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Effect of Toxicity of the Cyanobacterium (Blue green algae) *Oscillatoria rubescens* on blood and liver of the rats

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ABSTRACT. The effect of the cyanobacterium *Oscillatoria rubescens* eells extract on blood and liver of Wistar rat was investigated. Rats were divided into three groups and were injected intrapertioneally (i.p.) with weekly reciprocal dnses of *Oscillatoria rubescens* extract as 17.5, 35 and 70 mg/ rat, respectively for 7 weeks. Rats injeeted with 17.5 mg exhibited changes in WBC, platelets and MCHC associated with a depletion in blood glucose. Meanwhile, urea, triglycerides, creatinine, AST&ALT were increased in the serum of rats. Rats injected with 35 mg weekly showed mild decrease in RBC, blood hemoglobin (Hb) HCT, blood platelets and total leucocytic count. The serum of this group showed an increase in the levels of cholesterol, triglyceride and marked increase in AST and ALT. Rats received 70 mg were the mostly affected group and showed signs of acute eellular and physiological damage involving oligocythemia, leucoytosis, marked increase in serum urea, cholesterol, triglycerides, creatininc, AST, ALT, HCT, MCHC, and MCV while platelets showed abnormal increase. It is suggested that the crude eellular extract of *Oscillatoria rubescens* particularly at doses of 70mg aod ahove has an inhibitory action on haemopiesis. In addition, the abnormal pathophysiology observed here reflects the severe toxic effect from the crude extract of the cyanohacteria on both liver and kidney.

Introduction

Toxin-producing cyanobacteria pose a world-wide health threat to both humans and animals due to their increasing presence in both drinking and recreational water (Gehringer *et al.*, 2003). Microcystins released by cyanobacteria have severe toxic effects on liver, kidney, blood and other organs in humans and animals (Milutinovic *et al.*, 2003; Fleming *et al.*, 2003). Among these potent cyanobacteria is *Oscillatoria* sp., which is considered to be the most dominant species in natural freshwater habitat. (Reynolds & Bellinger, 1992; Namikosh *et al.*, 1992).

Oscillatoria sp. inhabits freshwater of rivers, ponds, streams and lakes world-wide (Bruno *et al.*, 1992). Mohsen and Al-Amoudi (1989) isolated several species of cyanobacteria from fresh water in Makkah, Saudi Arabia and suggested the toxic effeet of such bacteria. Jamel Al-Layl and Jamal Al-lail (1993) reported the first record on toxic

cyanobacteria including *Oscillatoria* sp., *Microcystis* sp. and yellow *Microcysits* sp. from Makkah area, the western province of Saudi Arabia. Arif (1997) mentioned the presence of *Oscillatoria* spp., *Synechococcus* sp. and *Calothrix* sp. in Bani Malek, Gizan province, Saudi Arabia.

Ingestion of contaminated water with cyanobacteria is very risky for humans and livestocks because of the possible accumulation of such toxins in different organs and tissues in vertebrate bodies. One of the most affected organs is liver which develops malignancy in the matter of few months (Ito *et al.*, 1997; Martin, 1998; Fleming *et al.*, 2002). Liver damage caused by cyclic heptapeptides leads to profound changes in the pathophysiology of liver enzymes including ALT and AST. The severity of liver damage occurrs due to the oxidative shock in hepatocytes which not only inhibits the liver enzyme ALT and AST but also significantly suppresses all other enzymes involved in liver function (Li *et al.*, 2003; Guzman *et al.*,2003).

The present study aims to evaluate the effect of crude extract of *Oscillatoria rubescens* on blood and liver of rats.

Material & Methods

Collection of water samples

Water samples were collected from different locations in Makkah area as Al-Sharay and Umm Alkodad farm sites. Samples were collected in one liter acid-washed polyethylene bottles at a depth ranging from 25cm up to 3 meters from concrete basin at the sites of collection. Bottles were exposed to direct sunlight to enhance the growth of cyanobacteria.

Identification of different cyanobacteria species

Twelve species were identified from collected sample according to the key proposed by Streble & Krauter (1978). Among the identified populations of cyanobacteria, five species belonging to the genus *Oscillatoria* were the most dominate. The five identified species were *O. agardhii, O. bervis, O.limnetica, O. tenuis and O. rubescens*, respectively.

Isolation, Purification and growing of O. rubescens.

