

Studies on Side Effects of Violacene, The Halogenated Monoterpene, On Albino Rats

Abd EL-Wahab,R.A. *, Bursic,V. and Lazić,S.

*Plant Protection Research Institute,Agriculture Research Center,EGYPT.

[*rania-proline@hotmail.com](mailto:rania-proline@hotmail.com)

ABSTRACT

Violacene is a halogenated monoterpene isolated from hexane extract of the red alga, *Plocamium cartilagineum*. It's highly acaricidal activity with LC50(200 ppm). So we studied the side effects of this compound against albino rats, which treated with LD50(10000 mg/kg) and 2XLD50(20000 mg/kg). The histopathological and haematological studies were done for both treated and control animals. All changes if occurred in some organs were temporarily.

INTRODUCTION

Plocamium cartilagineum This deep pink to rose-red alga is common on upper and lateral surfaces of rocks in the mid to lower intertidal zone and will extend into subtidal regions. It is abundant at Tunnel Beach where it tolerates being covered by sand. It has a flattened, cartilaginous thallus with successive branchlets, the outer ones branching from one side only, like a comb. Many of the branchlets are slightly curved (the genus is derived from the Greek word "plokos" meaning "curl"). It often grows from prostrate stoloniferous branches. Several types of chemicals called halogenated monoterpenes have been isolated from *P. cartilagineum* which have insecticidal properties against aphids, tomato moths (Argandoña, et al.2000). These compounds are also being investigated for antimicrobial activity as well as cancer treatments. Halogenated monoterpene, originally isolated from the marine red alga *Plocamium cartilagineum*, exhibits potent anti-algal activity as well as antimycobacterial activity towards *M. tuberculosis* H37Rv with an MIC of 32 µg ml⁻¹ and *M. avium* (MIC 64 µg ml⁻¹).Copp,2003.

Moreover, Violacene proved its acaricidal efficacy against the red spider mite, *Tetranychus urticae*, it showed that it could be used in control programs at wide scale but the safety wanted to be assessed on non target organisms.

So this paper liked to scope on the side effects of the most effective halogenated monoterpene violacene extracted from *Plocamium sp.* On the albino rats under laboratory conditions.

MATERIALS AND METHODS

Extraction and Isolation

Plocamium cartilagineum (Linnaeus) Dixon was collected from the Mediterranean sea coast of Egypt at a depth of 15cm. The dried alga was extracted exhaustively with acetone at room temperature to give a dark residue. This extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane: EtOAc (4:1), and was further separated by filtration chromatography to give violacene, according to Argandoña *et al.* 2000.

Animals

45 albino rats of both sexes, (150-200g), were obtained from Veterinary College, Mansoura University, Egypt. They were fed with growers mash and water *ad libitum*. The rats were divided into 3 groups (2 treatments + Control). Each treatment had 15 rats divided in 3 replicates.

Treatments

The main halogenated monoterpene as acaricide from hexane extract of the red alga *Plocamium cartilagineum* is violacene, with $LC_{50}=200$ ppm of *Tetranychus urticae*. Toxicity studies were carried out *in vivo* and administration was by intraperitoneal route. Both LD_{50} and $2XLD_{50}$ of albino rats were determined according to Miller and Tainter, 1944. Each group was placed in the clean cage and injected with its specific dose. They were observed and all changes were recorded through 14 days after treatments.

Biochemical analysis

On the 14th day of experimental period, the blood (~5 ml) was collected from retro-orbital vein using fine capillary tube and centrifuged at 5000rpm for 15min (Varley *et al.*, 1991). The serum was separated and collected into non-heparinized tubes for the following investigation: cholesterol, urea, protein, glucose, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and electrolytes (sodium and potassium).

Haematological studies

The blood samples before centrifugation that were collected into heparinized tubes were used for the estimation of haemoglobin, red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelets, polymorphs, lymphocytes and eosinophils (Ringler and

Dabich, 1979; Dacie and Lewis, 1991). All of biochemical and haematological parameters were measured in the histopathology Dept., Veterinary College, Mansoura University, Egypt.

