Results

Results

Cytogenetic investigation:

Various chromosomal aberrations are observed in the bone marrow cells of male rats treated with ciprofloxacin and protected with pelgaronium graveolens at different periods of time. Only Structural types of aberrations and chromosomal stickness are noticed.

The numbers of chromosomes present in bone marrow Cells of rats were 42 chromosomes (Fig 6).

Structural aberrations:

The Structural aberrations include chromatid deletion (Fig7), Fragmentation (Fig8), Centromeric attenuation (Fig9), end to end association (Fig10), Centric fusion (Fig11), break (Fig12) gap (Fig13) and chromosomal ring (Fig14).

Chromosomal stickness:

The stickness is considered as assort of chromosomal agglutination of unknown nature wich resulted in sticky appearance of chromosomes stickness may give arise to sticky adhesions between two or more chromosomes, and formation of sticky bridges at anaphase (Fig 11).

Fig (6) Normal metaphase spread in bone marrow cells of rats.
(Giemsa stain X1000)

Fig (7) Metaphase spreads showing deletion (D) in bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000)

Fig (8) Metaphase spreads Showing fragment (F) in bone marrow Cells of rats treated with Ciprofloxacin.

(Giemsa stain X1000)

Fig (9) Metaphase spreads showing Centromeric attenuation (c att) in bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000)

Fig (10) Metaphase spreads showing end to end association (E&E) of chromosomes of bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000)

Fig (11) Metaphase spreads of chromosomes showing centric fusion (CF) of bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000)

Fig (12) Metaphase spreads of chromosomes showing break (B) in bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000)

Fig (13) Metaphase spreads of chromosomes showing gap (G) in bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000).

Fig (14) Metaphase spreads of chromosomes showing Chromosomal ring in bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000).

Fig (15) Metaphase spreads of chromosomes showing stickness in bone marrow cells of rats treated with ciprofloxacin.

(Giemsa stain X 1000).

1. Deletion:

Table (1), fig (16) shows the mean value of chromatid deletions in 50 metaphases spread of male rats treated with Ciprofloxacin and pelargonium at different periods (24 hrs; 3 & 6 days) it represents a significant difference between control and treated animal groups at all duration and also between protected animal with pelargonium and treated animal groups.

Also, administrated that Pelargonium make high protective role at 24 hrs, 3 days and 6 days against treatment with ciprofloxacin.

2. Fragmentation:

Table (2), Fig (17) shows the mean value of Chromatid fragmentation / 50 metaphases spread of bone marrow cells of male rats treated with Ciprofloxacin and pelargoium at different periods of time.

It Cleared a significant difference between control and all treated groups. Also, Pelargonium make high protection role at 24 hrs, 3 days and 6 days.

3. Centromeric attenuation:

Table (3), Fig (18) shows the mean value of centromeric attenuation / 50 metaphases spread of bone marrow cells of male rats treated with ciprofloxacin and pelargonium.

It indicated that a significant difference between control and treated animals and also Cleared the protective effect of pelargonium at 24 hrs, 3 days and 6 days against treated groups.

4. End to end association:

Table (4) , Fig (19) shows the mean value of end to end association/50 metaphases spread of bone marrow cells of male rats. It represents a significant increase in the treated groups than control groups.

5. Centric fusion:

Table (5), Fig (20) shows the mean value of centric fusion/50 metaphases spread of bone marrow cells of male rats. It represents a significant increase in the mean value of centric fusion in treated groups than control and the role of protection of pelargonium is clear in protected groups against treated one.

6. Break :

Table (6) , Fig (21) shows the mean value of chromatid break /50 metaphases spread of bone marrow cells of male rats treated with Ciprofloxacin and Pelargonium at different periods of time. It represents a significant difference between control groups and treated animals, it also shows the protective role of pelargonium at 24 hrs, 3days and 6 days against ciprofloxacin.

7. gap:

Table (7) , Fig (22) shows the mean value of chromatid gaps / 50 metaphases spread of bone marrow cells of male rats treated with ciprofloxacin and protected with pelargonium.

It represents a significant increase in all treated animal than control one.

8. Chromosomal ring:

Table (8) , Fig (23) shows the mean value of chromosomal ring / 50 metaphases spread of bone marrow cells of male rats treated with Ciprofloxacin and pelargonium at different periods of time (24 hrs, 3days & 6days).

It represents a significant increase in all treated groups than control.

Pelargonium make high protection at 24 hrs; 3 days and 6 days.

There is a decline in the effect with increasing duration time.

9. Chromosomal Stickness:

Table (9), Fig (24) shows the mean value of chromosomal stickness / 50 Metaphases spread of bone marrow cells of male rats treated with ciprofloxacin and protected with pelargonium.

It represents a highly significant difference between control and all treated animals and also shows decrease in the mean value of protected groups but not reach to the normal stage again.

10. Mitotic index:

Table (10), Fig (25) shows the mean value of mitotic index of bone marrow cells of rats treated with ciprofloxacin and protected with pelargonium. It represented a significant difference between control and treated animals. The value of mitotic index decreased in treated animals but increased in protected animals.

11. Average of chromosomal abnormalities:

Table (11), Fig (26) shows average of chromosomal abnormalities observed in bone marrow cells of male rats treated with ciprofloxacin and protected with pelargonium at different periods of time (24 hrs; 3 days and 6 days).

It represents a significant increase in all treated groups than control.

Pelargonium make high protection at (24 hrs; 3 days and 6 days).

Molecular Biology

Determination of DNA

1. In Liver:

1 2 3 4 5 6 7 8 9 10 11

Fig (27) DNA damage in liver of rats treated with ciprofloxacin and protected with pelargonium graveolens for 24 hrs. lane 1 : DNA ladder ; lane 2 : Control; lane 3,4,5 : treated with ciprofloxacin ; lane 6,7,8 : treated with ciprofloxacin and protected with pelargonium; lane 9,10,11 : protected with pelargonium only (positive control).

