RESULTS

I-Chromosomes:

Many chromosomal aberrations are observed in the bone marrow cells of mice *(Mus musculus)* which treated with Mannitol as control +ve, MMC, Oxyplex (it is used as a protective substance), and MMC with Oxyplex respectively. These treatments were acute (1/10 LD50) or chronic (1/200 LD50). In the first case the anesthesia was after (6 hrs, 24 hrs, 48, hrs and 5days). In the second case the anesthesia was after (2 weeks and 4 weeks).

Both numerical and structural types of aberrations were designated in comparison with normal metaphase spread of control group (Figure 2).

A-Structural aberrations:

These types of aberration related to the abnormalities of chromosomes that occur in the somatic cells contain one or more abnormal chromosomes. They referred to the changes in the arrangement of chromosomal breakage followed by recombination in an abnormal order. They summarized as; Chromatid deletion (Figure 3), Chromatid break (Figure 4), Chromatid gaps (Figure 5), Fragmentation (Figure 6), End to End association (Figure 7), Centric fusion (Figure 8), and Centromeric attenuation (Figure 9).

B-Numerical aberrations:

Numerical aberrations mean that the somatic cells contain an abnormal number of chromosomes. They usually arise when the chromosomes or chromatids associated in such adhesions move to opposite spindle pole. Stickiness may give rise to sticky adhesions between two or more chromosomes, and formation of sticky bridges at anaphase (Figure 10).

Fig. (2): Normal metaphase spread in bone marrow cells of *(Mus musculus).*

(Giemsa stain x 1000)

Fig. (3): Metaphase spread showing deletion (D) in bone marrow cells of *(Mus musculus)*.

(Giemsa stain x 1000)

Fig. (4): Metaphase spread showing chromatid break (B) in bone marrow cells of *(Mus musculus)*.

(Giemsa stain x 1000)

Fig. (5): Metaphase spread showing chromatid gap (G) in bone marrow cells of *(Mus musculus*).

(Giemsa stain x 1000)

Fig. (6): Metaphase spread showing fragmentation (F) in bone marrow cells of (Mus musculus).

(Giemsa stain x 1000)

Fig. (7): Metaphase spread showing end to end association (EE) in bone marrow cells of (*Mus musculus*).

(Giemsa stain x 1000)

Fig. (8): Metaphase spread showing centromeric attenuation (CA) in bone marrow cells of *(Mus musculus*).

(Giemsa stain x 1000)

Fig. (9): Metaphase spread showing centric fusion (CF) in bone marrow cells of (*Mus musculus*).

(Giemsa stain x 1000)

Fig. (10): Metaphase spread showing stickiness (S) in bone marrow cells of (*Mus musculus*).

(Giemsa stain x 1000)

Acute treatment

A-Structural aberration:

1-Deletion:

Table (2) and Figure (11) show the mean values of chromatid deletion in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively.

The statistical analysis indicate that, there is a very highly significant difference between the control and animals treated by MMC (acute treatment 1/10 LD50) after two periods of treated (24 hrs and 48, hrs). But after 6 hrs and 5days the recorded results were significant between them.

But there is no significant difference between the control and groups of animals which treated by Mannitol, Oxyplex, and MMC with Oxyplex respectively) in the whole trial period.

2-Fragmentation:

Table (3) and Figure (12) show the mean values of chromatid fragment in 50 metaphases spread in bone marrow cells of (*Mus musculus*) treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively, at many periods of treatments (6 hrs, 24 hrs, 48 hrs and 5days).

The results designate the mean values are very highly significant between the control values and the MMC treatments

after (24hrs and 48hrs) of treatments. On the other hand there isn't significant different in the results of the group MMC itself. And there is highly significant difference between control group and MMC after 6hrs and 5days of treatment.

Also, there aren’t significant differences between the control and the other groups of treatment or among the groups themselves.

3-Centric fusion:

Table (4) and Figure (13) show the mean values of Centric fusion in 50 metaphases spread in bone marrow cells of *(Mus musculus).* The results symbolize that, there is a significant difference between the control -ve and the MMC treatment after 24hrs of treatment.

On the other hand there is non significant different between the remaining groups with the control at all periods of treatments, or also among each group results themselves.

4- Chromatid breaks:

Table (5) and Figure (14) show the mean values of Chromatid breaks in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Manitol, MMC, Oxyplex, and MMC with Oxyplex respectively, At many times of treatments (6 hrs, 24 hrs, 48 hrs and 5days).

The recorded results apparent indicated that there are significant, highly significant and very highly significant differences between control results and the MMC group results.

5-Centromeric attenuation:

Table (6) and Figure (15) show the mean values of Centromeric attenuation in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively, at many times of treatments (6 hrs, 24 hrs, 48 hrs and 5days).

As usual there is highly significant difference between the control and MMC results. On the other hand there is non significant difference between control and the other groups.

6-Chromatid gaps:

Table (7) and Figure (16) show the mean values of Chromatid breaks in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively, at many times of treatments (6 hrs, 24 hrs, 48 hrs and 5days).

There is significant difference between the control results and MMC treatment at periods (6 hrs, 24 hrs and 5days).

7-End to end association:

Table (8) and Figure (17) show the mean values of End to end association in 50 metaphases spread in bone marrow cells of *(Mus musculus)*.

The statistical analysis refers to that there are significant differences between control result and MMC treated animals at all periods of treatments. On the contrast there is no significant difference between the control and other groups or among the groups results themselves.

B-Numerical aberrations:

1-Chromatid stickiness:

Table (9) and Figure (18) show the mean values of Chromatid stickiness in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively, at many times of treatments (24 hrs, 48 hrs and 5days).

The results point to that there are significant difference between control and MMC treated animals at all times of treatment. And there is non significant difference recorded between control and other groups of treatment or among all groups themselves.

Table (10) summarize the average of chromosomal abnormalities observed in bone marrow cells of *(Mus musculus)* treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively, at many times of treatments (6 hrs, 24 hrs, 48 hrs and 5days).

Mitotic index:

Table (11) and Figure (19) show the mean values of mitotic index (number of dividing cells per 1000 cells) in bone marrow cells of *(Mus musculus)* treated with Manitol, MMC, Oxyplex, and MMC with Oxyplex respectively, at many times of treatments (6 hrs, 24 hrs, 48 hrs and 5days).

It is clear that there is very highly significant difference between the control and the treated animals by MMC. On the other hand the mean values at all durations in the group Oxyplex treatment are higher than in the control group.

Chronic treatment

A-Structural aberration:

1-Deletion:

Table (12) and Figure (20) show the mean values of chromatid deletion in 50 metaphases spread in bone marrow cells of (*Mus musculus*) treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively. At chronic treatments, two duration periods of treatments (2 weeks and 4 weeks) with very low concentrations.