Statistical Analysis

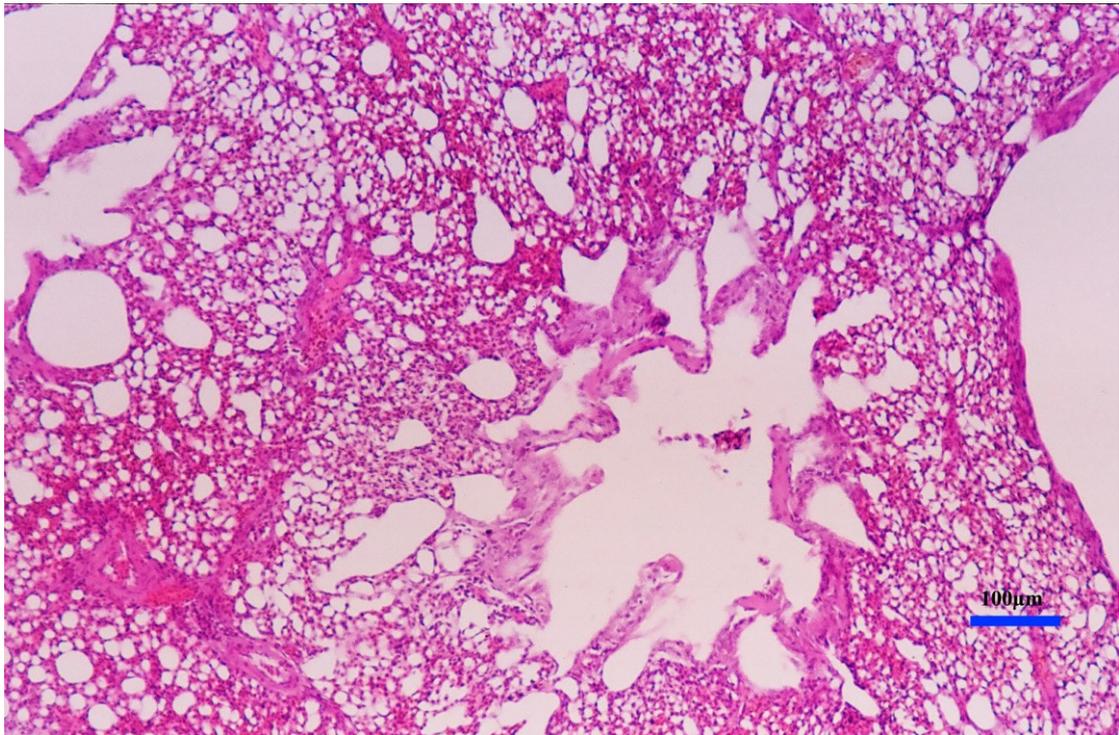
The statistical significance between the groups was analyzed by means of an Analysis of variance (ANOVA). Results were expressed as mean \pm SD. A probability level of less than 0.05 was considered significant.

RESULTS

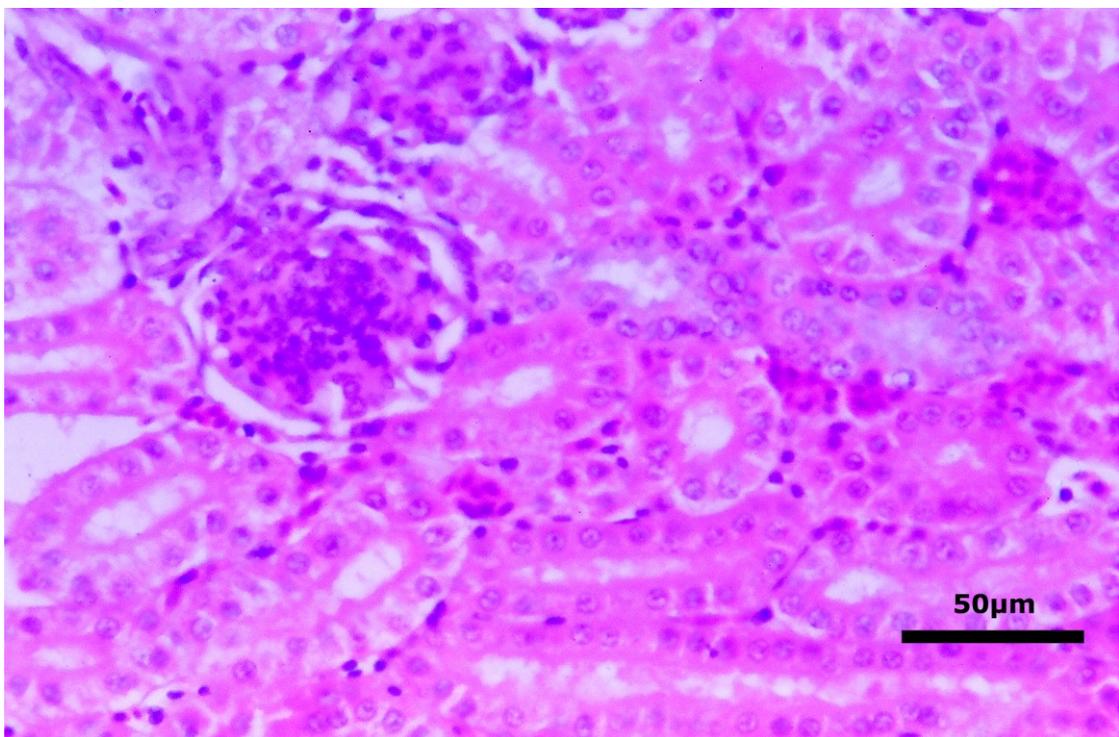
Data of this research showed that histopathological defects resulted from the treatments in vivo and administration by intraperitoneal route with LD50 and its double values as showed in FIGS.(2-10), are not permanent. So it could be said that the use of violacene, the halogenated monoterpene as acaricide from hexane extract of the red alga *Plocamium cartilagineum* is safe. Especially that even with highly values of LD50's, no serious changes observed even of the biochemical or haematological parameters, resp. as shown in Tables(1) and (2), resp.