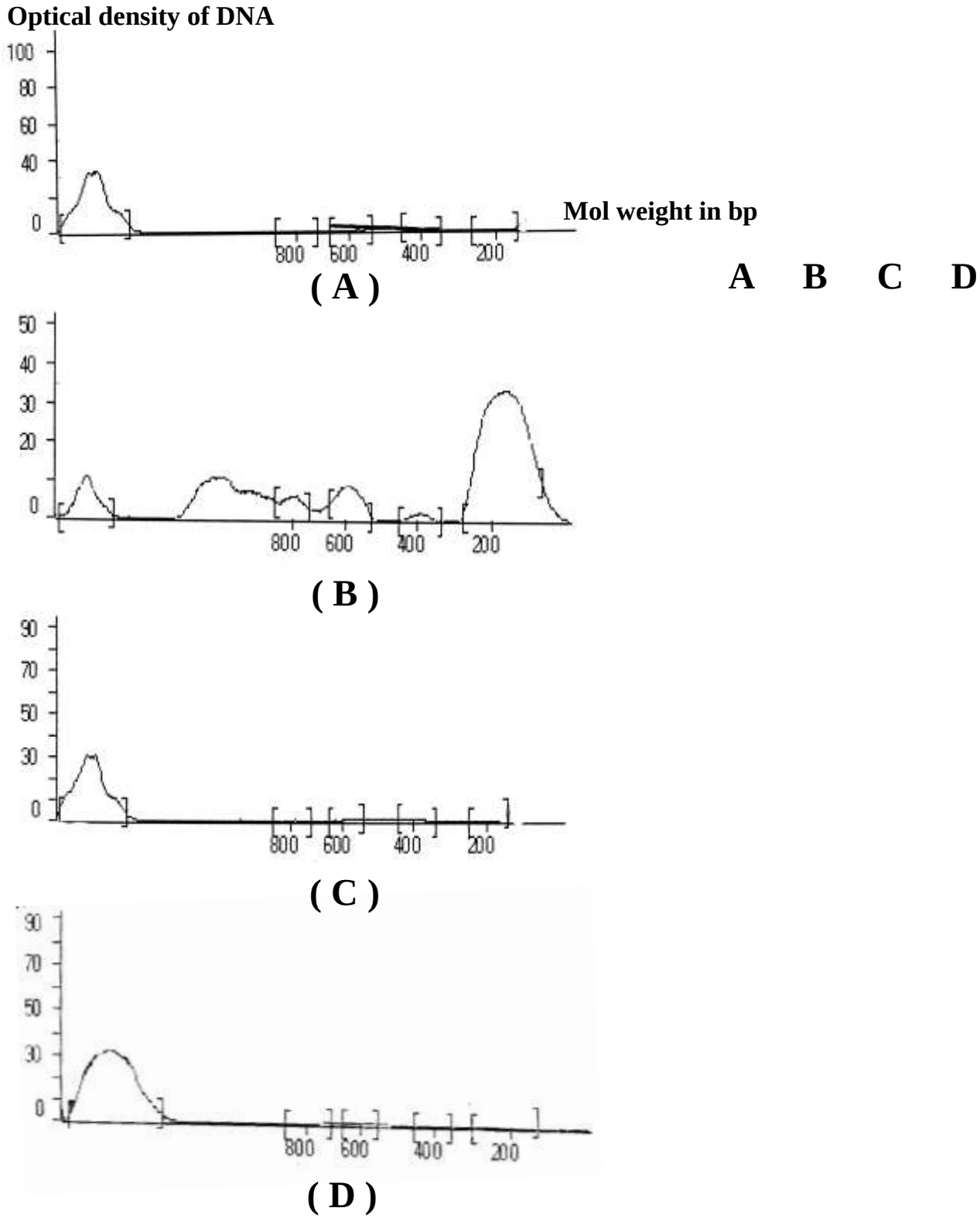
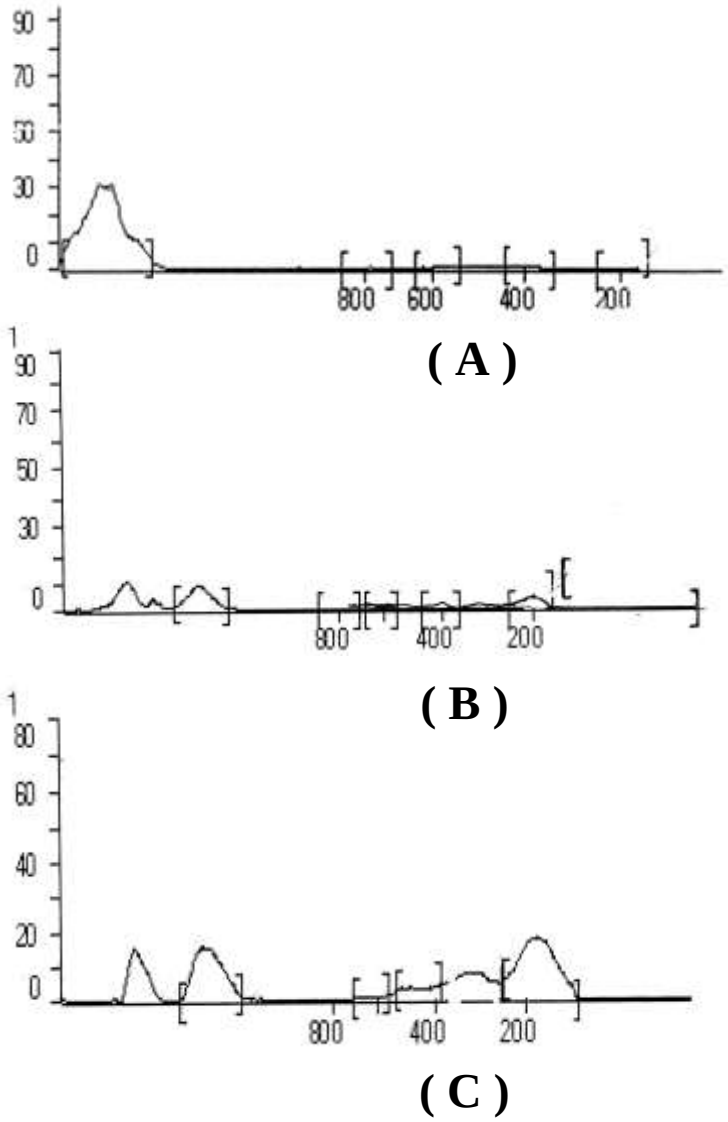


Fig (28) Histograms of optical density of intact and apoptotic fragments of DNA at 200,400 and 600 bp in liver of rats treated with ciprofloxacin (B) and protected with pelargonium (C) (D) : protected with pelargonium only ; (A) : control for 24 hrs.

Table (12) Optical density of intact and apoptotic fragments of DNA at 200 , 400 , 600 bp in liver of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs.

	Control	**Treated**	**Treated and Protection**	**Protection only**
Intact DNA	35.614	12.699	30.31	33.12
DNA at 600bp	1.34	10.51	2.51	1.45
DNA at 400bp	1.32	5.91	1.82	1.15
DNA at 200bp	1.00	36.312	1.312	1.02

Fig (29) DNA damage in liver of rats treated with ciprofloxacin and protected with pelargonium for 3 days lane 1 : control ; lane 2 , 3 , 4 5, 6 : treated with Ciprofloxacin; lane 7 : DNA ladder; lane 8,9,10: treated with Ciprofloxacin and protected with pelargonium.



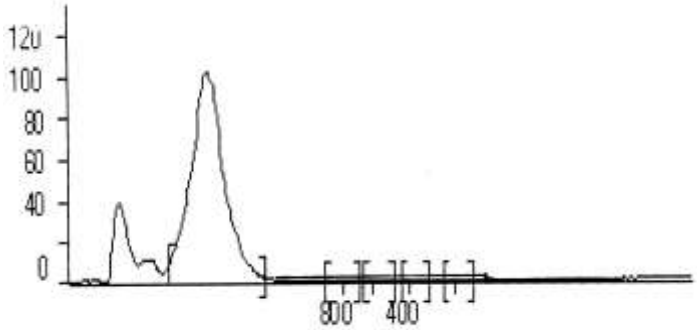
A B C

Fig (30) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200, 400 and 600 bp in liver of rats treated with ciprofloxacin (B) and protected with pelargonium (C) ; (A) : control for 3days.