After two weeks and four weeks of treatment, the mean value of the deletion in the treated animals by Oxyplex is the lowest value of all groups, so there is highly significant difference between it and control and other groups of treatment.

After 4weeks of treatment, the mean value of the deletion in the treated animals by MMC is the highest value of all groups, so there is significant, highly significant and very highly significant difference between it and control and all groups of treatment. Also, there is significant between the control and the treatment by MMC with Oxyplex.

2-Fragmentation:

Table (13) and Figure (21) show the mean values of chromatid fragment in 50 metaphases spread in bone marrow cells of (*Mus musculus*) treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively. At chronic treatments, two duration periods of treatments (2 weeks and 4 weeks) with very low concentrations.

After two weeks and 4weeks of treatment, the mean value of MMC treatment is the highest value when it compared with control or other groups. Then there is significant and highly significant difference between the MMC results and the other values.

On the other hand, after 4weeks of treatment; the mean value of Oxyplex treatment is the smallest value in the table. So there is significant difference when it compared with the others.

3-Centric fusion:

Table (14) and Figure (22) show the mean values of centric fusion in 50 metaphases spread in bone marrow cells of (*Mus musculus*) treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively. At chronic treatments, two duration periods of treatments (2 weeks and 4 weeks) with very low concentrations.

The statistical analysis indicated that there is significant and highly significant difference between the MMC treatment

and others groups after two periods of treatment (two weeks and 4weeks).

4- Chromatid breaks:

Table (15) and Figure (23) show the mean values of chromatid breaks in 50 metaphases spread in bone marrow cells of (*Mus musculus*) treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively.

The statistical analysis indicates that there is highly and very highly significant different between the MMC treatment and other groups.

5-Centromeric attenuation:

Table (16) and Figure (24) show the mean values of centromeric attenuation in 50 metaphases spread in bone marrow cells of (*Mus musculus*) treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively, after (2 weeks and 4 weeks) with very low concentrations.

The results illustrate that there is significant difference between the MMC value and both Oxyplex, and MMC with Oxyplex values after two weeks of treatment, but after four weeks of treatment there is significant difference between the MMC value and Oxyplex value.

6-Chromatid gaps:

Table (17) and Figure (25) show the mean values of chromatid gabs in 50 metaphases spread in bone marrow cells of (*Mus musculus*) treated with Mannitol, MMC, Oxyplex, and MMC with Oxyplex respectively for (2 weeks and 4 weeks) with very low concentrations.

The statistical analysis indicated that there is no significant difference between the control and other groups of treatment.

7-End to end association:

Table (18) and Figure (26) show the mean values of end to end association in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Manitol, MMC, Oxyplex, and MMC with Oxyplex respectively at (2 weeks and 4 weeks) with very low concentrations.

The results reported that there is no significant difference between the control and other groups at all periods of durations.

B-Numerical aberrations:

1-Chromatid stickiness:

Table (19) and Figure (27) show the mean values of chromatid breaks in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Mannitol, MMC, Oxyplex, and MMC + Oxyplex respectively at (2 weeks and 4 weeks).

The results point to that there is significant difference between MMC treated animals and Oxyplex group after two weeks of treatment. Also after four weeks of treatment there is significant difference between MMC treated animals and MMC with Oxyplex group, and very highly significant difference between MMC treated animals and Oxyplex group. Also, there is significant difference between control value and MMC treated animals after four weeks of treatment.

Mitotic index:

Table (20) and Figure (28) show the mean values of chromatid breaks in 50 metaphases spread in bone marrow cells of *(Mus musculus)* treated with Mannitol, MMC, Oxyplex, and MMC + Oxyplex respectively, at chronic treatments, of (2 weeks and 4 weeks). The results show that there is high and very highly significant difference among most of groups.

II-Sperms:

The sperm in the normal case consists of head and tail, figure (29). The abnormalities appear in the head region are mostly related to size or shape of the head. If the hook is absent this is called ***lack hook*** aberration, figure (30). The head may be reduced in size; it is called ***amorphous*** aberration, figure (31). The head might be shown on unusual curvature or ***banana like shape***, figure (32).

Fig. (29): Normal sperm from a control group of *(Mus musculus)*.

(Eosin stain x 1000)

Fig. (30): Sperm head with a lost pointed hook (lack hook) in *Mus musculus* treated by MMC.

(Eosin stain x 1000)

Fig. (31): Amorphous sperm head in *Mus musculus* treated by MMC.

(Eosin stain x 1000)

Fig. (32): Banana like sperm head in *Mus musculus* treated by MMC.

(Eosin stain x 1000)

Table (21) and figure (33) summarize the total abnormalities (Amorphous, Banana like, No hook and Folded) in the shape of sperms of *(Mus musculus)* per 1000 sperms after exposed to Mannitol treatment (which used as control +ve group), at many times of treatments (6 hrs, 24 hrs, 48 hrs and 5days).

The results designate that the difference between the control –ve group result and the animals which treated by Mannitol at all durations of treatment is not significant.

On the other hand, the animals (which treated by MMC) recorded very high significant difference with the control group, after all duration periods of treatment. These results are indicated in table (22) and figure (34).

Also there is no significant difference between the control group and the treated animals by Oxyplex except that there is significant difference after 24hrs of this treatment where the mean value of sperms aberrations is less than the mean value of control ones. These results are shown in the table (23) and figure (35). And also all means values of sperms abnormalities are less in case of Oxyplex treatment than the control -ve ones.

Table (24) and figure (36) summarize the abnormality in the shape of sperms of *(Mus musculus)* per 1000 sperms after exposed to MMC + Oxyplex treatment at (6 hrs, 24 hrs, 48 hrs and 5days).

The observed results show that there is high significant difference between the control and these treated animals after 6hrs of treatment and there are significant difference between the control and these treated animals after 24, 48hrs and 5days of treatment . But all mean values are less than that treated with only MMC.

After two weeks of treatment with Mannitol, MMC, Oxyplex and MMC with Oxyplex respectively; it is clear that the results recorded no significant difference between control group and Mannitol group, on the other hand the treated animals by Oxyplex shows significant difference with control group where the mean value of sperms aberrations is less than the mean value of control ones. Also the animals treated by MMC with Oxyplex recorded significant difference with the control group. But in case of MMC group, there is very highly significant difference compared with control one. These results summarized in tables (25) and figures (37).

After four weeks treatment with Mannitol, MMC, Oxyplex and MMC with Oxyplex respectively; it is clear that the results recorded no significant difference between control group and Mannitol, or Oxyplex group. On contrast in case of MMC there was very highly significant difference. Also there is significant difference between control group and MMC + Oxyplex one values. These results summarized in tables (26) and figures (38).

III-Molecular observations:

Determination of DNA damage and apoptosis in liver and spleen of *Muss musculus* animals treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex respectively.