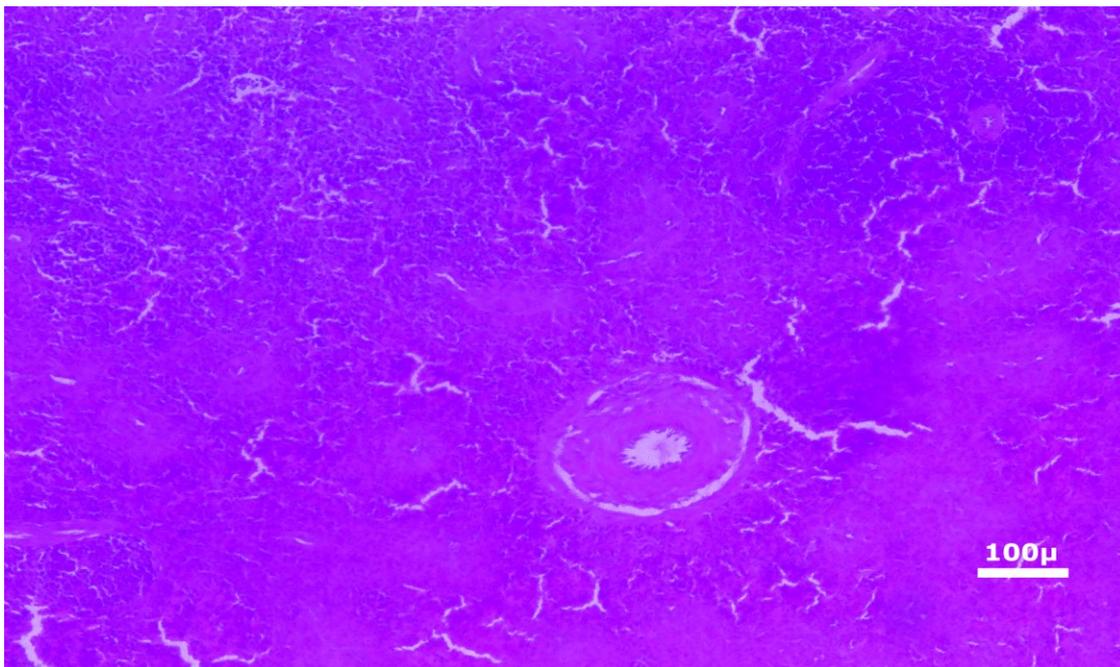
FIG.(1) : *Plocamium cartilagineum* (Linnaeus) Dixon