Since the cyanobacterium *O. rubescens* was the most dominant, it has been selected for the purpose of the present study. Isolation and purification were carried out in accordance to Daft (1988). Original water sample conraining populations of cyanobacteria were suspended in 100 ml of synthetic culture medium of CT medium (Watanabe & Nozaki, 1994). The homogenate was then cnltured and incubated at 28°C ± 2.0 and permited to grow at light intensity of 2000 lux.

Preparation of cell free extract (CFE)

As the biomass density reached its maximum growth (3-4 weeks), cells were harvested and allowed to pass through 5µm mesh disposable filter, (Millipore, USA). The harvested cells were washed with distilled water, centrifuged at 15000 xg for 20 minutes and kept frozen at -20 ° C (Van der westhuizen & Eloff, 1983, 1985 Park *et al.*, 1993). Frozen dried cells (200mg) were mixed with 8ml sterilized distilled water and then broken

by ultra-sonication for 5 minutes on icc –bath followed by centrifugation at 15000 rpm for 40 minutes. The supernatant (CFE) were used for toxicity tests (Watanabe & Oishi, 1985 and Jamel Al-Layl, 1996).

Experimental animals

Adult males of Wistar Albino rats (*Rattus norvegicus*) aged 3 months and weighing 350 ± 10 g were kindly obtained from animal house, King Fahd Medical Research Center. Thirty two rats were housed (4 per cage) at a constant temperature of 25°C with periods of 12 / 12 hr (light/dark). Rats were divided into four groups subjected to reciprocal doses of cell free extract of the cyanobacterium O. rubescens as follows:

1- Group I were injected a dose of 17.5 mg (1/16 dilution).
2- Group II were injected a dose of 35 mg (1/8 dilution).
3- Group III were injected a dose of 70 mg (1/4 dilution).
4- Group IV were injected a dose of 1 ml distilled water (controls).

All animals were injected intrapertioneally. The first case of death was recorded at the end of the third week.

Haematological and serological studies

Blood samples were collected from both treated and control rats by cardiac puncture on weekly basis for seven weeks according to Waynforth (1980). The haematological parameters investigated were red blood cell (RBC's) count and shape, haemoglobin content (Hb), haematocrite value (HCT) ,mean cell haemoglobin concentration (MCHC)and white blood cell count (WBC's). Serum glucose, albumin, cholesterol, triglycerides, creatinine .bilirubin, SGOT, and SGPT were measured to evaluate the pathophysiological changes induced by such toxins.

Histopathological studies

Animals were dissected at the end of experiment and random pieces of liver were taken, fixed in 10% buffered formaline then subjected to routine histology with haematoxyline and eiosin , examined under microscope and photographed.

Results

Haematological Studies

Pathophysiological changes in blood of rats injected with doses of 17.5,35 and 70 mg/rat are presented in Tables (1) through Table (7). Platelets were the most affected with a significant decrease in number from the beginning of the second week. Decrease reached its peak at the seventh week in rats injected with 70 mg of the cellular crude extract. Leukocytes exhibited significant increase in number, reached its maximum at the seventh week with the dose of 70mg/rat. Suppressive effect of crude extract on haemopiesis in the second and third groups was observed from the beginning of the third week. A significant depletion on both erythrocytic count and its haemoglobin content ($P<0.05$). The rest of haematological parameters were increasing and decreasing in a fluctuant manner.

Table (1). Serum analysis of the 1st week trail of rats exposed to hepatotoxins from whole cell of the toxic cyanobacterium *Oscillatoria rubescens*.