Table (13) Optical density of intact DNA and apoptotic fragments of DNA at 200, 400 and 600 bp in liver of rats treated with Ciprofloxacin and protected with pelargonium for 3 days.

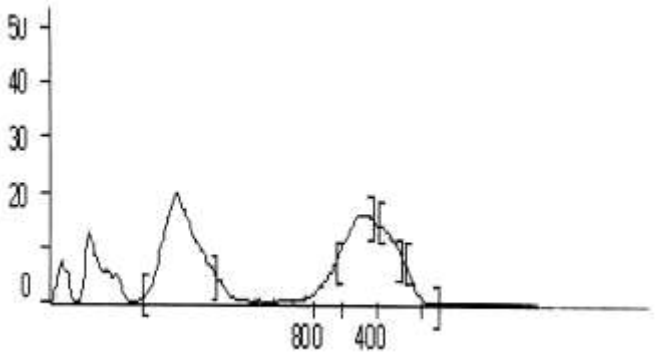
	Control	**Treated**	**Treated and Protection**
Intact DNA	32.63	10.12	18.31
DNA at 600bp	1.43	2.45	4.28
DNA at 400bp	1.32	3.83	8.46
DNA at 200bp	1.00	8.51	20.81

Fig (31) DNA damage in liver of rats treated with ciprofloxacin and protected with pelargonium for 6 days. Lane 1 : control; lane 2,3,4,5: treated with ciprofloxacin and protected with pelargonium lane 6: DNA ladder ; lane 7 ,8,9,10,11,12 : treated with ciprofloxacin.



(A)

A B C



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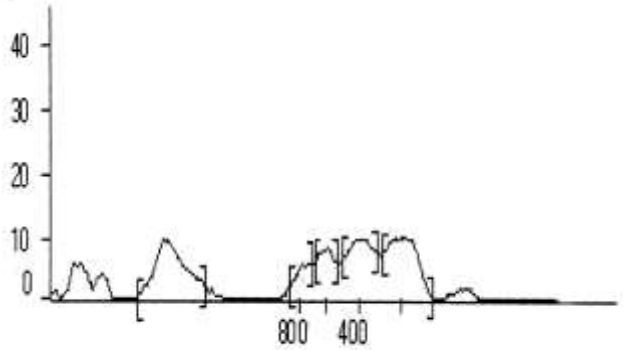


(C)

Fig (32) Histograms of optical density of intact and apoptotic fragments of DNA at 200 , 400 , and 600 bp in liver of rats treated with ciprofloxacin (C) and protected with pelargonium (B) for 6 days ; (A) control.

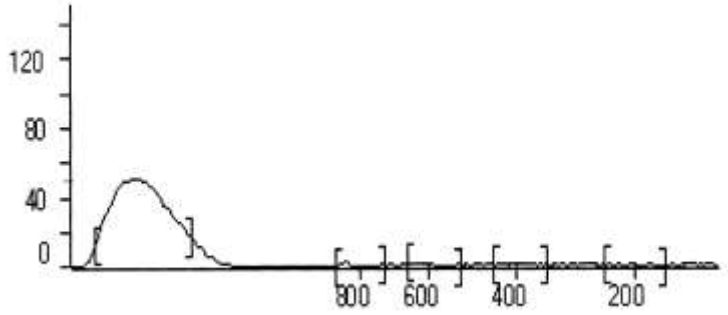
Table (14) Optical density of intact and apoptotic fragments of DNA at 200,400,600 bp in liver of rats treated with ciprofloxacin and protected with pelargonium for 6 days.

	Control	**Treated**	**Treated and Protection**
Intact DNA	105.32	12.67	22.11
DNA at 600bp	1.41	10.13	18.32
DNA at 400bp	1.31	12.32	15.63
DNA at 200bp	1.00	13.35	5.31

2) In spleen:

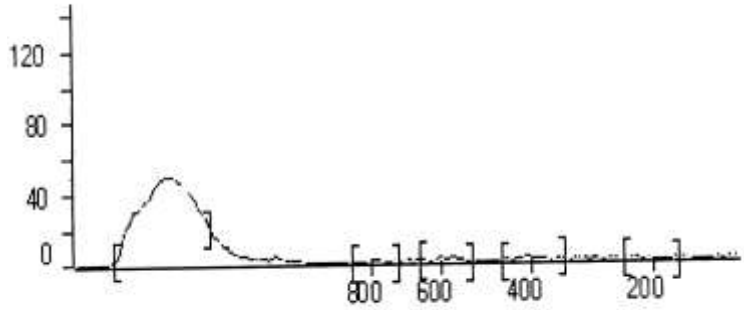
1 2 3 4 5 6 7 8 9

Fig (33) DNA damage in spleen of rats treated with ciprofloxacin and protected with pelargonium for 24hrss ; lane 1 : control ; lane 2 , 3 : treated with ciprofloxacin and protected with pelargonium ; lane 4,5,6,7,8 : treated with ciprofloxacin ; lane 9 : DNA ladder.



A B C

(A)



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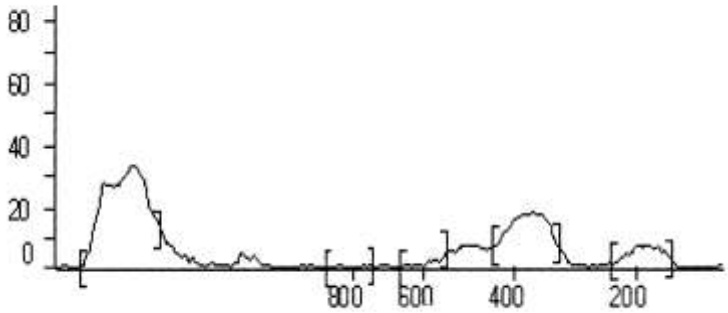


(C)

Fig (34) Histograms of optical density of intact and apoptotic fragments of DNA at 200,400 and 600 bp in spleen of rats treated with ciprofloxacin (C) and protected with pelargonium (B) for 24hrs ; (A): control.

Table (15) Optical density of intact and apoptotic fragments of DNA at 200, 400, 600 bp in spleen of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs.

	Control	**Treated**	**Treated and Protection**
Intact DNA	52.32	35.72	50.03
DNA at 600bp	4.52	5.92	4.81
DNA at 400bp	3.32	22.91	3.92
DNA at 200bp	2.01	8.36	2.54

Fig (35) DNA damage in spleen of rats treated with ciprofloxacin and protected with pelargonium for 3days ; lane 1,2 , 3 : control; lane 4,5,6 : treated with ciprofloxacin only ; lane 7,8,9,10 : treated with ciprofloxacin and protected with pelargonium.