In all figures 39, 41, 43, 45, 47 and 49 (liver), And in all figures from 51, 53, 55, 57, 59 and 61 (spleen), lane 1 represents that DNA genome control –ve samples, from all treated mice with different durations (6hrs, 24hrs, 48hrs, 5days, 2weeks and 4 weeks, which has high molecular weight, so that they often remained in the wheels of the agarose gel electrophoresis. But the ladder in these all figures lane 6, it is separated into bands with different lengths.

In treated liver:

Figures (from 39 to 50) and tables (from 27 to 32) elucidate the damage and the optical density of DNA of liver cells of mice treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex, with different durations (6hrs, 24hrs, 48hrs, 5days, 2weeks and 4 weeks respectively.

The results refer to that; nearly there are no damages in DNA of mice treated with Mannitol and Oxyplex at all durations of treatment (often as the control results).

MMC causes several damages (necroses) in DNA of hepatocytes when it was compared with control. And damage

decreased in DNA of hepatocytes of mice which treated with both MMC and Oxyplex together.

Hepatocytes DNA damage of mice treated with MMC increased when compared with control, so the optical density of apoptosis bands of DNA at (180 bp and its multiples 360, 540 and 720 bp) bp show a very high significant increase than control. On the other hand intact DNA decreased sharply than control.

Oxyplex makes highly protection against treatment with MMC, so the optical density of apoptosis bands of DNA decreased and intact DNA increased in protected groups than treated group with MMC only.

DNA of hepatocytes of mice treated with Mannitol or Oxyplex showed nearly similar results of DNA as control.

Figure (39) elucidate the damage DNA of liver cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 6hrs. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

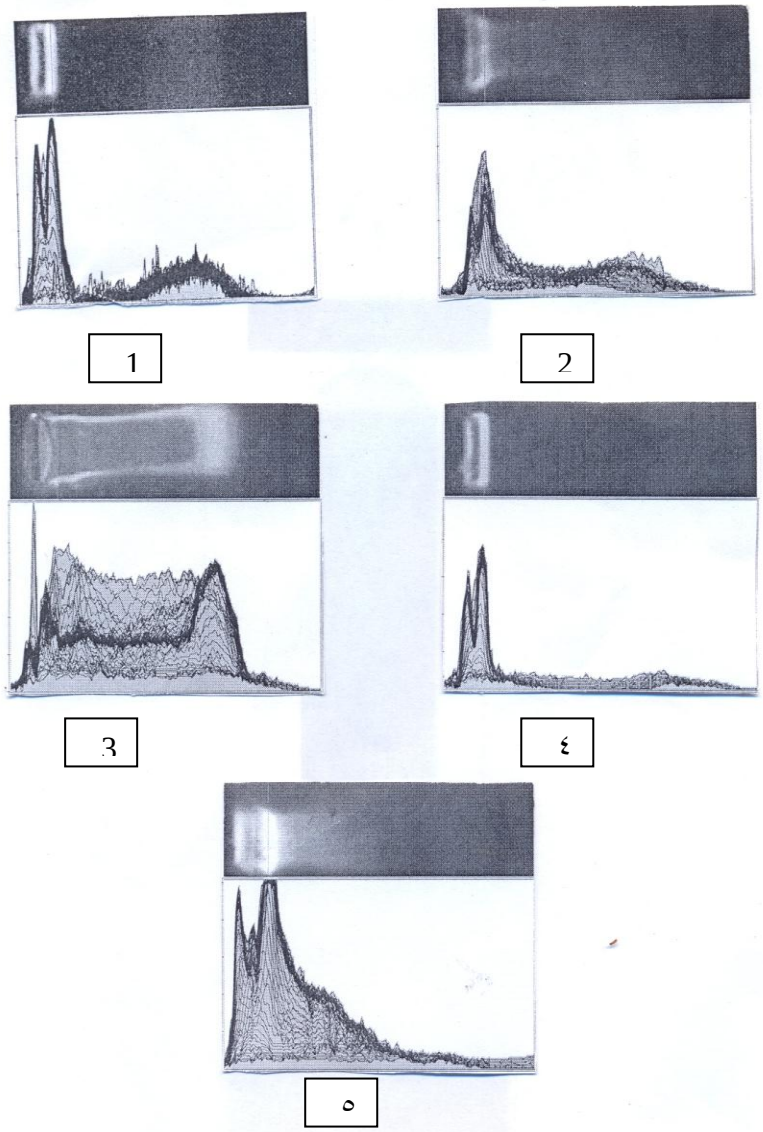

Figure (40) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 39) by using biogene software.

Table (27) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in liver of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 6hrs (respectively).

	L1	L2	L3	L4	L5
Band 1	_	193	200	193	255
Band 2	_	_	220	_	_
Band 3	_	_	_	_	_
Band 4	_	_	_	_	_

Figure (41) elucidate the damage DNA of liver cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 24hrs. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