Test	Control	1/4 Dilution	1/8 Dilution	1/16 Dilution
WBC (1000/µl)	7.2+ 0.77	7.56±0.33	7.12±0.38	7.55±0.75
RBC (1000//µ)	7. 3 ± 0.23	7.36± 0.43	7.49±0.29	7.53±0.47
Hb (g/d1)	13.94± 0.41	13.28±0.47	13.94±0.91	14.56±0.72
HCT %	40.92± 0.54	38.58±1.67	40.67±0.92	39.75±0.59
MCV (fL)	51.45± 0.45	51.69± 0.25	53.84±0.52	51.69±0.35
MCHC (g/d1)	31.02± 0.36	33.79± 0.24*	31.64±0.76	32.15±0.59
MCH (pg)	17.91± 0.24	19.32±0.12*	18.72± 0.32	17.87±0.38
Platelets (1000//µl)	984 ± 29.06	784 ±12.79*	999.9±28.45*	978.24±32.27
Glucose (mg/dl)	126.90± 3.01	117.0±2.12*	123.62±1.81*	123.12±1.29
Cholesterol (mg/dl)	61.13± 2.48	63.25±1.25	59.43±1.17	58.25±0.59
Triglyeeride (mg/dl)	71.25± 1.30	76.50± 1.40	72.00±1.89	74.62±1.31
Liver function				
Total protein (g/dl)	6.68±0.38	6 .57±0.16	6.91±0.06	6.47±0.10
Albumin (g/dl)	3.46±0.25	3.74± 0.11	3.57±0.07	3.45±0.08
Total bilirubin (mg/dl)	0.17±0.01	0.19± 0.07	0.18±0.06	0.17± 0.55
AST (ull)	35.38±1.24	57.88± 1.38*	50.00±0.65*	41.65±0.45
ALT (ull)	36.12±1.55	51.38± 1.02*	51.50±1.12*	42.38±1.66
Kidney function				
Urea (mg/dl)	22.35±1.56	38.98±0.65*	41.82±1.45*	34.39±0.67*
Creatinine (mg/dl)	0.54± 0.32	0.82±0.12	0.75±0.42*	0.55±0.14

(*) Significant at P<0.05 with sindent t-test compared to control.

Table (2). Serum analysis of the 2nd week trail of rats exposed to hepatotoxins from whole cell of the toxic cyanobacterium *Oscillatoria rubescens*.

Test	Control	1/4 Dilution	1/8 Dilution	1/16 Dilution
WBC (1000/µl)	7.31±0.67	7.21±0.24	7.92±0.54	7.42±0.19
RBC (1000//µ)	7.63 ± 0.18	7.72±0.18	7.19±0.130	7.37±0.96
Hb (g/d1)	13.94±0.35	15.21±0.27	14.02±0.24	14.62±0.92
HCT %	40.92±0.66	44.11±0.88	40.22±0.65	39.82±0.43
MCV (fL)	51.48±0.77	53.96±0.84	52.67±0.42	51.74±0.93
MCHC (g/d1)	35.16±0.26	35.28±0.25	34.94±0.76	33.89±0.34
MCH (pg)	19.51±0.27	19.15±0.10	18.81±0.23	19.56±0.24
Platelets (1000//µl)	972.4 ±26.56	732.25+25.40*	881.2±26.6*	885.00±36.25
Glucose (mg/dl)	127.50±2.11	122.38±1.50	119.50±1.22	122.12±1.69
Cholesterol (mg/dl)	60.12±2.68	73.25±1.44	58.25±0.77	58.35±0.29
Triglyceride (mg/dl)	70.75±1.00	87.62±2.08	76.38±1.27	74.62±1.31
Liver function				
Total protein (g/dl)	6.78±0.28	6.38±0.10	6.71±0.11	6.69±0.08
Albumin (g/dl)	3.82±0.15	3.44± 0.09	3.33±0.07	3.55±0.06
Total bilirubin (mg/dl)	0.12±0.00	0.12± 0.00	0.12±0.00	0.12± 0.00
AST (ull)	35.38±1.24	49.75± 1.38*	56.25±1.25*	37.38±0.68
ALT (ull)	36.12±1.55	45.75± 0.92*	58.75±1.66*	47.38±0.80*
Kidney function				
Urea (mg/dl)	24.75±1.46	38.88±0.95*	41.62±1.15*	39.50±0.87*
Creatinine (mg/dl)	0.53± 0.02	0.8±0.03	0.78±0.02	0.55±0.03

(*) Significant at P<0.05 with student t-test compared to control.

Table (3). Serum analysis of the 3rd week trail of rats exposed to hepatotoxins from whole cell of the toxic cyanobacterium *Oscillatoria rubescens.*