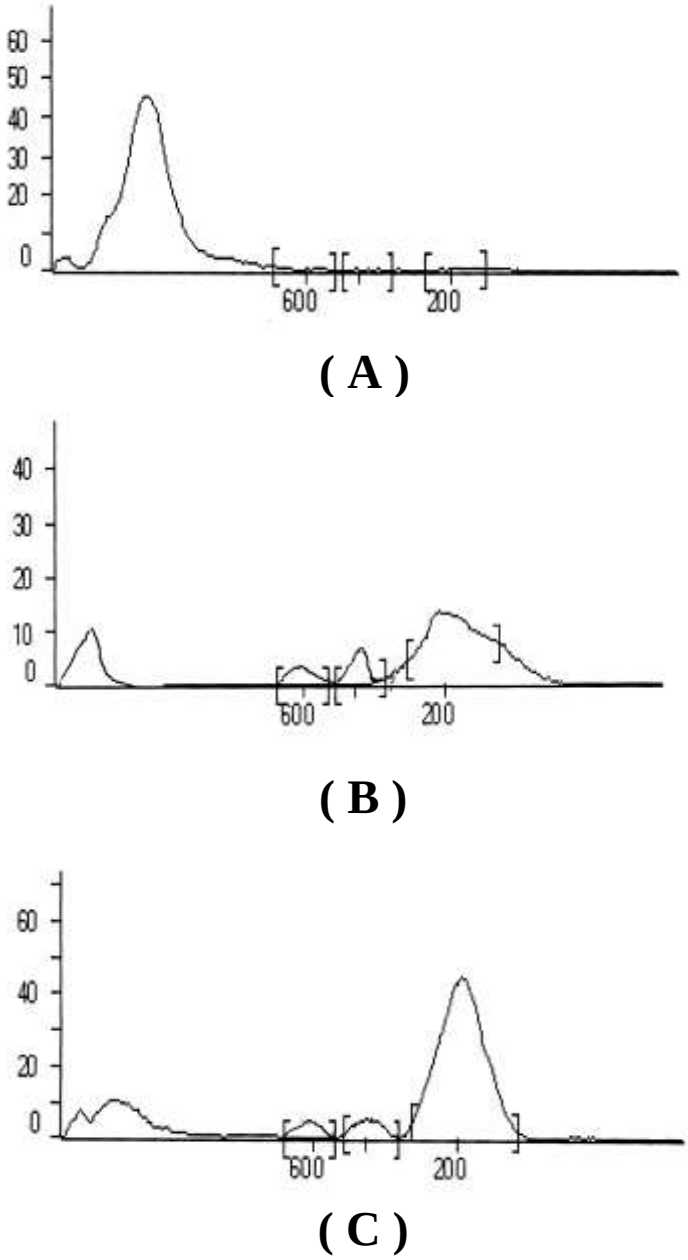
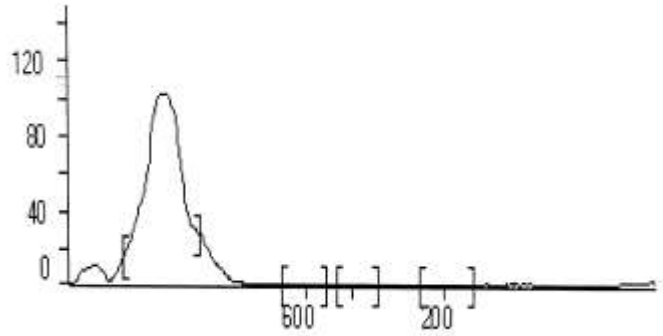


Fig (36) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200,400 and 600 bp in spleen of rats treated with ciprofloxacin (B) and protected with pelargonium (C) for 3 days; (A) : control.

Table (16) Optical density of intact and apoptotic fragments of DNA at 200,400,600 bp in spleen of rats treated with ciprofloxacin and protected with pelargonium for 3days.

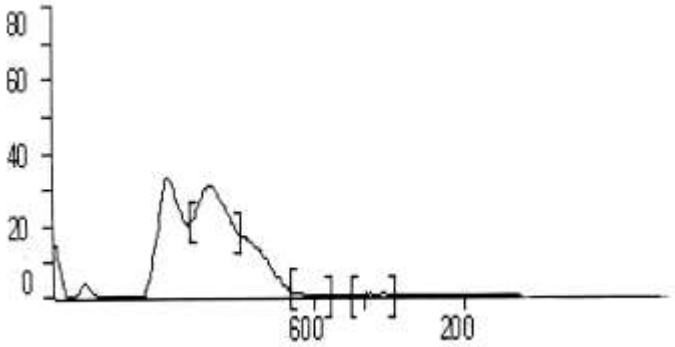
	Control	**Treated**	**Treated and Protection**
Intact DNA	45.38	10.95	12.83
DNA at 600bp	2.01	2.92	7.82
DNA at 400bp	2.11	5.01	10.61
DNA at 200bp	1.12	15.31	48.53

Fig (37) DNA damage in spleen of rats treated with ciprofloxacin and protected with pelargonium for 6 days ; lane 1: control; lane 2,3,4,5 : treated with ciprofloxacin and protected with pelargonium; lane 6 : DNA ladder; lane 7,8,9,10,11,12 : treated with ciprofloxacin only.

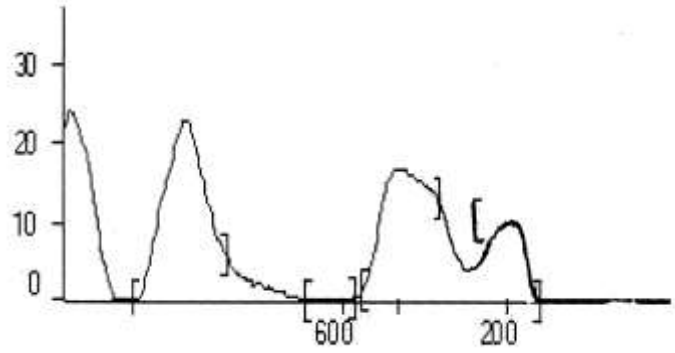


(A)

A B C



(B)



(C)

Fig (38) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200,400 and 600 bp in spleen of rats treated with ciprofloxacin (C) and protected with pelargonium (B) for 6 days ; (A): control.

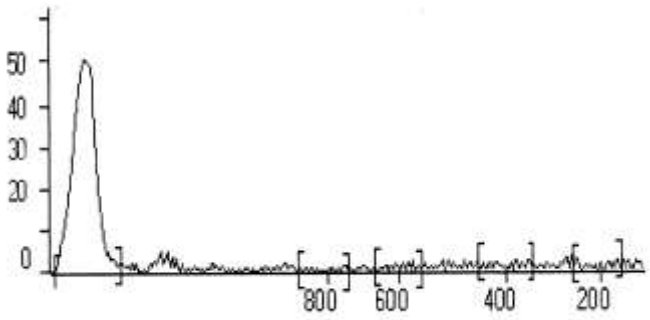
Table (17) Optical density of intact and apoptotic fragments of DNA at 200,400,600 bp in spleen of rats treated with ciprofloxacin and protected with pelargonium for 6 days.

