EFFECTS OF METHOMYL ON DNA, AN EXPERIMENTAL STUDY

BY

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ABSTRACT

Methomyl is a carbamate insecticide that is widely used. This study investigates the genotoxic effects of methomyl on mice. Methomyl was given intraperitoneally to mice in two doses. Groups I and III received methomyl as 2.5 mg /kg while groups II and IV received methomyl as 5 mg /kg. Groups I and II were used for the comet and alkaline elution assays, while groups III and IV were used for the micronucleus test. The comet and alkaline elution assays and the micronucleus test are markers for genetic damage , while apoptosis is a sequel of this damage. The comet assay and apoptosis were performed on peripheral lymphocytes, while alkaline elution assay was performed on liver and spleen. The micronucleus test was performed on polychromatic erythrocytes (PCEs). Methomyl was found to increase significantly DNA damage in peripheral lymphocytes, liver, spleen and polychromatic erythrocytes. This effect did not improve after 24h excpet in PCEs although it did not return to control levels. Increasing methomyl dose showed different responses in different tissues. It produced insignificant changes in the PCEs and the spleen, very high significant increase in DNA damage in the peripheral lymphocytes and in the liver and a significant decrease in apoptotic lymphocytes. This may be due to difference in repair effeciency, metabolic activity and / or cell division. So it is recommended to use a combination of tests to assess the genotoxicity of a chemical to decrease the incidence of erroneous results. Application of the micronucleus test and the comet assay as monitoring methods for high risk groups to exclude genotoxicity. These tests are rapid, simple and sensitive for measuring and analyzing DNA breakage.

INTRODUCTION

The ability of environmental toxicants and xenobiotics to adversely affect the body systems is of increasing concern (Holsapple et al., 1996). Since the human gene pool is our most precious heritage, it is vital to keep the mutagenic pressure as low as possible. The correlation between mutagenic and carcinogenic properties of chemical substances and our inability to cure mutations urgently cause scientists to

detect mutagenic chemicals (Vanparys et al., 1996).

Molecular epidemiology is increasingly applied in the studies of cancer risks associated with exposure to environmental carcinogens. Genetic markers are used to detect early biological responses in an attempt to link carcinogen exposure to initiating events in the carcinogenesis process. These include chromosomal aberrations and micronuclei (Wogan, 1992).

Genotoxic stress triggers a variety of biological responses including transcriptional activation of genes regulating cell survival (Aubracht et al., 1999). Toxicants can induce or inherence apoptosis or alternatively inhibit normal physiological apoptosis from occurring. Apoptosis is one mechanism for the removal of cells with DNA damage by toxicants. Removal of cells with DNA damage minimizes the chances for malignant transformation to occur (Horn et al., 2000).

Methomyl is a synthetic carbamate that was introduced in 1966 in the United States. It is now available throughout the world (Ellenhorn, 1997). It is listed in the highly hazardous group of pesticides in the WHO recommended classification on the basis of its rat acute oral LD 50 value (IPCS, 1994).). Human exposure occurs during the preparation and application of these products and from the ingestion of crop residues in foodstuffs. Methomyl appears to elicit weak or no response in microbial gene mutation assays, however, it is able to induce structural and numerical chromosomal aberrations in plants and animals and causes increase in micronulei and chromosomal aberrations in human lymphocytes (Bonatti et al., 1994).

By using the in vivo method, factors which may affect the induction of mutations like bioavailability, metabolic inactivation, DNA repair or cell proliferation are all taken into account (Myhr, 1991).

The aim of this study is to prove the genotoxic effect of methomyl on liver, spleen, bone marrow cells and peripheral lymphocytes. Employing a combination of different tests will reduce the risk of erroneous results and conclusions.

MATERIAL AND METHODS

I. Animals

One hundred and thirty-five mice, 8-10 weeks old and weighing 25-35 g, obtained from Animal House of Mansoura University were used in the present study. Animals were fed on ordinary food with free water supply.

Animals were divided into:

1- Control group: comprised 15 mice, each mouse was given 1mL distilled water

intraperitoneally, sacrificed by decapitation and blood samples were collected in test tubes containing heparin. Livers and spleens were removed and bone marrow from femur bones was aspirated by a syringe after cutting their ends, then flushed into test tubes containing calf serum.

2- Test groups: Four main groups (I, II, III and IV), 30 mice each. According to Bologensi et al. (1994) two doses of methomyl (5 and 10 mg/ kg) were used. The chosen doses did not show anticholinergic clinical effects. Animals from groups I and II were used for the comet and the alkaline elution assays and for apoptosis. While animals from group III and IV were used for the micronucleus assay.

Group I : Each mouse was given methomyl (2.5 mg /kg) intraperitoneally as a single dose. Then half the animals were sacrificed 4 h (subgroup I a) and the other half 24 h (subgroup I b) after the injection.

Group II : Each mouse was given methomyl (5 mg /kg) intraperitoneally as a single dose. Then half the animals were sacrificed 4 h (subgroup II a) and the other half 24 h (subgroup II b) after the injection.

Animals of group I and II were sacrificed by decapitation, blood samples were collected in test tubes containing heparin and liver and spleen from each animal were removed.

Group III: Each mouse was given two doses of methomyl (2.5 mg /kg each) intraperitoneally with 24 h interval. Then half the animals were sacrificed 6 h (subgroup III a) and the other half 24 h (subgroup III b) after the last injection .

Group IV : Each mouse was given two doses of methomyl (5 mg /kg each) intraperitoneally with 24 h interval. Then half the animals were sacrificed 6 h (subgroup IV a) and the other half 24 h (subgroup IV b) after the last injection.