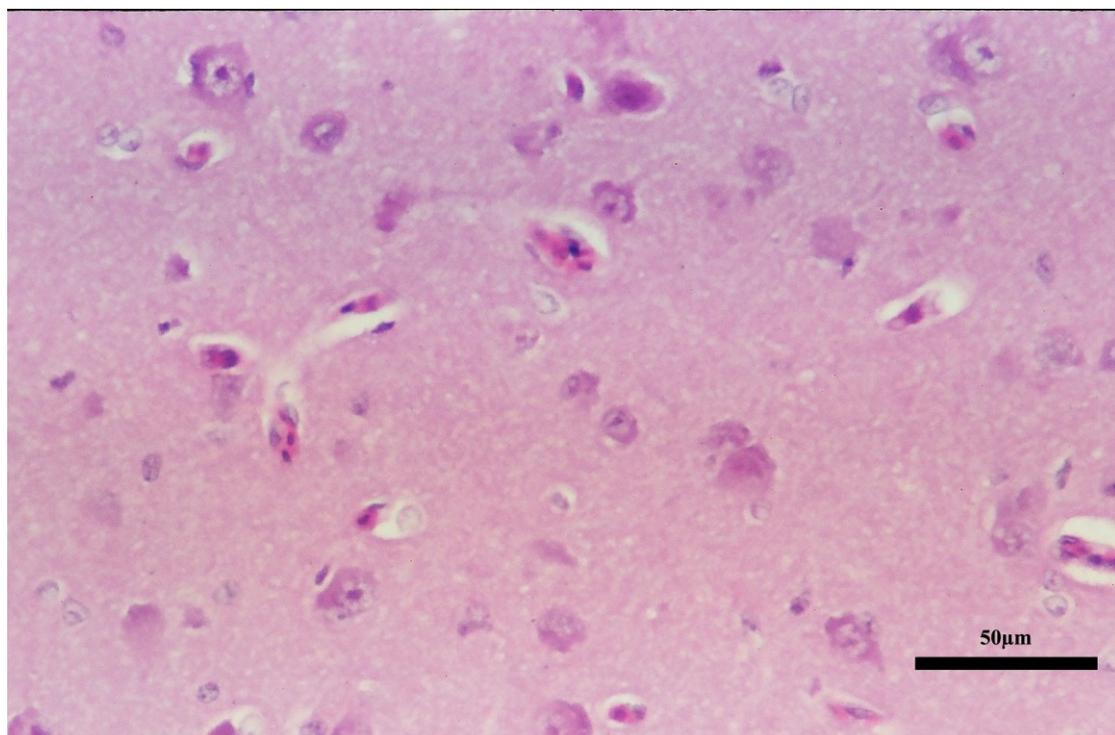
(FIG.2) : The lung showed normal histological appearance without any fluid in alveoli or pulmonary inflammation beside mild congestion of interalveolar capillary.(Control)



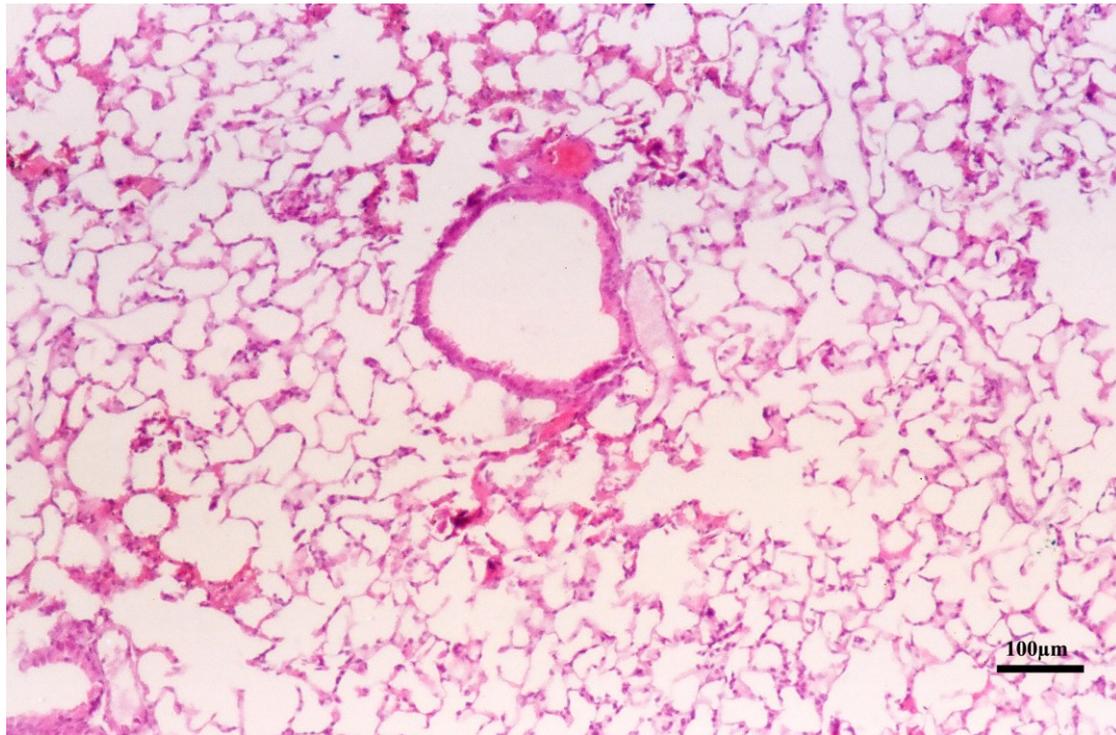
(FIG.3) : The kidney showed normal histological appearance without any necrosis or inflammation beside. (Control)