	Control	**Treated**	**Treated and Protection**
Intact DNA	105.12	22.53	32.62
DNA at 600bp	1.85	5.12	3.64
DNA at 400bp	1.61	18.43	3.99
DNA at 200bp	1.01	10.35	2.89

3. In lung:

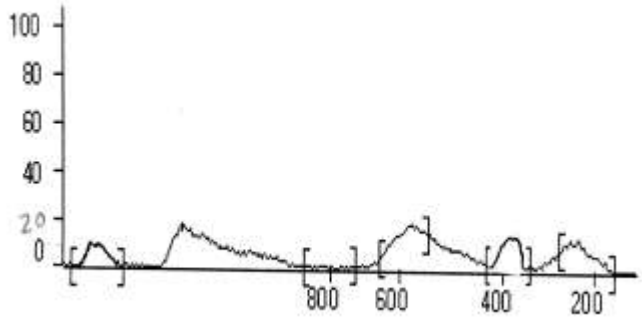
1 2 3 4 5 6 7 8 9

Fig (39) DNA damage in lung of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs ; lane 1,2,3 : control; lane 4,5,6 : treated with ciprofloxacin only; lane 7,8,9 : treated with ciprofloxacin and protect with pelargonium.

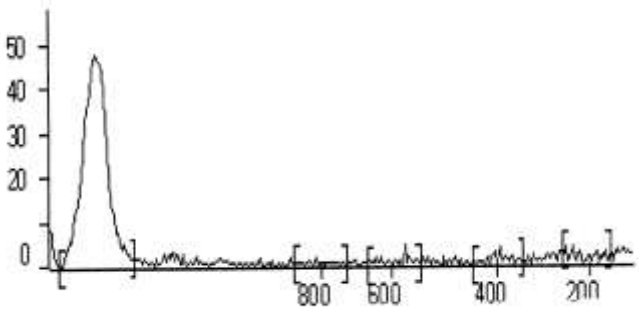


(A)

A B C



(B)



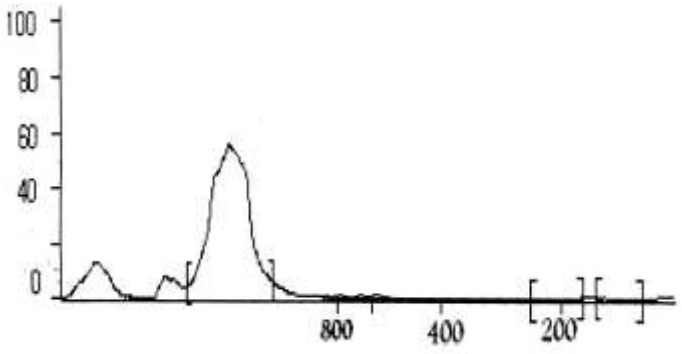
(C)

Fig (40) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200,400 and 600 bp in lung of rats treated with ciprofloxacin (B) and protected with pelargonium (C) for 24 hrs ; (A): control.

Table (18) Optical density of intact and apoptotic fragments of DNA at 200,400,600 bp in lung of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs.

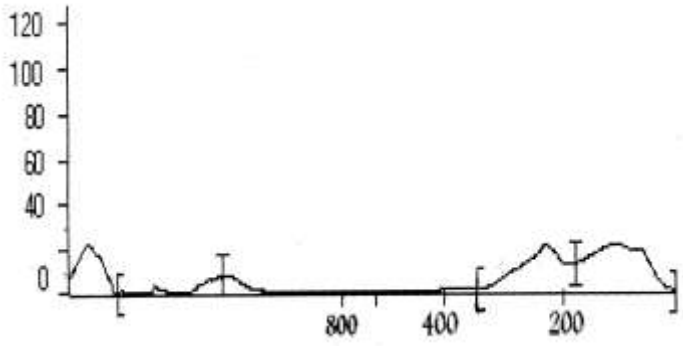
	Control	**Treated**	**Treated and Protection**
Intact DNA	50.11	10.84	48.51
DNA at 600bp	2.62	20.26	3.62
DNA at 400bp	2.41	16.31	3.51
DNA at 200bp	2.23	15.23	2.62

Fig (41) DNA damage in lung of rats treated with ciprofloxacin and protected with pelargonium for 3 days; lane 1: DNA ladder ; lane 2,3 : treated with ciprofloxacin and protected with pelargonium; lane 4,5 : treated with ciprofloxacin only; lane 6 : control.



C B A

(A)



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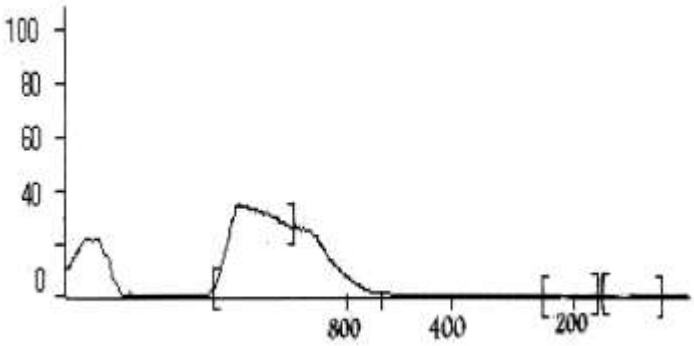


(C)

Fig (42) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200,400 and 600 bp in lung of rats treated with ciprofloxacin (B) and protected with pelargonium (C) for 3 days ; (A): control.

Table (19) Optical density of intact and apoptotic fragments of DNA at 200,400,600 bp in lung of rats treated with ciprofloxacin and protected with pelargonium for 3 days.

	Control	**Treated**	**Treated and Protection**
Intact DNA	55.34	6.31	35.62
DNA at 600bp	2.12	0.62	1.99
DNA at 400bp	1.52	3.53	0.60
DNA at 200bp	1. 03	28.34	0.31