Test	Control	1/4 Dilution	1/8 Dilution	1/16 Dilution
WBC (1000/µl)	7.11±0.47	11.71±1.45	8.56±0.83	8.35±0.63
RBC (1000//µ)	7.53 ± 0.18	6.76±2.23	6.78±0.34	7.36±0.34
Hb (g/d1)	14.54±0.31	13.75±0.48	14.14±0.43	14.44±0.07
HCT %	41.32±0.64	36.57±0.46	39.35±0.74	41.04±0.28
MCV (fL)	51.28±0.57	52.97±0.88	52.24±0.37	53.32±0.34
MCHC (g/d1)	31.12±0.66	333.73±0.46	31.45±0.59	32.33±0.29
MCH (pg)	18.51±0.27	18.14±0.37	18.38±0.22	19.61±0.74
Platelets (1000//µl)	972 ±26.56	749.88±29.38*	821.8±29.10*	861.45±37.56*
Glueose (mg/dl)	127.50±2.11	118.75±1.00	111.50±2.23	116.85±2.94
Cholesterol (mg/dl)	60.12±2.68	59.52±0.48	57.55±1.67	59.22±0.32
Triglyceride (mg/dl)	70.75±1.00	90.62±0.78*	87.34+3.55	78.92±0.73
Liver function				
Total protein (g/dl)	6.67±0.43	5.91±0.16	6.43±0.21	5.76±0.16
Albumin (g/dl)	3.81±0.54	3.22±0.02	3.29±0.17	3.33±0.43
Total bilirubin (mg/dl)	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00
AST (nll)	34.98±1.64	56.43±0.68*	61.17±0.73*	57.23±0.64*
ALT (ull)	37.18±1.75	53.46±1.78*	63.08±1.05*	52.97±2.23*
Kidney function				
Urea (mg/dl)	23.72±1.36	51.74±0.65*	46.55±1.46*	42.68±0.93*
Creatinine (mg/dl)	0.52± 0.12	1.10±0.05	0.55±0.02	0.66±0.12

(*) Significant at P<0.05 with student t-test compared to control.

Table (4). Serum aualysis of the 4th week trail of rats exposed to hepatotoxins from whole cell of the toxic cyanobacterium *Oscillatoria rubescens.*

Test	Control	1/4 Dilution	1/8 Dilution	1/16 Dilution
WBC (1000/µl)	7.12±0.47	12.89±0.54*	8.90±2.46	9.45±0.56*
RBC (1000//µ)	7.74 ± 0.18	5.43+0.44*	6.55±1.13	7.75±0.24
Hb (g/d1)	14.44±0.81	10.34±0.34*	13.61±0.78	15.34±0.53
HCT %	41.62±0.74	31.55±0.34*	39.78±0.57	40.53±0.55
MCV (fL)	52.85±0.57	54.20±0.88	51.76±0.48	52.75±0.34
MCHC (g/d1)	30.92±0.96	33.43±0.66	30.43±0.71	33.31±074
MCH (pg)	18.41±0.77	19.34±0.84	18.45±0.21	19.46±0.72
Platelets (1000//µl)	984+26.46	540.53±6173*	516.67±39.90*	779.70±31.42*
Glucose (mg/dl)	126.98±2.21	116.85±1.63	103.99±2.29	117.89±3.59
Cholesterol (mg/dl)	61.02±2.78	75.98±1.23	63.23±1.67	58.98±1.53
Triglyceride (mg/dl)	70.75±2.70	83.60±1.51	90.77±2.33	77.88±1.71
Liver funetion				
Total protein (g/dl)	6.78±0.28	6.71±0.06	5.79±0.06	6.76±019
Albumin (g/dl)	3.82±0.15	3.58±0.04	3.00±0.09	3.54±0.21
Total bilirubin (mg/dl)	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00
AST (ull)	35.38±1.24	54.75±0.82*	64.75±1.58*	69.95±0.43*
ALT (ull)	36.12±1.55	57.75±0.80	69.00±1.98	56.62±1.15
Kidney function				
Urea (mg/dl)	24.75±1.54	46.8±1.67*	48.79±0.60*	42.63±0.69*
Creatinine (mg/dl)	0.58± 0.12	0.91±0.06	0.88±0.02	0.70±0.02

(*) Significant at P<0.05 with student t-test compared to control.

Table (5). Serum analysis of the 5th week trail of rats exposed to hepatotoxins from whole cell of the toxic cyanobacterium *Oscillatoria rubescens*.