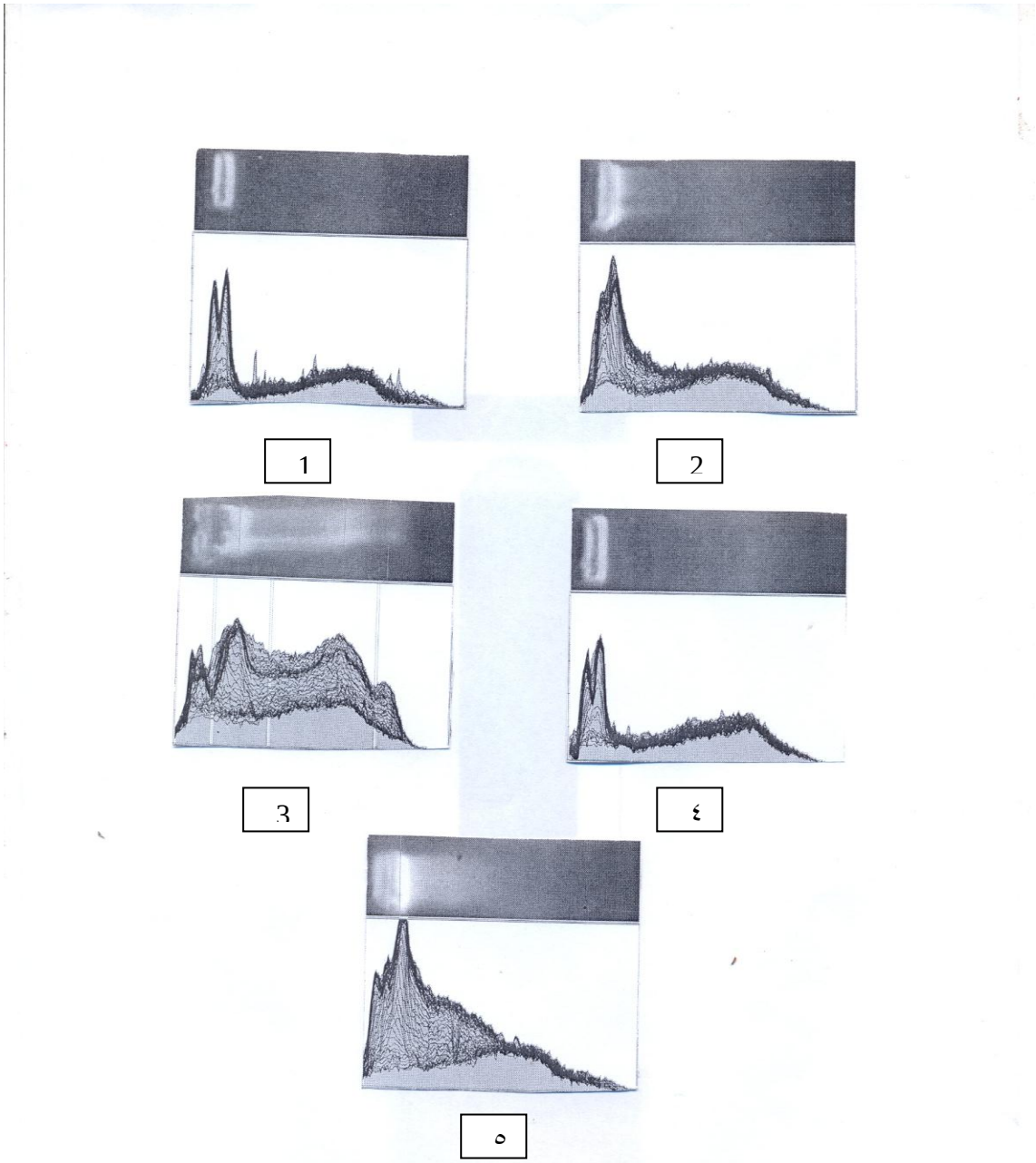

Figure (42) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 41) by using biogene software.

Table (28) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in liver of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 24hrs (respectively).

	L1	L2	L3	L4	L5
Band 1	200	230	151	191	255
Band 2	_	_	193	_	_
Band 3	_	_	174	_	_
Band 4	_	_	103	_	_

Figure (43) elucidate the damage DNA of liver cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 48hrs. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

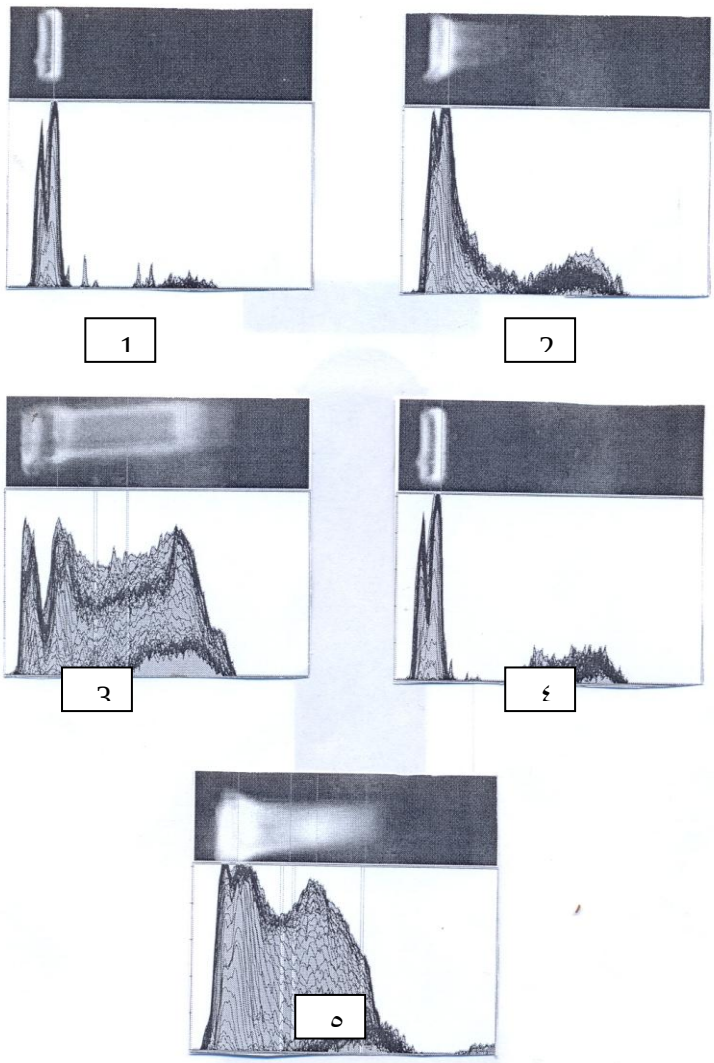

Figure (44) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 43) by using biogene software.

Table (29) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in liver of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 48hrs (respectively).

	L1	L2	L3	L4	L5
Band 1	240	255	217	240	255
Band 2	_	_	176	_	192
Band 3	_	_	120	_	236
Band 4	_	_	200	_	133

Figure (45) elucidate the damage DNA of liver cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 5days. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

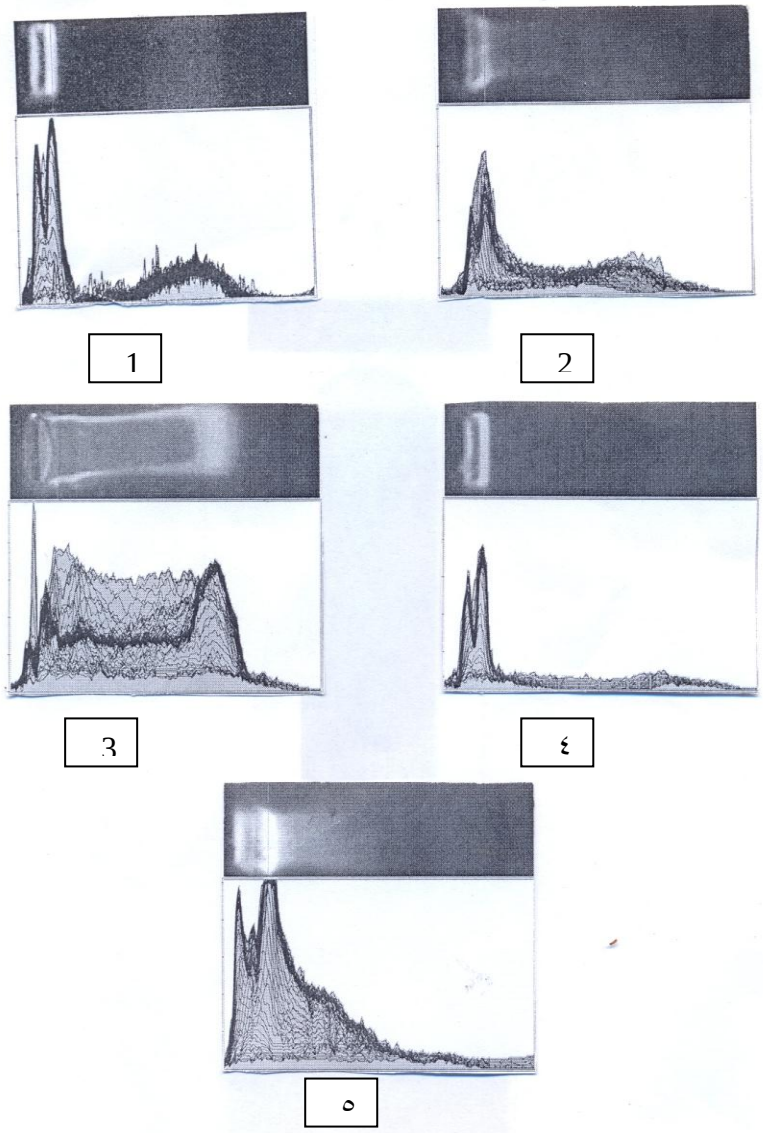

Figure (46) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 45) by using biogene software.