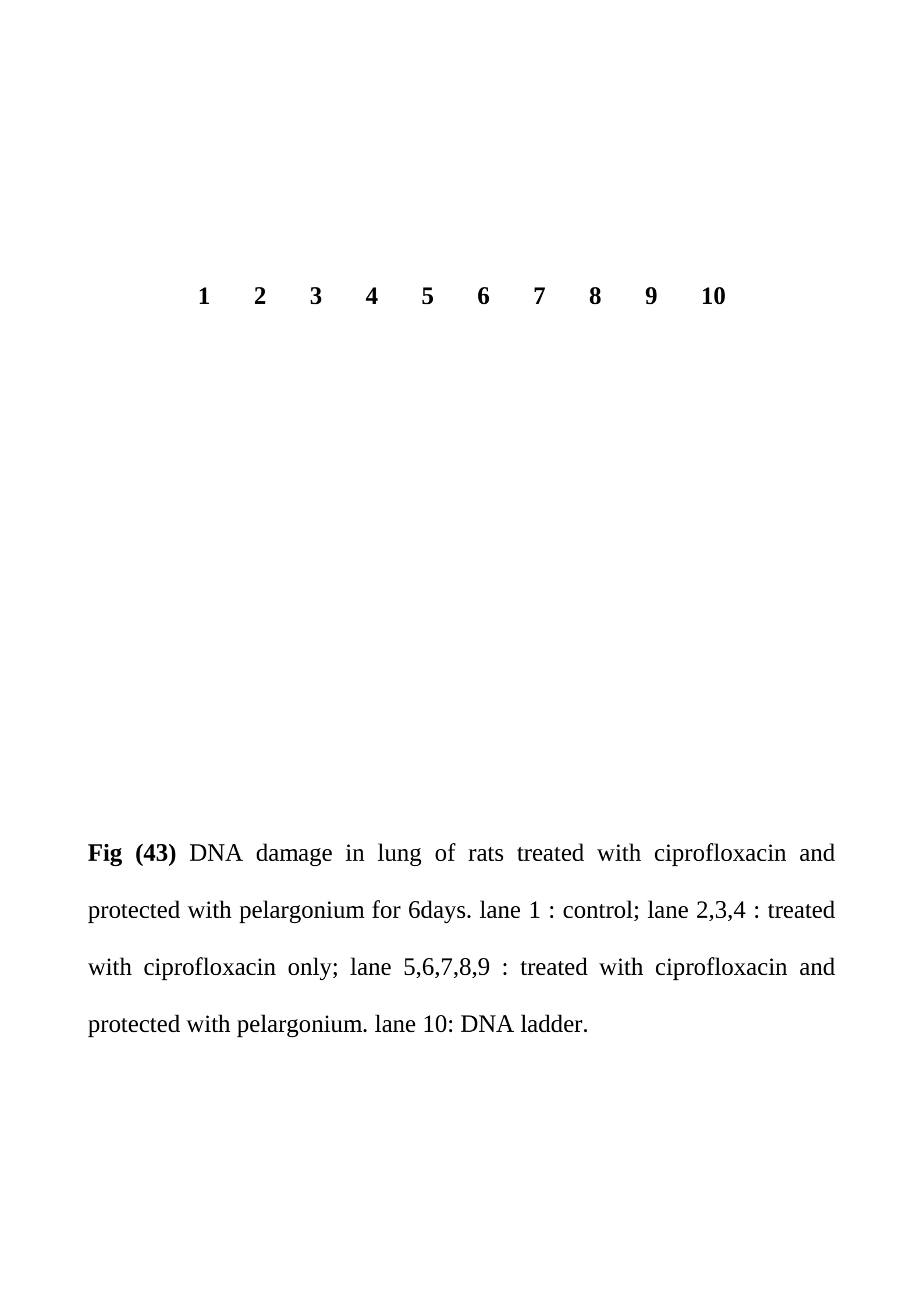
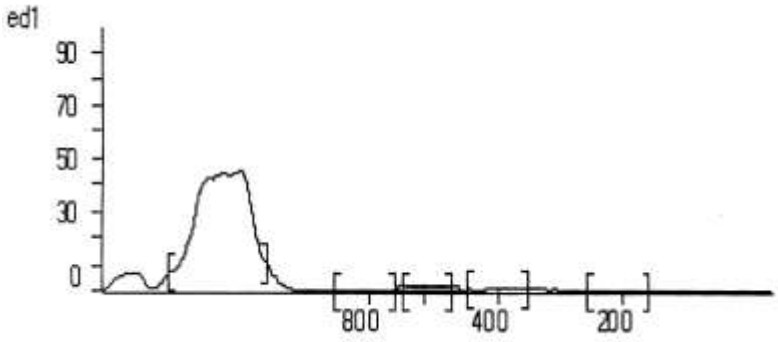
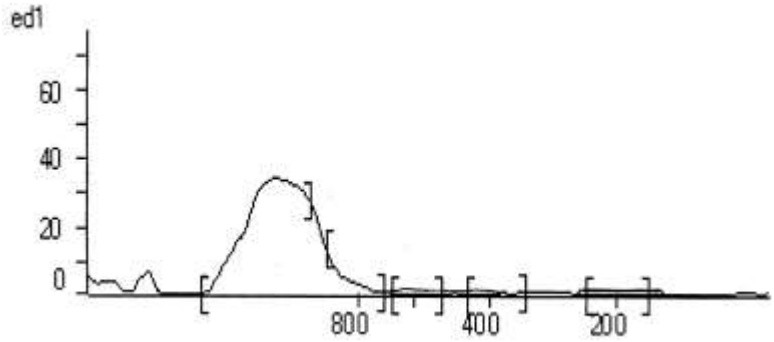


Fig (43) DNA damage in lung of rats treated with ciprofloxacin and protected with pelargonium for 6days. lane 1 : control; lane 2,3,4 : treated with ciprofloxacin only; lane 5,6,7,8,9 : treated with ciprofloxacin and protected with pelargonium. lane 10: DNA ladder.



(A)

A B C



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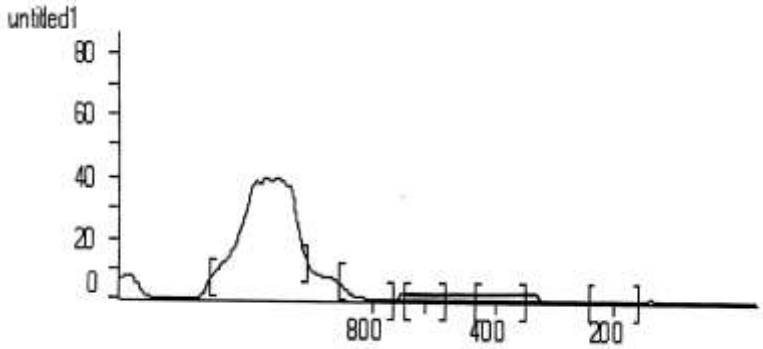


(C)

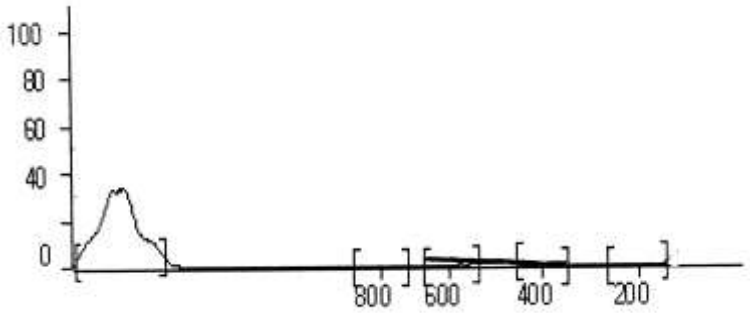
Fig (44) histograms of optical density of intact DNA and apoptotic fragments of DNA at 200,400 and 600 bp in lung of rats treated with ciprofloxacin (B) and protected with pelargonium (C) for 6 days; (A): control.