Test	Control	1/4 Dilution	1/8 Dilution	1/16 Dilution
WBC (1000/µl)	7.32±0.83	13.50±0.45*	8.94±0.34	9.87±0.12*
RBC (1000//µ)	7.49± 0.23	5.78±0.15*	5.94±0.15*	7.35±0.82
Hb (g/d1)	14.76±0.78	10.93±0.10*	12.30±0.09*	14.99±0.59
HCT %	41.55±0.56	31.87±0.66*	37.77±0.58	40.85±0.90
MCV (fL)	51.67±0.89	52.37±0.29	51.56±0.34	52.57±0.75
MCHC (g/d1)	31.38±0.76	32.10±0.64	30.01±0.26	32.85±0.34
MCH (pg)	18.42±0.43	19.18±0.64	18.54±0.28	18.98±0.20
Platelets (1000//µl)	985 ±26.57	576.5±53.86*	735.55±19.17*	757.54±29.34
Glucose (mg/dl)	126.90±2.66	118.97±1.39*	113.44±3.32*	108.59±2.93*
Cholesterol (mg/dl)	61.45±3.78	82.78±2.38*	59.56±3.67*	60.79±0.44
Triglyceride (mg/dl)	69.67±1.90	91.17±0.82*	84.90±2.13*	79.69±0.72*
Liver function				
Total protein (g/dl)	6.66±0.89	5.89±0.11	5.54±0.06*	6.67±0.18
Albumin (g/dl)	3.79±0.67	3.34±0.11	2.91±0.06*	3.65±0.23
Total bilirubin (mg/dl)	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00
AST (ull)	34.99±2.46	62.86±1.03*	67.96±1.67*	62.88±0.85*
ALT (ull)	35.92±2.62	63.76±1.13*	73.60±1.49*	60.38±0.86*
Kidney function				
Urea (mg/dl)	23.95±2.35	51.12±0.30*	49.63±1.42*	43.75±0.67*
Creatinine (mg/dl)	0.54± 0.02	1.14±0.06*	0.84±0.11*	0.81±0.02*

(*) Significant at P<0.05 with student t-test compared to control.

Table (6). Serum analysis of the 6th week trail of rats exposed to hepatotoxins from whole cell of the toxic cyanobacterium *Oscillatoria rubescens*.

Test	Control	1/4 Dilution	1/8 Dilution	1/16 Dilution
WBC (1000/µl)	7.18± 0.39	13.12±0.26*	10.39±0.34*	10.02±0.34*
RBC (1000//µ)	7.54 ± 0.32	5.79±0.76*	6.10±0.18	7.34±0.10
Hb (g/d1)	14.53±0.64	11.83±0.28*	12.86±0.23	14.65±0.26
HCT %	41.43±0.11	33.96±0.77*	40.89±0.36	40.30±0.19
MCV (fL)	51.33±0.64	54.67±0.53	52.32±0.35	52.10±051
MCHC (g/d1)	31.18±0.47	32.95±0.67	31.31±0.27	32.62±0.47
MCH (pg)	18.82±0.33	19.83±0.86	19.12±0.04	19.79±0.26
Platelets (1000//µl)	958 ±43.56	574.0±63.9*	657.5±17.7*	726.25±23.14*
Glucose (mg/dl)	126.40±3.49	122.64±1.19	112.60±2.33*	113.5±2.17*
Cholesterol (mg/dl)	61.82±2.35	82.56±0.93*	58.95±2.32	61.88±0.72
Triglyceride (mg/dl)	72.05±1.07	96.75±1.52*	79.22±2.74*	78.80±0.46*
Liver function				
Total protein (g/dl)	6.86±0.51	6.25±0.09	5.79±0.26	6.95±0.27
Albumin (g/dl)	3.38±0.42	3.444±0.08	3.86±0.03	3.65±0.16
Total bilirubin (mg/dl)	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00
AST (ull)	36.72±1.32	65.28±0.88*	64.96±1.48*	59.02±1.64*
ALT (ull)	35.90±1.55	67.95±0.75*	61.12±0.51*	54.12±0.80*
Kidney function				
Urea (mg/dl)	25.05±1.26	85.25±1.10*	47.00±0.96*	46.25±0.45*
Creatinine (mg/dl)	0.53± 0.02	1.12±0.06*	0.88±0.03*	0.79±022*

(*) Significant at P<0.05 with student t-test compared to control.

Table (7). Serum analysis of the 7th week trail of rats exposed to hepatotoxins from whole cell of the toxic cyanobacterium *Oscillatoria rubescens*.