Table (30) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in liver of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 5days (respectively).

	L1	L2	L3	L4	L5
Band 1	215	213	139	208	255
Band 2	_	_	197	_	106
Band 3	_	_	203	_	_
Band 4	_	_	_	_	_

Figure (47) elucidate the damage DNA of liver cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 2 weeks. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

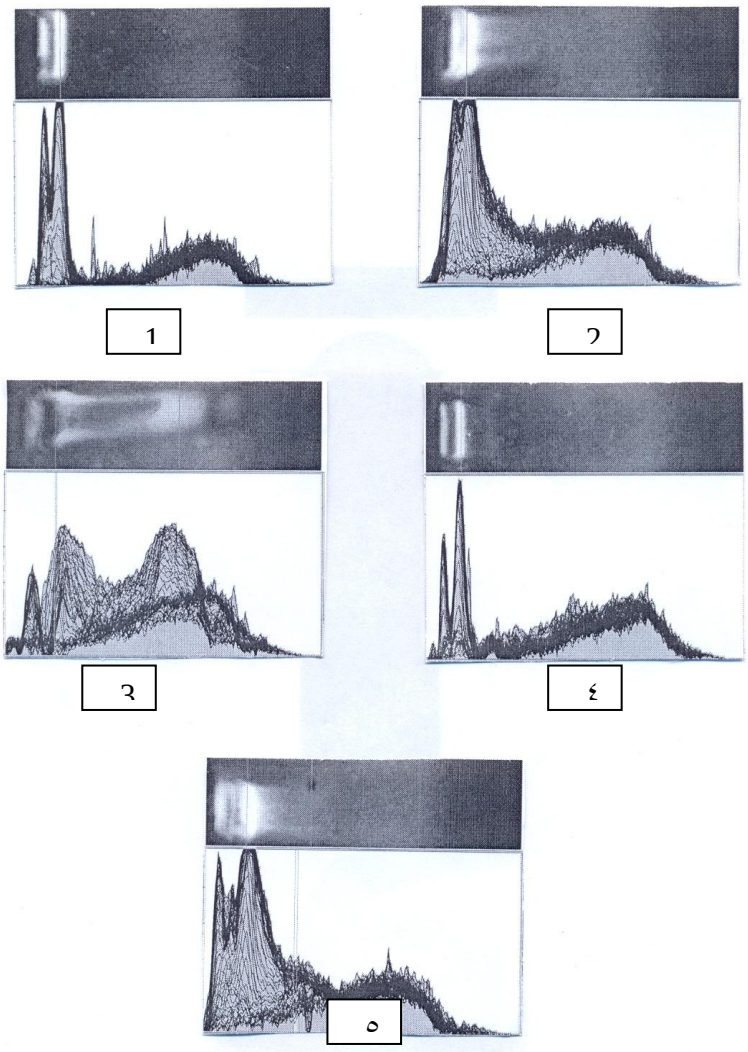

Figure (48) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 47) by using biogene software.

Table (31) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in liver of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 2 weeks (respectively).

	L1	L2	L3	L4	L5
Band 1	223	255	153	241	254
Band 2	_	_	203	_	117
Band 3	_	_	_	_	_
Band 4	_	_	_	_	_

Figure (49) elucidate the damage DNA of liver cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 4 weeks. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

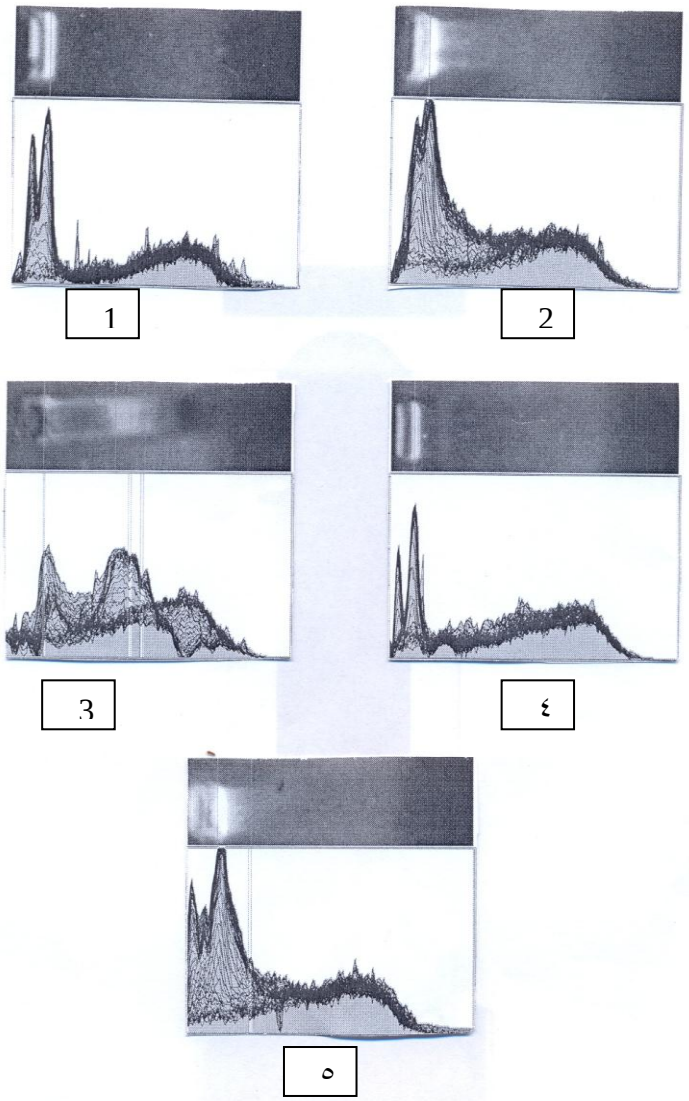

Figure (50) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 49) by using biogene software.

Table (32) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in liver of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 4weeks (respectively).