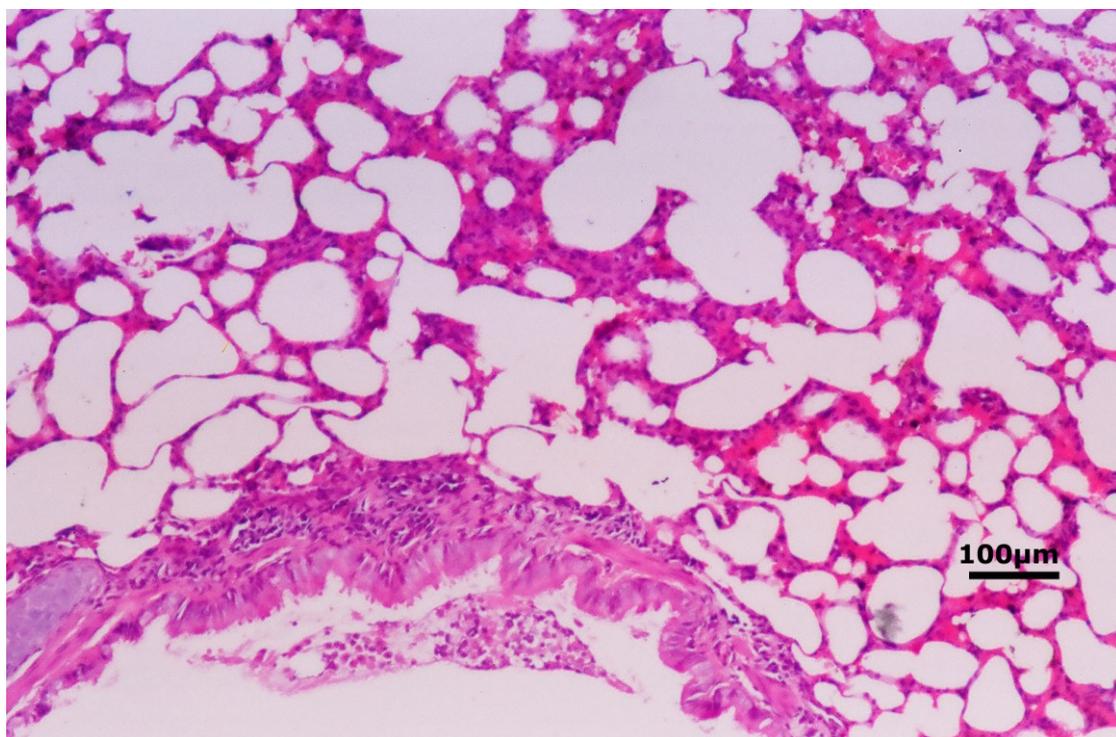
(FIG.4) : The spleen showed normal histological structure of red and white pulp.(Control)



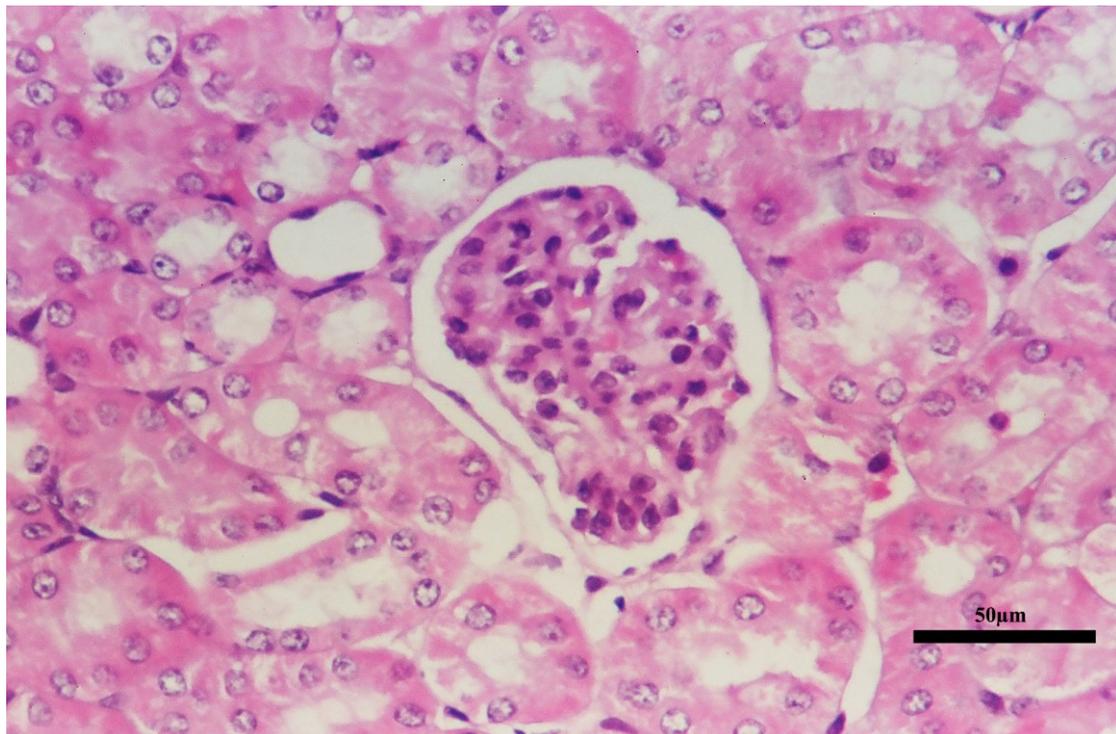
(FIG.5) : The brain showed little mild-degenerative changes in neurons beside normal neuropil without any abnormality.(Control)



