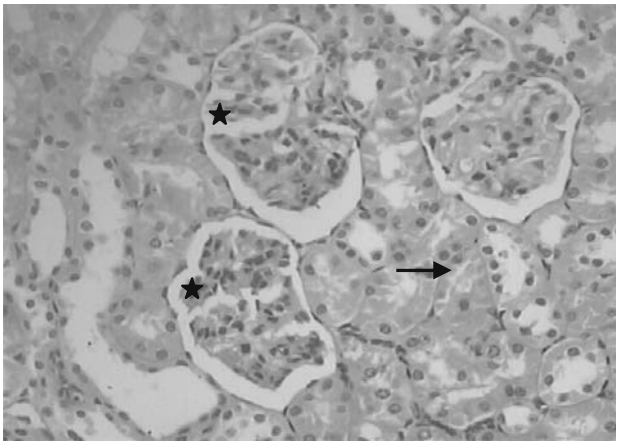

Fig. 6. Section of kidney of rat treated by $^{1}/_{10}$ LD_{50} clove essential oil showing renal corpuscle the lobulated renal corpuscles (asterisk) and the desquamation of the epithelial cells of the renal tubules (arrow) (H & E stain –X 150).

cytic infiltration and fibrosis at house sparrow kidney treated by 1/4 LD_{50} of camphor leaves extract, while kidney palm dove showed cloudy swelling.

Conclusions

It could be concluded that the both tested essential oils attack more than one biochemical active site and act on a physiological system, leading to serious metabolic and cellular damage. These effects may influence the use of the oils from eucalyptus *Eucalyptus globules* L. and clove *Eugenia caryophyllus* as insecticides. Also, more studies are needed to confirm the insecticidal activity of these oils. Moreover, the comparative study showed that clove essential oil has a lower toxicity than eucalyptus essential oil.

References

1. DUKE S.O., BAERSON S.R., DAYAN F.E., RIMANDO A.M., SCHEFFLER B.E. United States department of agricultural research service on natural products for pest management. Pest Manage. Sci., **59**, 708, **2003**.
2. JOVETIC S. Natural pyrethrins and biotechnological alternatives. Biotechnol. Dev. Monit., **21**, 12, **1994**.
3. SCHMUTTERE H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann. Rev. Entomol., **35**, 271, **1990**.
4. WEINZIERL R.A. Botanical insecticides, soaps and oils. In: biological and biotechnological control of insect pests. Rechcigl, J. E. and N.A. Rechcigl (Eds.). Lewis Publisher, New York, ISBN: 1566704790, pp: 1-12, **2000**.
5. BLASKE V., HERTEL H. Repellent and toxic effect of plant extracts on subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol., **94**, 1200, **2001**.
6. SABBOUR M., ISMAIL I. A. The combined effect of microbial control agents and plant extracts against potato tuber moth *Phthorimaea operculella* zeller. Bull. NRC, Egypt, **27**, (4), 459, **2002**.
7. MAGD EL-DIN M. Biological effects of some medicinal and aromatic plants on the cowpea weevil *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). J. Egypt. Ger. Soc. Zool. (42 E), Entomology 1-10, **2003**.
8. SOLIMAN M. M. M., SALLAM A. A., MANSOUR F. A. Insecticidal activity of *Francoeria crispa* (Forssk.) extract on the cotton leafworm, *Spodoptera littoralis* (Boisd.). J. Pest Cont. & Environ. Sci. **11**, (1), 121, **2003**.
9. ATTIA Z. A. Evaluation of the efficiency of some plant extracts on European corn borer *Ostrinia nubilalis*. Thesis M.Sc. Faculty of Agriculture Al-Azhar Univ. Cairo, Egypt, **2002**.
10. GUENTHER E. The essential oils. D. van Nostfend company, New York, Toronto, Londan. **1**, **1973**.
11. FINNEY D. J. Probit analysis, 3rd edn. Cambridge: Cambridge Univ. Press, **1971**.
12. SUN Y.P. Toxicity index: an improved method of comparing the relative toxicity of insecticides. J. Econ. Ent., **34**, 45, **1950**.
13. SCHALM O.W. Veterinary Hematology. 4th Ed., Loa and Fibiger, Philadelphia, pp. 21-86, **1986**.
14. Bayer Corporation, Sera Pak. Reagent kits for *in vitro* diagnostic, USA, **1997**.
15. CARLETON M.A., DRURY R.A.B. Carleton's histological technique. 3rd ed., Oxford University Press, New York, Toronto, **1967**.
16. SNEDECOR G.W., COCHRAN W.G. Statistical Methods. 7th (Ed). State university press, Ames, Iowa, **1980**.
17. WHO. The WHO recommendation classification of pesticides by hazard and guideline to classification. World Health Organization, Geneva, **2005**.
18. GROMYSZ M. 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., **1993**.
19. JAMEL Al-LAYL K.M.S. Toxicological and histopathological effects of the Cyanobacterium *Oscillatoria rubescens* on blood and liver of the white albino rats. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt, **12**, (2), 821, **2004**.
20. CHOUDHARI C. V., DESHMUKH P. B. Acute and subchronic toxicity study of *Semecarpus anacardium* on haemoblobin percent and RBC count of male albino rat. J. Herb. Med. Toxicol. **1**, (1), 43, **2007**.
21. TORDIOR W.F., VAN HEEM STRA-LEQUIN E.A. Field studies monitoring exposure and effects in the deffelopment of pesticides. Elsevier, Amsterdam, Oxford, New York, 207, **1980**.
22. ARISE R. O., MALOMO S. O., ADEBAYO J. O., IGUNNU A. Effects of aqueous extract of *Eucalyptus globules* on lipid peroxidation and selected enzymes of rat liver. J. Med. Plant Res. **3**, (2), 77, **2009**.
23. WITTAWASKUL P., PANTHONG A., KANJANAPOTHI D., TAESOTHIKUL T., LERTPRASERTSUKE N. Acute and subacute toxicities of saponin mixture isolated from *Schefflera leucantha* Viguier. J. Ethnopharma., **89**, 115, **2003**.
24. MUKINDA J.T., SYCE J.A. Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents, J. Of Ethnopharma. **112**, 138, **2007**.
25. OBICI S., OTOBONE F.J., SILVASELA V.R., ISHIDA K., SILVA J.C., NAKAMURA C.V., CORTEZ DAG., AUDI E.A. Preliminary toxicity study of dichloromethane extract of *Kielmeyer coriacea* stems in mice and rats. J. Ethnopharma. **115**, 131, **2008**.
26. EL-MAHROUKY F., SANAD A.S., KHIDER F. Effect of methomyl and camphor leaves ethanol extract on some transaminases enzymes and total protein in birds. J. Agric. Sci. Mansoura Univ., Egypt. **26**, (10), 6437, **2001**.
27. JANSSEN W. Forensic Histopathology. Spring-Verlag, Berlin, NY, pp. 314-315, **1984**.
28. RUHA A.M., GRAME K.A., FIFLD A. Late seizure following ingestion of Vicks vapoRub. Acad Emerg Med. **10**, 691, **2003**.
29. ABDEl-GAWAD A., EL-MAHROUKY F., KHIDR F. Histological effects of camphor leaves ethanol extract in some organs of house sparrow and palm dove. J. Agric. Sci. Mansoura Univ., Egypt. **26**, (10), 6451, **2001**.