Table (20) Optical density of intact and apoptotic fragments of DNA at 200,400,600 bp in lung of rats treated with ciprofloxacin and protected with pelargonium for 6 days.

	Control	**Treated**	**Treated and Protection**
Intact DNA	45.31	32.82	42.56
DNA at 600bp	1.95	3.05	2.01
DNA at 400bp	1.05	2.62	1.53
DNA at 200bp	0.31	1.92	0.95

4. In bone marrow:

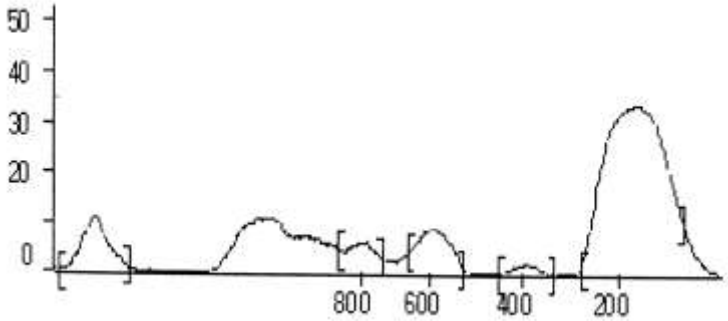
Fig (45) DNA damage in bane marrow of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs , 3 days and 6 days ; lane 1 :DNA ladder; lane 2: control; lane 3: treated with ciprofloxacin for 24 hrs ; lane 4: treated + protection for 24 hrs; lane 5: treated with ciprofloxacin for 3 days; lane 6,7: treated + protection for 3 days lane 8: treated with ciprofloxacin for 6days ; lane 9,10: treated + protection for 6 days; lane 11: protected with pelargonium only.



A B C D

(A)

(B)



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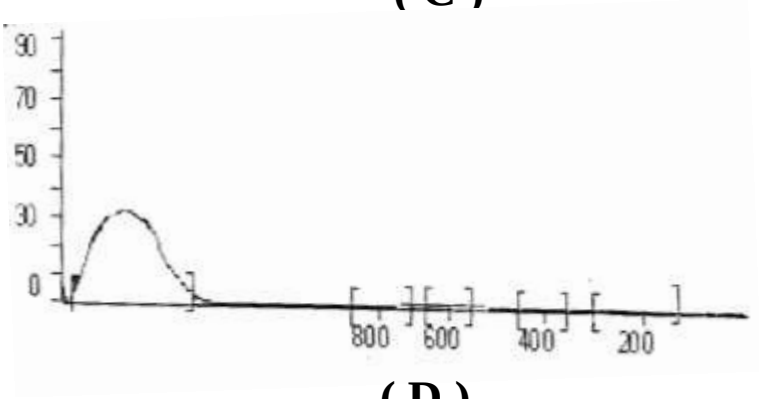
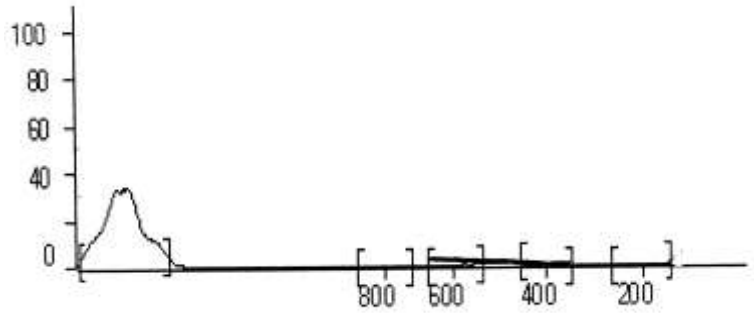


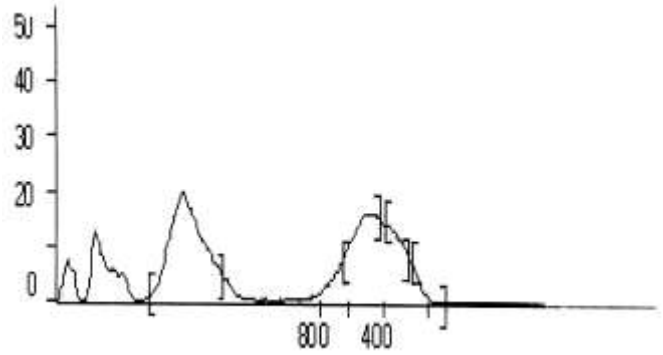
(D)

Fig (46) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200,400 and 600 bp in bone marrow of rats treated with ciprofloxacin (B) and protected with pelargonium (C) for 24hrs; (A): control; (D): protected with pelargonium only.



A B C

(A)



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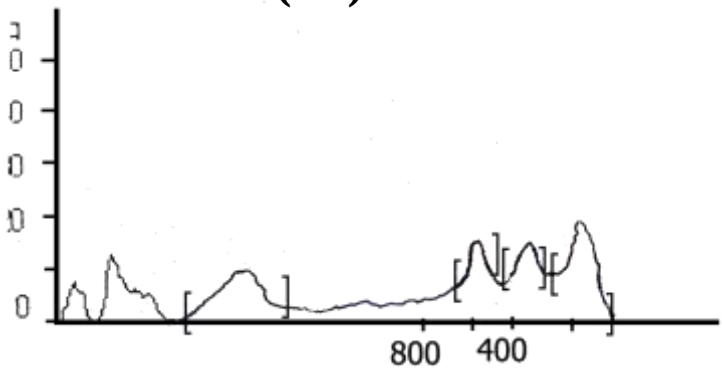
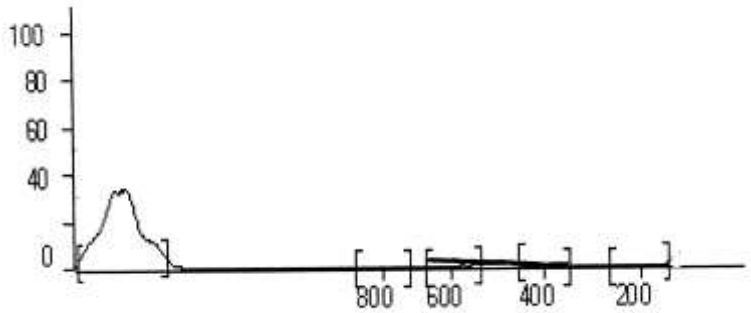


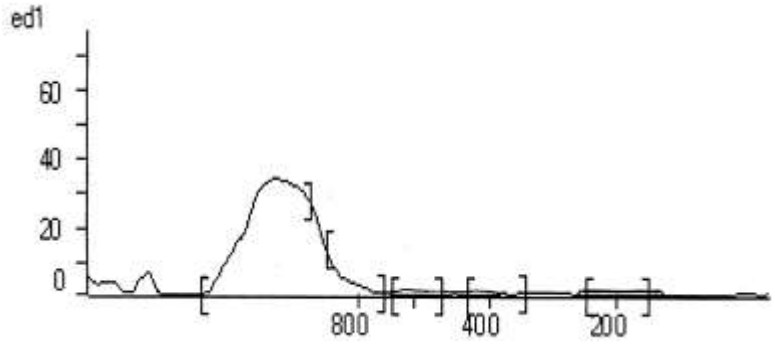
(C)

Fig (47) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200 , 400 and 600 bp in bone marrow of rats treated with ciprofloxacin (C) and protected with pelargonium (B) for 3 days; (A): control.