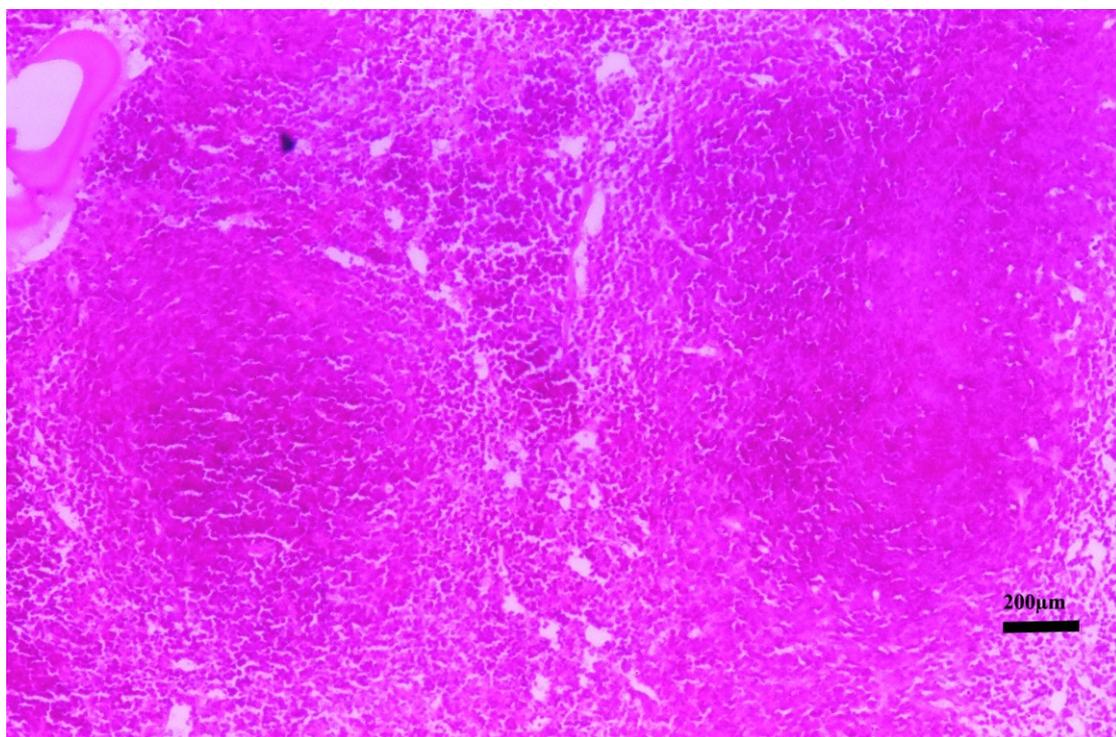
(FIG.6) : The liver showed little mild degenerative changes.The portal areas showed normal histological structure without evidence of fibrosis or congestion. (Control)