	L1	L2	L3	L4	L5
Band 1	225	255	154	213	251
Band 2	_	_	195	_	_
Band 3	_	_	_	_	_
Band 4	_	_	_	_	_

In treated spleen:

Figures (from 51 to 61) and tables (from 33 to 38) elucidate the damage and the optical density of DNA of spleen cells of mice treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex, with different durations (6hrs, 24hrs, 48hrs, 5days, 2weeks and 4 weeks respectively. Nearly, there are no damages in DNA of mice treated with Mannitol and Oxyplex at all durations of treatment (often as the control results).

MMC causes several damages (necroses) in DNA of spleen cells compared to control. The damage decreased in DNA of spleen cells of mice which treated with both MMC and Oxyplex.

The results elucidate that spleen cells DNA damage of mice treated with MMC increased when compared with control, so the optical density of apoptosis ands of DNA at (180 bp and its multiples 360, 540 and 720 bp.) show a very high significant increase than control. On the other hand intact DNA decreased sharply than control.

Oxyplex makes highly protection against treatment with MMC, so the optical density of apoptosis bands of DNA decreased and intact DNA increased in protected groups than treated group with MMC. DNA of spleen cells of mice treated with Mannitol or Oxyplex shows nearly similar results of DNA as control.

Figure (51) elucidate the damage DNA of spleen cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 6hrs. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

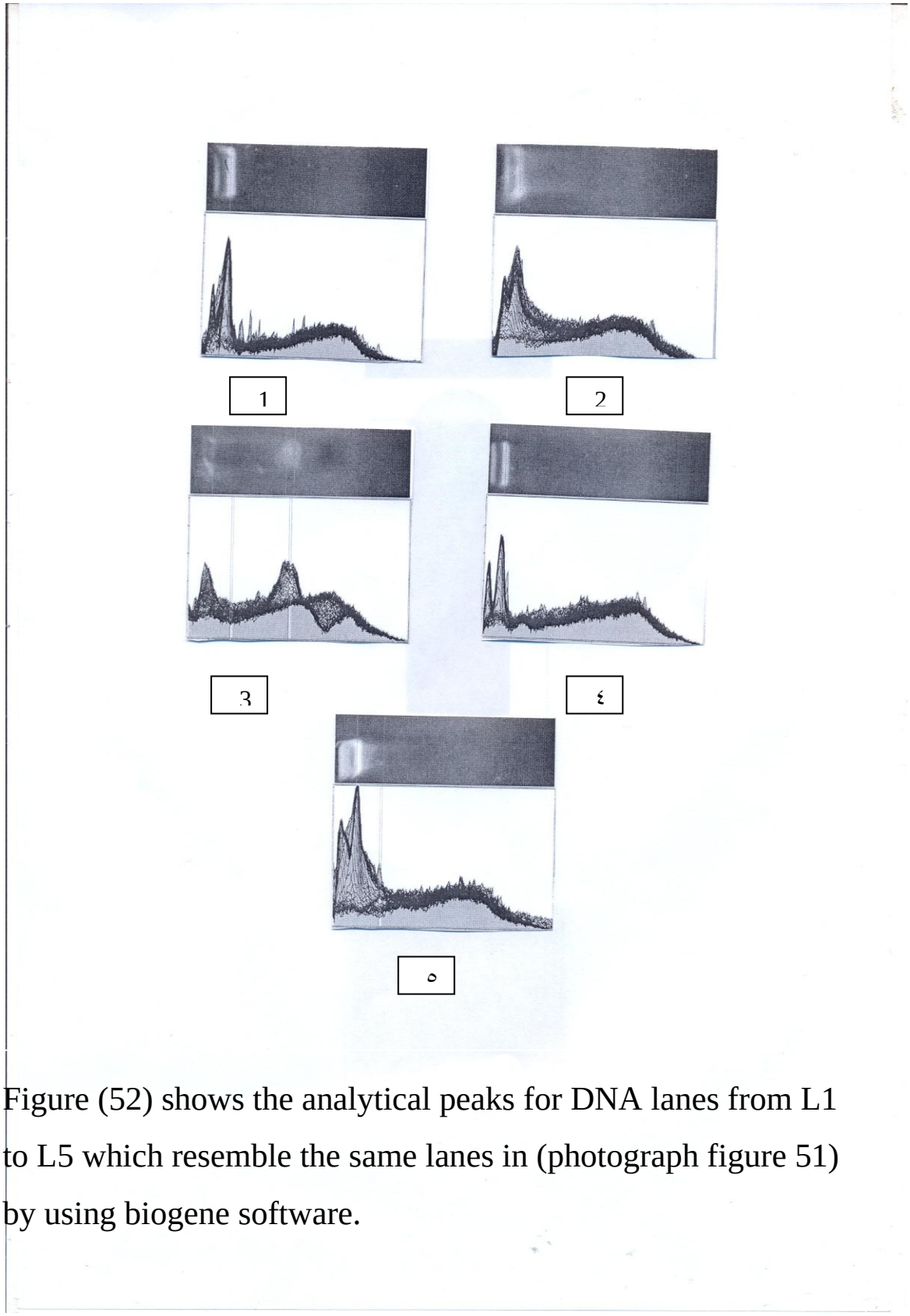

Figure (52) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 51) by using biogene software.

Table (33) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in spleen of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 6hrs (respectively).

	L1	L2	L3	L4	L5
Band 1	213	201	134	185	252
Band 2	_	_	147	_	_
Band 3	_	_	_	_	_
Band 4	_	_	_	_	_

Figure (53) elucidate the damage DNA of spleen cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 24 hrs. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