Test	Control	1/4 Dilution	1/8 Dilution	1/16 Dilution
WBC (1000/µl)	7.43 ± 0.37	13.59±0.64*	11.53±0.23*	11.20±0.64*
RBC (1000//µ)	7.22 ± 0.24	5.62±0.26*	6.25±0.09	7.29±0.74
Hb (g/d1)	14.64±0.58	12.41±0.29*	11.95±0.15*	13.78±0.22
HCT %	41.47±0.48	36.98±0.38	39.79±0.65	39.86±0.65
MCV (fL)	51.73±0.75	53.43±0.91	51.96±0.99	51.48±0.46
MCHC (g/d1)	31.34±0.86	34.11±0.43	32.88±0.34	3135±0.40
MCH (pg)	18.11±0.33	20.64±0.55	19.83±0.13	19.03±0.78
Platelets (1000//µl)	976 ±26.40	528.25±68.38*	621.02±19.03*	671.25±23.90
Glueose (mg/dl)	127.53±2.31	118.35±0.25*	105.85±2.61*	111.50±1.63*
Cholesterol (mg/dl)	60.62±2.64	84.69±0.88 *	77.35±1.67*	62.01±0.53
Triglyeeride (mg/dl)	70.85±1.50	112.6±1.24*	86.93±1.94*	79.78±0.56
Liver function				
Total protein (g/dl)	6.48±0.28	6.18±0.04	6.06±0.06	6.81±0.04
Albumin (g/dl)	3.05±0.15	3.64±0.06	3.36±0.06	3.60±0.05
Total bilirubin (mg/dl)	0.11±0.00	0.11±0.00	0.11±0.00	0.11±0.00
AST (ull)	35.86±1.24	115.38±2.00*	102.62±1.31*	54.12±1.03*
ALT (ull)	36.42±1.55	84.62±0.91*	72.50±0.65*	49.21±1.01*
Kidney function				
Urea (mg/dl)	23.95±1.43	65.85±1.71*	51.93±1.14*	49.75±0.57*
Creatinine (mg/dl)	0.57± 0.02	1.56±0.07*	0.89±0.10*	0.67±0.05*

(*) Significant at P<0.05 with student t-test compared to control

Blood biochemistry

Glucose level in the three treated groups showed fluctuated decrease in the first three weeks and stable decrease in the last three weeks without exception. Serum eholesterol increased significantly (P<0.05) in the last four weeks in the third group. Serum triglycerides increased significantly in the last four weeks in the three groups (P<0.05).

As presented in Table 5, 6 and 7 both serum urea and creatinine exhibited prominent increase even from the 1st week (P<0.05) and this reveals that renal stress was indueed particularly in the third group at the end of the 7th week. Surprisingly, the values increased three times fold compared to that of the control group. It is obvious that the toxin content in the crude extract caused a fall in glomerular filtration that subsequently raised the level of serum creatinine uremia. This suggests the induction of renal damage or renal toxicity and probably would lead to renal failure.

Total proteins have not been affected except in the 2nd group in the second and fifth weeks. Serum albumin showed significant fluctuated decrease (P<0.05) in the three groups. However, rats of group two were the only affected by such decrease (hypoalbuminaemia). One of the causes of hypoalbuminaemia is hepatopathy associated with tissue damage in liver. This also suggests that such toxins induced liver damage. Total bilirubin, however, showed no changes in the three groups. Here the author cannot find an explanation for such absolute stable values.

Effect of O. Oscillatoria rubescens on blood enzymes

Both AST (previously known as GOT) and ALT (previously known as GPT) raised significantly (P<0.05) in serum of the three groups without exception. The elevated level in

these two enzymes reflects the extreme toxic effect of the microcystin toxins. Interestingly, the levels of these two enzymes have increased two folds in value in the second and the third groups. This suggests that the chronic effect of such toxin would cause extreme damage in liver of treated rats. According to the rule that the higher the AST and ALT values, the greater the damage in liver, as has been observed clearly in pilot experiment, a dose more than 70 mg/rat for more thau one week probably becomes lethal.

Effects on rat liver

Chronic treatment with cell extract induced noticeable pathophysiological effect on rat. The most common histopathological effect was congestion of liver with dilated blood sinusoids. This was associated with nuclear atrophy and degeneration of hepatic cords. What supports the phenomenon of congestion liver is the appearance of multiple hemorrhagic and so leaky foci among liver parenchyma. This leads to promoting deposition of collagen fibers around both portal veins and biliary tracts. Meanwhile, the biliary passages wall exhibited deformity and primary atresia.

Discussion

Microcystins are the most frequently cyclic heptapeptides produeed by different genera of cyanobacteria and act as hepatotoxins for both humans and animals. Cyanobacteria induce a wide variety of pathophysiological symptoms. Among these potent harmful cyanobacteria is *O. rubescens*, which showed significant effect on blood parameters, liver and kidney of treated rats. The present results of biochemical analyses run parallel to that described by Oudra *et al* (2002), who observed high toxicity in mice injected intraperitoueally with bloom sample of *Microcytis* microcystins. The microcystin produeed by *O. rubescens* had a very defective action on blood platelets. Siegelman *et al* (1984) also reported the reduction of platelets (thrombocytopenia) in treated rats. The present findings revealed reduction in numbers of both RBCs and platelets due to suppressive and toxic effect on bone marrow and subsequently on haematopoiesis. Since platelets are syuthesized in bone marrow, so the double suppressing actions on RBCs and platelets would be explained. As there is a reciprocal relationship between oxidation of glucose and fatty acids, it may be concluded that the depletion in oxidation of glucose and glycogen in liver cells leads to promoting oxidation of fatty acids. This subsequently will yield more cholesterol and triglycerides.