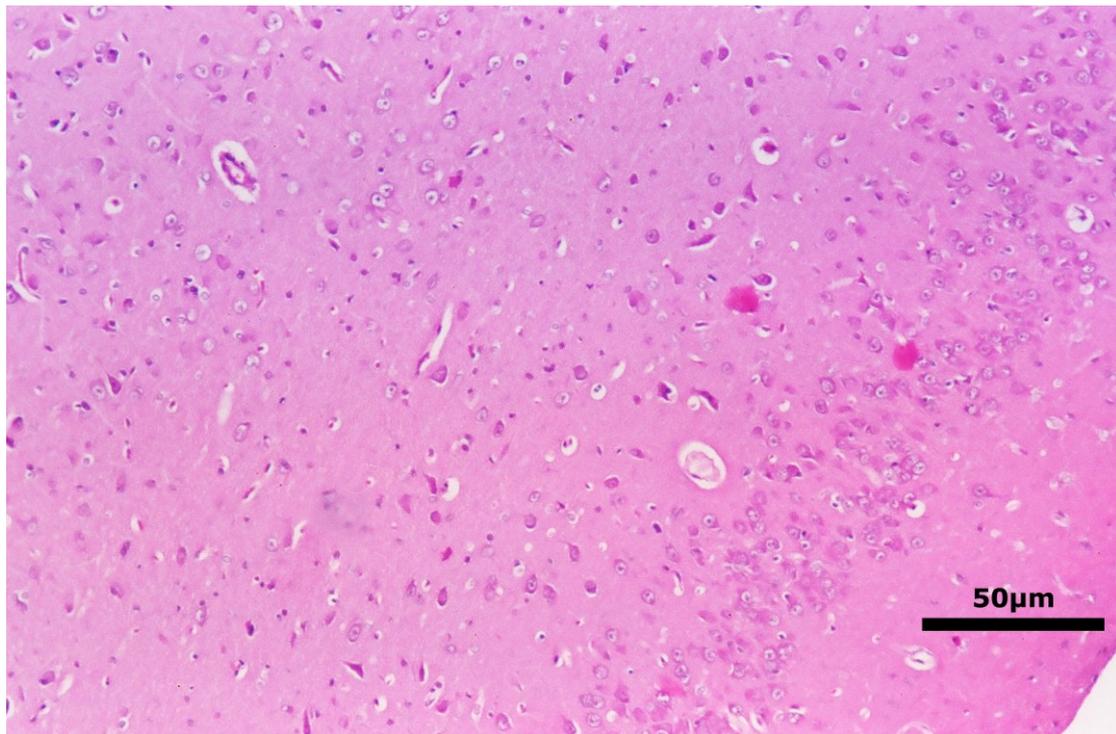
(FIG.7) : The lung showed little mild degenerative changes.The portal areas showed normal histological structure without evidence of fibrosis or congestion.(Both Treat.1 & 2).



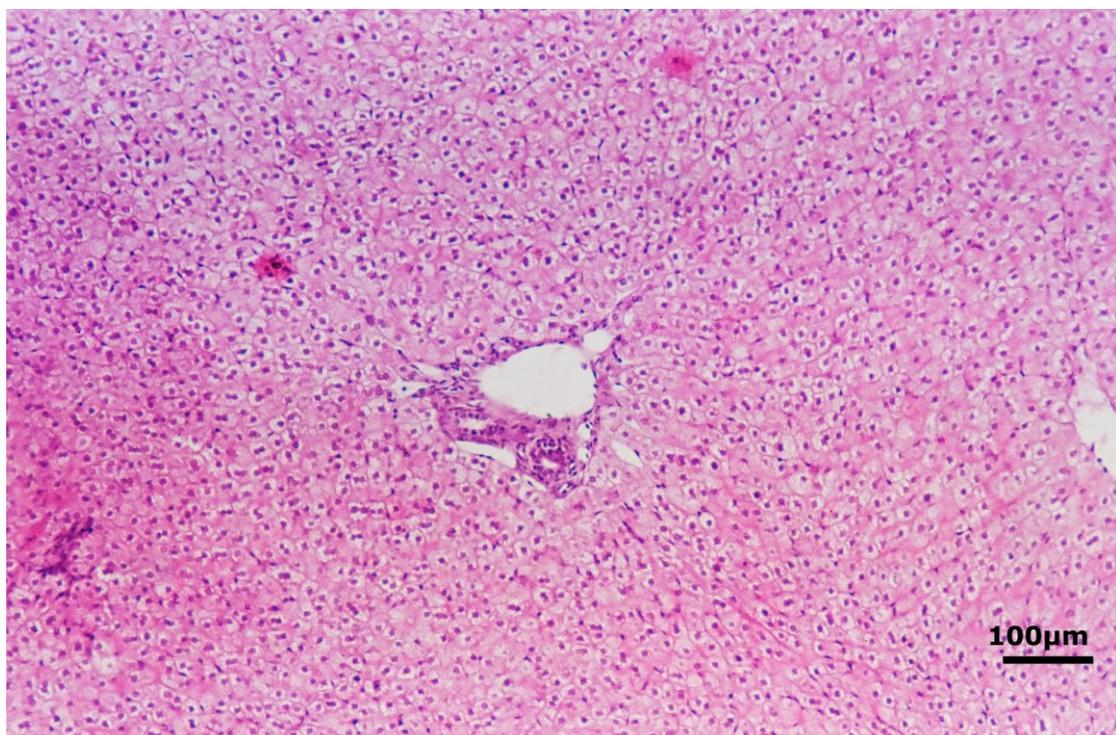
(FIG.8) : The kidney normal glomeruli, beside slight cloudy swelling in some renal tubular epithelium. (Both Treat.1 & 2).