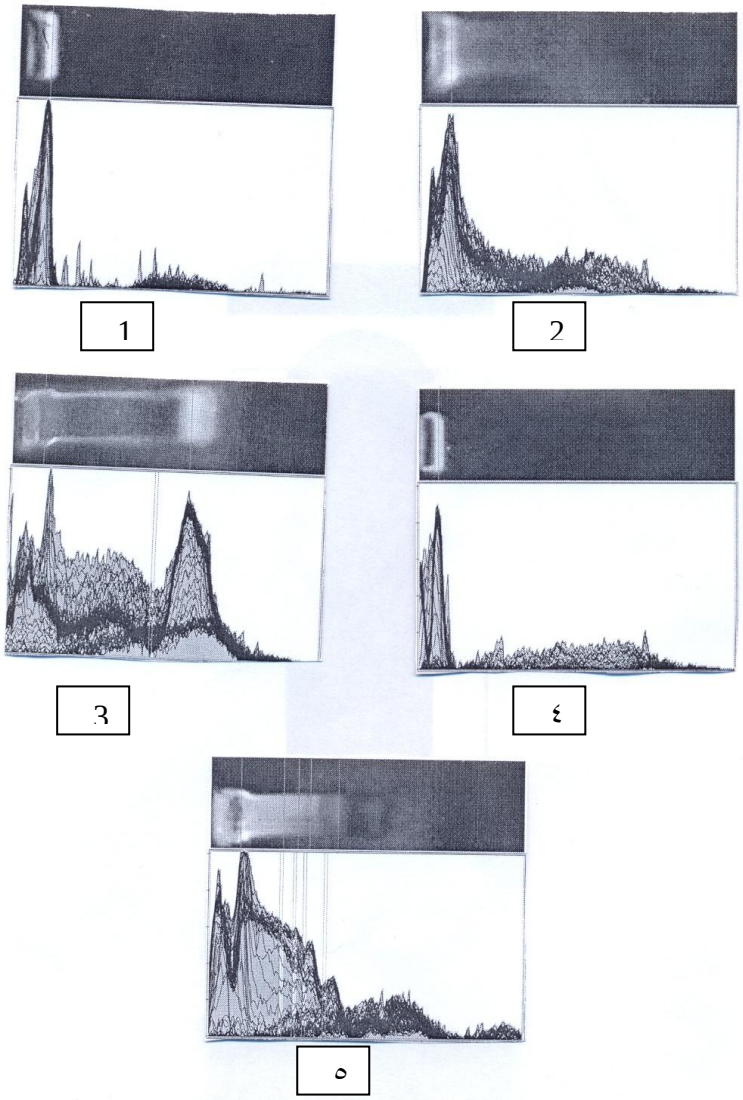

Figure (54) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 53) by using biogene software.

Table (34) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in spleen of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 24hrs (respectively).

	L1	L2	L3	L4	L5
Band 1	238	231	221	238	249
Band 2	_	_	241	_	151
Band 3	_	_	_	_	133
Band 4	_	_	_	_	84

Figure (55) elucidate the damage DNA of spleen cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 48 hrs. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

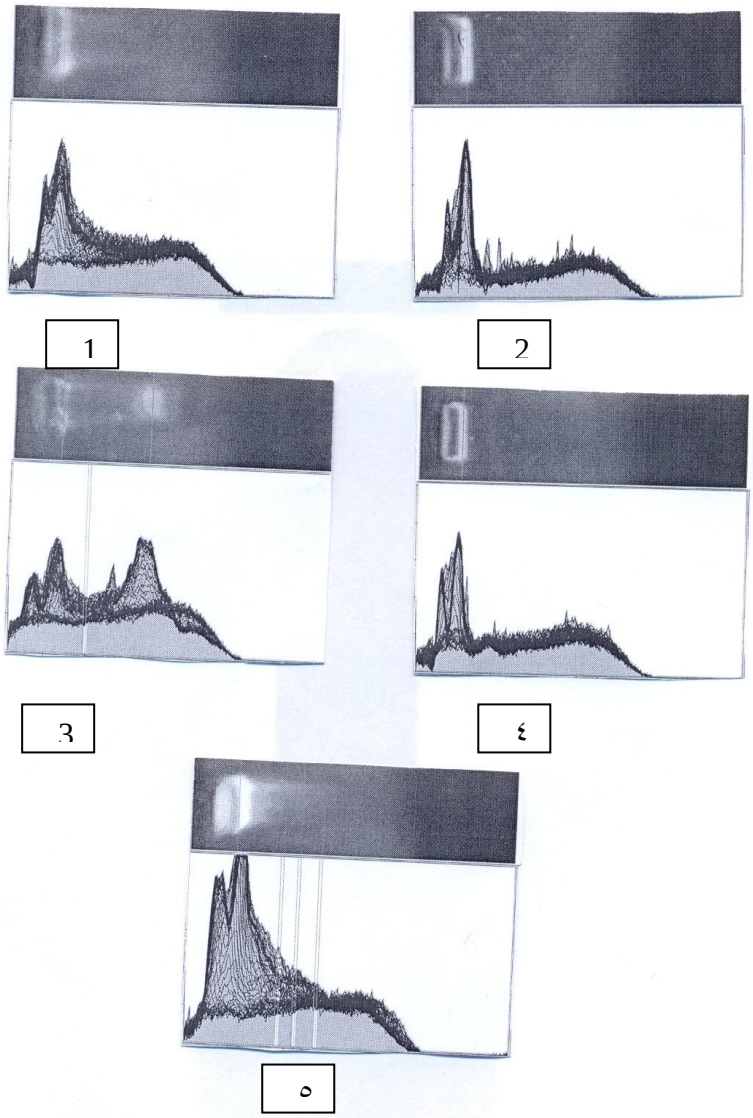

Figure (56) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 55) by using biogene software.

Table (35) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in spleen of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 48 hrs (respectively).

	L1	L2	L3	L4	L5
Band 1	222	214	153	167	253
Band 2	_	_	223	_	_
Band 3	_	_	_	_	_
Band 4	_	_	_	_	_

Figure (57) elucidate the damage DNA of spleen cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 5days. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

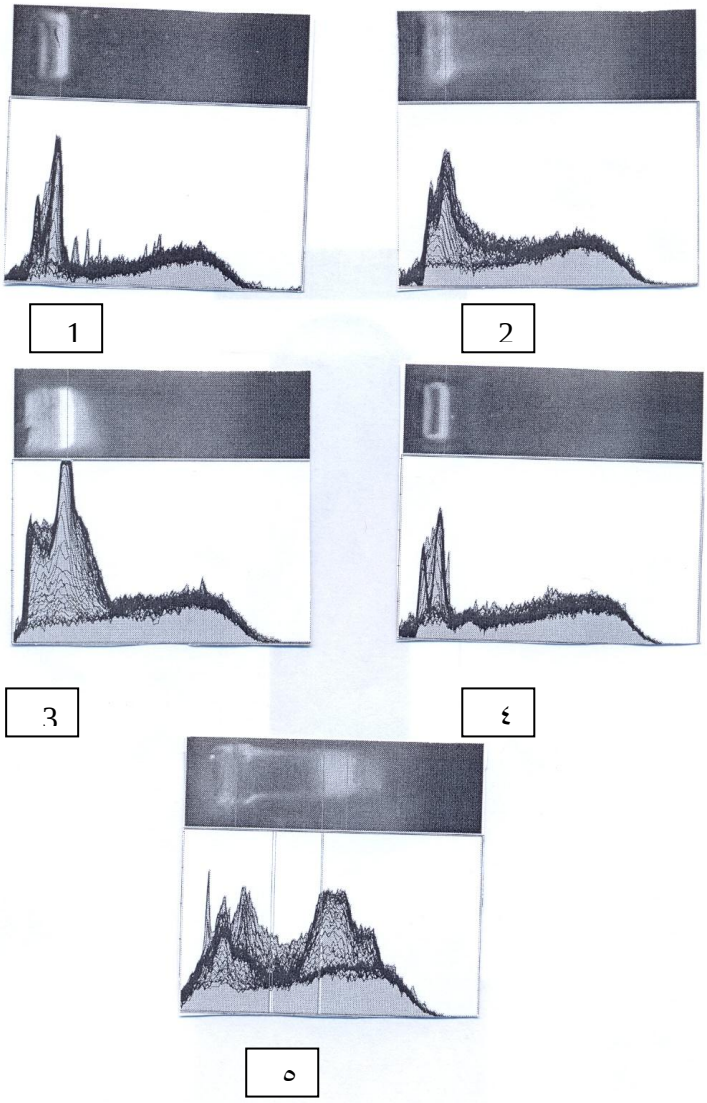

Figure (58) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 57) by using biogene software.