Guzman *et al* (2003) reported that toxic inhibitory shock caused by microcystin released by cyanobacreria leads to disturbiug liver enzymatic activity particularly in sublethal doses. Li *et al* (2003), confirmed the same findings in fish hepatocytes. They reported that microcystins induce oxidative shock in hepatocytes. The mechanism of toxicity is similar in both fish and rat hepatocytes. As a result, it was suggested that the toxic effect is nearly the same in all vertebrates including humans. The intrahepatic hemorrhage and necrosis induced by microcystin was confirmed by Milutinovic *et al* (2003), especially in chronic treatment with such toxius in treated rats. As a result, the present finding runs parallel to that reported earlier by many workers. Uraemia observed in treated rats was induced by intoxication with microcystin producing hepatotoxicity and nephrotoxicity. Urea is filtered by renal glomeruli, which are collapsed due to microcystins circulated in blood. As a result, renal damage due to chronic toxicity elevated both serum urea and creatinine in blood of treated rats. These finding are compatible with that of

Milutinovic *et al* (2003). They revealed also that pathological changes induced by microcystin LR appeared more sever than those induced by microcystin YR. The potential toxic effect of cyanobacteria is extending to plant. Gehringer *et al* (2003), observed a significant decrease in root development in seeds of *Lepidium sativum* treated with microcystin LR.