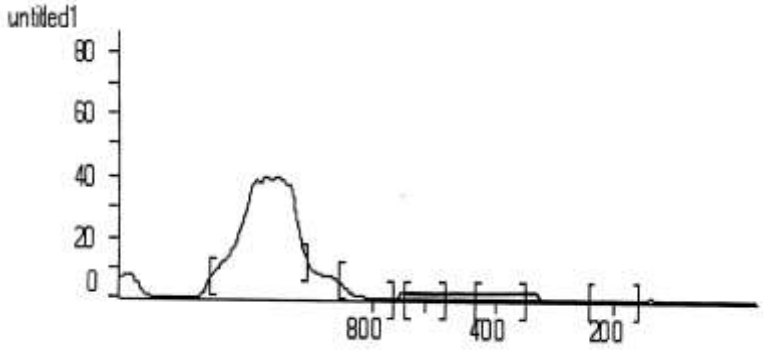


A B C

(A)



(B)



(C)

Fig (48) Histograms of optical density of intact DNA and apoptotic fragments of DNA at 200 , 400 and 600 bp in bone marrow of rats treated with ciprofloxacin (B) and protected with pelargonium (C) for 6 days; (A): control.

Table (21) Optical density of intact and apoptotic fragment of DNA at 200 , 400 , 600 bp in bone marrow of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs , 3 days and 6 days.

	Control	T 24 hrs	T + P 24 hrs	T 3 days	T + P 3 days	T 6 days	T + P 6 days	P **Control (+)**
Intact DNA	36.51	10.21	12.98	10.77	22.21	33.88	42.56	33.11
DNA at 600bp	1.53	2.54	10.61	12.13	18.42	3.55	2.01	1.42
DNA at 400bp	1.33	3.82	5.95	16.52	15.81	2.82	1.53	1.12
DNA at 200bp	0.92	8.61	36.21	20.62	5.41	1.99	0.96	1.05

T= Ciprofloxacin (0.09 mg/gm of rats).

P= Pelargonium graveolens (0.2 mg/gm of rats).

In liver :-

Figs (27, 28) & table (12) show the damage and optical density of DNA of liver cells of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs it represents that:

- Ciprofloxacin caused sever damage in DNA of hepatocytes when was compared with control and damage decreased in DNA of hepatocytes of rats which drinked pelargonium and ciprofloxacin together.
- DNA of hepatocytes of rats have pelargonium only shows similar a results of DNA as control (without damage).

Figs (29, 30) & table (13) show the damage and optical density of DNA of liver cells of rats treated with ciprofloxacin and protected with pelargonium for 3 days and it represent that:

- Damage in DNA hepatocytes of rats treated with ciprofloxacin increased when was compared with control and 24 hrs.
- The protective role of pelargonium against the effect of ciprofloxacin so the optical density of apoptotic bands of DNA at 200 , 400 , 600 bp and intact DNA increased in protected groups.

Figs (31 , 32) & table (14) show the damage and optical density of DNA of liver cells of rats treated with ciprofloxacin and protected with pelargonium for 6 days, they represent that:

- Damage in DNA of hepatocytes of treated groups increased when was compared with control so optical density of DNA increased at 200 , 400 and 600 bp and decreased in intact DNA.
- Damage in DNA of hepatocytes decreased when was compared with 24 hrs , 3 days.
- Pelargonium make highly protection against treatment with ciprofloxacin so optical density of DNA decreased at 200 and 400 bp and increased at 600 bp and intact DNA protected groups.

In spleen:

Figs (33 , 34 , 35 , 36) & tables (15 , 16) show the damage and optical density of DNA of spleen of rats treated with Ciprofloxacin and protected with pelargonium for 24 hrs and 3 days, they represents that:

- Damage in DNA of spleen of rats in treated group increased when was compared with control.
- Damage in DNA of spleen of rats in protected groups decreased against treated one.
- damage in DNA of spleen of rats in treated groups for 3 days increased than treated groups for 24 hrs.

Figs (37 , 38) , table (17) show DNA damage in DNA of spleen of rats treated with ciprofloxacin and protected with pelargonium for 6 days and represent that:

- Damage in DNA of spleen of rats in treated groups increased in compared with control.
- Damage in treated groups for 6 days decreased than treated one for 3 days so the optical density of intact DNA was 22.53 , 10.95 respectively.

In Lung:

Figs (39 , 40) & table (18) show damage and optical density of DNA of lung of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs and represents that :

- Sever damage in DNA of lung of treated animals when compared with control:
- Protective role of pelargonium against treatment with observed was indicated in optical density of protected groups at 200 , 400 , 600 bp and intact DNA near to optical density of control in the same regions.

Figs (41 , 42) & table (19) show damage and optical density of DNA of lung of rats treated with ciprofloxacin and protected with pelargonium for 3 days and represents that:

- Apoptosis in DNA of lung was increased in treated animals the control.
- Pelargonium gives a highly protection role against ciprofloxacin.
- Damage in DNA treated groups for 3 days (Apoptosis) decreased than treated groups for 24 hrs (Necrosis) and that cleared in optical density table (19).

Figs (43 , 44) & table (20) show damage and optical density of DNA of lung of rats treated with ciprofloxacin and protected with pelargonium for 6 days and represents that:

- Very little damage in DNA of lung of treated animals when compared with control.
- Highly protective role of pelargonium in protected groups so DNA of lung of protected animals were near to control and that cleared in optical density of DNA of lung protected animals control at 200 , 400 , 600 bp and intact DNA.
- The damage in DNA of lung in treated groups for 3 days decreased than that for 24 hrs (necrosis) and for 3 day (apoptosis).

Bone marrow:

Figs (45 ; 46; 47 & 48) & table (21) show the damage and optical density of DNA of bone marrow cells of rats treated with ciprofloxacin and protected with pelargonium for 24 hrs , 3 days and 6 days and represents that:

- Sever damage in DNA (necrosis) of bone marrow cells of rats treated with ciprofloxacin for 24 hrs.
- Damage decreased in DNA of bone marrow cells in protected animals (apoptosis).
- Damage decreased in treated groups for 3 days (apoptosis) than treated groups for 24 hrs (necrosis).
- The protective role of pelargonium against treated groups for 3 days.
- A very little damage in DNA of bone marrow cells of rats treated with ciprofloxacin for 6 days and protected groups without damage near to control.

- The damage in DNA of bone marrow cells of treated groups for 6 days decreased than the damage in DNA of bone marrow cells of treated groups for 24 hrs and 3 days , and the optical density of DNA of bone marrow cells cleared all these results.