(FIG.9) : The spleen showed normal lymphoid follicles without any abnormalities. (Both Treat.1 & 2).



(FIG.10) : The brain(white matter) moderate astrocytosis with normal brain architecture. (Both Treat.1 & 2).



(FIG.11) : The liver showed hydropic degeneration in hepatocyte with mild round cell infiltration in portalarea. (Both Treat.1 & 2).

Table (1): Effects of violacene extracted of the red alga *Plocamium cartilagineum* on biochemical parameters in rats.

Parameters	Control	Treat.1 ¹	Treat.2 ²
Glucose (mg/dl)	92.826 ± 2.49	101.02 ± 2.06	99.118 ± 1.59
Urea (mg/dl)	43.08 ± 0.48	39.30 ± 0.49**	37.53 ± 0.62**
Cholesterol (mg/dl)	65.53 ± 1.74	66.04 ± 0.78	69.68 ± 0.86
Protein (g/dl)	6.17 ± 0.9	7.20 ± 0.10*	7.61 ± 0.26*
Phosphate (mg/dl)	7.00 ± 0.93	6.91 ± 0.07	6.16 ± 0.11
Creatinine (mg/dl)	0.60 ± 0.04	0.58 ± 0.01	0.59 ± 0.007
Sodium (meq/L)	136.43 ± 2.7	140.55 ± 1.17	140.72 ± 1.44
Potassium (meq/L)	3.22 ± 0.10	3.45 ± 0.14	4.14 ± 0.06*
AST (IU/L)	43.79 ± 0.97	45.62 ± 1.70	47.73 ± 1.11*
ALP (IU/L)	32.79 ± 0.68	33.62 ± 0.77	34.86 ± 0.66

Values represent mean ± SD, significance relative to control: *P<0.05; **P<0.01.
Treat.1¹: Treatment with LD50 value, Treat.2²: Treatment with 2XLD50 value

Table (2): Effects of violacene extracted of the red alga *Plocamium cartilagineum* on haematological parameters in rats.

Parameters	Control	Treat.1	Treat.2
Haemoglobin (g%)	10.65 ± 0.82	13.785 ± 0.76**	11.72 ± 0.05
RBC (10 ⁶ /mm ³)	4.30 ± 0.11	4.41 ± 0.10	4.52 ± 0.57
PCV (%Volume)	41 ± 1.34	41.05 ± 1.32	42.14 ± 1.79
Platelets (10 ⁵ /mm ³)	2.99 ± 0.16	3.00 ± 0.74	3.02 ± 0.09
WBC (10 ³ /mm ³)	9744 ± 64	9779 ± 42**	9982 ± 21**
Polymorphs (%)	55.56 ± 1.93	57.11 ± 0.69**	57.95 ± 1.99**
Lymphocytes (%)	36.57 ± 0.97	36.72 ± 0.70	38.37 ± 0.97**
Eosinophils (%)	0.40 ± 0.09	0.43 ± 0.03	0.50 ± 0.06**

Values represent mean ± SD, significance relative to control: *P<0.05; **P<0.01.
Treat.1¹: Treatment with LD50 value, Treat.2²: Treatment with 2XLD50 value

REFERENCES

- Argandoña,V., Del Pozo,T., San-Martín,A. and Rovirosa,J.2000. Insecticidal activity of *Plocamium cartilagineum* monoterpenes. Bol. Soc. Chil. Quim., 45 (3) :1-6.
- Copp,B.2003. Antimycobacterial natural products. Nat. Prod. Rep., 20: 535–557.
- Dacie JV, Lewis SM. Practical Haematology. Churchill Livingstone, New York: 1991. pp. 52–56. 67–69.
- Miller LC, Tainter ML. Estimation of LD50 and its errors by means of log probit graph paper. Proc. Soc. Exp. Biol. Med. 1944 ;57:261–264.
- Ringler DH, Dabich L. Haematology and Clinical Biochemistry. In: Baker J, Lindsey JR, Weisbroth SH, editors. The Laboratory Rat. London: Academic press; 1979. pp. 105–118.
- Varley, H., Gewenlock ,A.H.and Bell, M.Practical Clinical Biochemistry. Delhi : CBS Publishers & Distributors; 1991. pp. 741–742.