Table (36) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in spleen of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 5 days (respectively).

	L1	L2	L3	L4	L5
Band 1	219	203	163	225	255
Band 2	_	_	245	_	_
Band 3	_	_	185	_	_
Band 4	_	_	_	_	_

Figure (59) elucidate the damage DNA of spleen cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 2 weeks. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

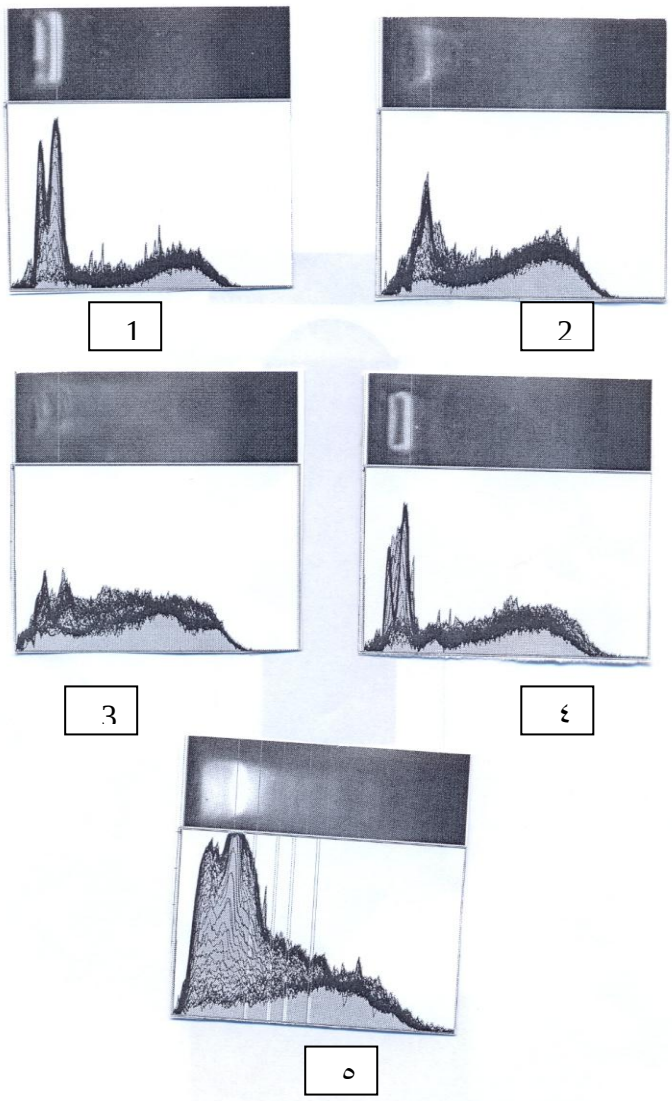

Figure (60) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 59) by using biogene software.

Table (37) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in spleen of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 2 weeks (respectively).

	L1	L2	L3	L4	L5
Band 1	246	185	124	213	255
Band 2	_	_	_	_	211
Band 3	_	_	_	_	122
Band 4	_	_	_	_	97

Figure (61) elucidate the damage DNA of spleen cells of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 4 weeks. Lane1: control, lane 2: Mannitol, lane 3: MMC, lane 4: Oxyplex, lane 5: MMC with Oxyplex, and lane 6: ladder

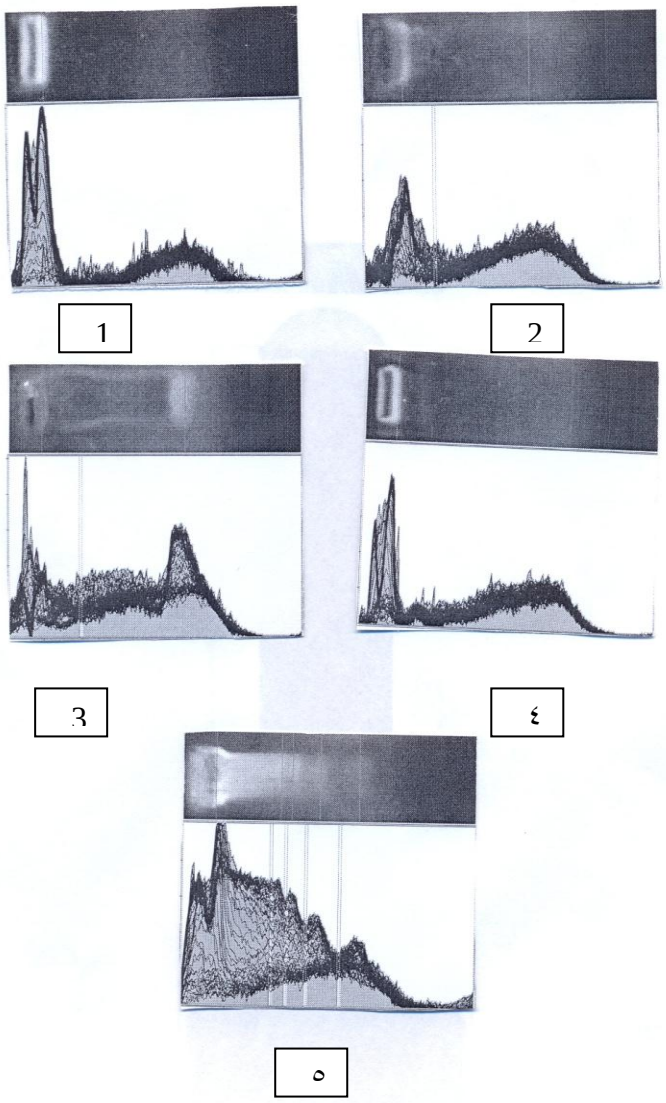

Figure (62) shows the analytical peaks for DNA lanes from L1 to L5 which resemble the same lanes in (photograph figure 61) by using biogene software.

Table (38) optical density of intact and apoptotic bands of DNA appeared and located at 180 bp and its multiples 360, 540 and 720 bp in spleen of *Mus Musculus* treated with Mannitol, MMC, Oxyplex and MMC with Oxyplex after 4 weeks (respectively).

	L1	L2	L3	L4	L5
Band 1	243	186	127	203	249
Band 2	_	_	221	_	176
Band 3	_	_	_	_	128
Band 4	_	_	_	_	98