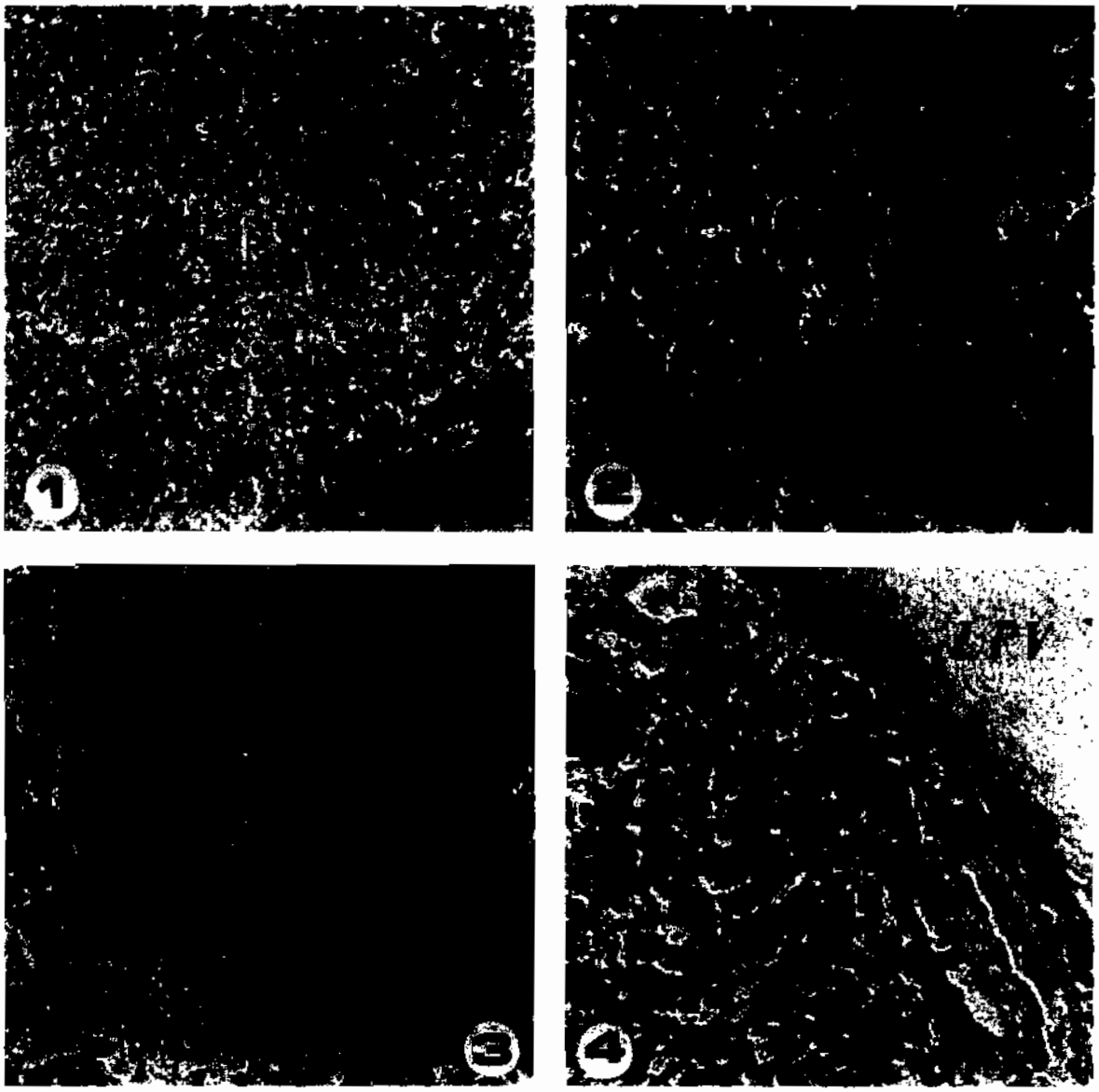

Plate I

Hepatotoxic effects induced by whole cell extract of *O. rubescens.*

1- Control liver. Arrow is pointed to aetive mitosis. The letters PV refer to portal vein, X100.

2- The dilated blood sinusoidal (S) with nuclear atrophy. Note also that liver parenchyma has lost its architecture. The letters CV refer to central vein, X100.

3- Marked hemorrhage (H) in foci adjacent a portal vein (PV). Note also blood sinusoid dilatation (S) accompanied by hepatic cords atrophy. The letter (F) refers to a fatty vacuole which is frequently observed, X100.

4- Disorganizatiou in hiliarly tracts wall (bt) with moderate fibrosis and their walls and so around a large portal vein (LPV). The phenomenon of leaky liver (L) was observed. X100.

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تأثير سمية البكتريا الزرقاء (الطحالب الخضراء المزرقة) أوسيلاتوريا روبسنس علي دم وكبد الجرذ را تس نورفيجيكس

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المستخلص. تم دراسة تأثير المستخلص الخلوي للبكتريا الزرقاء *أوسيلاتوريا روبسنس* على دم وكبد الفئران البيضاء ، حيث تم تقسيم الفئران إلى ثلاث مجموعات وتم حقنها بجرعات أسبوعية تحت الغشاء البريتوني بالمستخلص الخلوي، وكانت قيم الجرعات تمثل ١٧,٥ و ٣٥ و ٧٠ مليجرام / فأر لمدة سبعة أسابيع متتالية. أظهرت الفئران التي حقنت بجرعة ١٧,٥ مليجرام بعض التغيرات على كرات الدم البيضاء، الصفائح الدموية، متوسط تركيز الهيموجلوبين الخلوي مع انخفاض معدل الجلوكوز في الدم. بينما زاد معدل كل من اليوريا، والدهون الثلاثية، الكرياتينين والأنزيمات AST&ALT في السيرم للفئران المحقونة. كذلك أوضحت الدراسة أن الفئران المحقونة بجرعة ٣٥ مليجرام أسبوعياً أظهرت انخفاضًا في عدد كرات الدم الحمراء وارتفاعًا في معدل الكوليسترول والدهون الثلاثية مع ارتفاع ملحوظ في مستوى الأنزيمات AST&ALT. وتبين من خلال الدراسة أيضاً أن الفئران التي حقنت بجرعة ٧٠ مليجرام أسبوعياً كانت الأكثر تأثراً حيث ارتفعت بشكل معنوي معدلات اليوريا، الدهون الثلاثية، الكوليسترول، الكرياتينين الصفائح الدموية علاوة على ارتفاع في معدلات AST, ALT. HCT, MCHC, MCV. وعلى ذلك فإن هذه القراءات تشير إلى أن المستخلص الخلوي الخام للبكتريا الزرقاء *أوسيلاتوريا روبسنس* خاصة عند معدل الجرعات التي تزيد على ٧٠ مليجرام له تأثير مثبط على مكونات الدم ، وبالتالي يستدل من الدراسات الفسيولوجية على التأثير المتوقع على أنسجة الكبد والكلي.