After the animals of groups III and IV were sacrificed by decapitation, femur bones of each mouse were cut at their ends, and bone marrow was aspirated by a syringe and flushed into test tubes containing calf serum.

II. Methods:

1) Lymphocyte separation and culture (Boyum, 1976).

2) Single cell - gel electrophoresis assay (comet assay) (Singh et al., 1988):

It is based on embedding of cells in agarose and alkaline lysis of labile DNA at sites of damage by alkaline buffer (pH 12.3). The alkalinity causes DNA denaturation. This unwound DNA is able to migrate out of the cell during electrophoresis and can be visualized by ethidium bromide. In damaged cells, breaks appear as fluorescent tails extending from the core

towards the anode. The tail length reflects the amount of DNA breakage in the cell. It was evaluated in 100 randomly selected cells per sample.

3) Alkaline elution of organs (liver and spleen). (Cavanna et al., 1979):

The alkaline elution assay measures the rate of DNA elution through a filter membrane under alkaline conditions. The alkalinity causes DNA denaturation. The amount of DNA single strand breaks (SSB) or lesions converted to SSB under alkaline conditions are estimated on the basis of the increase in DNA elution rate. The filter allows SSB to pass while large DNA molecules are retained on the filter. Results were expressed as elution rate constant, K (mL^{-1}):

$$K\ (mL^{-1}) = \frac{\text{Fraction of DNA retained on the filter}}{\text{eluted volume}}$$

4) Micronuclei (MN) test for bone marrow (Schmid, 1975):

The mouse bone marrow micronucleus test, an in vivo assay that detects damage to the chromosomes or the mitotic apparatus of immature red blood cells found in bone marrow. During cell division, undamaged chromosomes give rise to normal daughter nuclei, but if the chromosomes are broken or the mitotic apparatus of the cell is damaged, chromosome fragments may be incorporated in secondary nuclei instead of into the main nucleus. Secondary nuclei are much smaller than the main nucleus and are referred to as micronuclei. When erythroblasts develop into polychromatic erythrocytes (PCEs), the main nucleus is extruded but any micronuclei that are present remain behind. Thus, an increase in the number of micronucleated PCEs in animals treated with the test article extract is an indication of the presence of a genotoxin. Micronuclei were scored under microscope at a magnification of X 200. A total of 1000 cells / slide were examined.

5) Assessment of apoptosis:

a- Morphological assessment of apoptosis using different stains.

1- Giemsa stain (Gregory et al., 1991) : Cells were examined for decreased size, chromatin aggregation, condensed and fragmented nuclei, and cytoplasmic vaculation. The percentage of apoptotic cells was calculated.

2- Acridine orange stain (Whyte et al., 1990): Lymphocytes exhibiting bright green fluorescent condensed chromatin located at the nuclear membrane and those with fragmented nuclei were interpreted as apoptotic cells and expressed as a percentage of the total cell number. Viable lymphocytes appeared with green diffusely stained intact nuclei.

b- DNA fragmentation (Bursch et al., 1992 modified by Shin et al., 2000): Cells undergoing programmed cell death exhibited DNA fragmentation and appeared as ladder like pattern.

6) **Statistical analysis** was done using Statistical Package for Social Science Program version 10, (SPSS) (1999). Significance was considered when p value is less than 0.05.

RESULTS

I. The comet assay :

Table (1): It shows the effects of methomyl on DNA of peripheral lymphocytes compared to control measured by the comet assay.

Statistical analysis of these data reveals very high significant increase in the percentage of the comet assay in all test groups compared to control ($P<0.001$).

Time relation: There are statistically insignificant increases in the percentage of the comet assay when subgroups Ia and IIa are compared to subgroups Ib and IIb respectively ($P>0.05$).

Table (2) It shows the effect of increasing methomyl dose on the percentage of the comet assay of test groups.

Dose - response relationship

Statistical analysis shows very high significant increase in the percentage of the comet assay by increasing the methomyl dose both after 4h (a) and after 24h (b) ($P<0.001$).

II. Alkaline elution for the spleen:

Table (3): It shows the effects of methomyl administration on the DNA of spleen cells compared to control.

Statistical analysis of these data reveals very high significant increase in the elution rate constant when control is compared to all subgroups ($P<0.001$).

Time relation:

There is statistically significant increase in elution rate constant when the mean values for subgroups Ia and IIa are compared to subgroups Ib and IIb respectively ($P<0.05$).

Table (4): It shows the effects of increased methomyl dose on the alkaline elution rate constant for the splenic cells in test groups.

Dose - response relationship:

Statistical analysis shows insignificant increases in the elution rate constant when the mean values for subgroups Ia and IIa and subgroups Ib and IIb are compared to each other both after 4h (a) and after 24h (b) ($P > 0.05$).

III. Alkaline elution for the liver

Table (5): It shows the effects of methomyl on DNA of liver cells compared to the control.

Statistical analysis of the results reveals very high significant increases in elution rate constant in all subgroups when compared to control (P<0.001).

Time relation:

There is statistically insignificant increase in elution rate constant when subgroups Ia and IIa are compared to subgroups Ib and IIb respectively (P>0.05).

Table (6): It shows the effects of increasing methomyl dose on the alkaline elution rate constant for the liver in test groups.

Dose- response relationship:

Statistical analysis reveals significant increase in elution rate constant after 4h when subgroup Ia is compared to subgroup IIa (P < 0.05). However, there is insignificant increase in elution rate constant after 24h when subgroup Ib is compared to subgroup IIb (P > 0.05).

VI. The micronucleus test (MNT):

Table (7): It shows the effects of methomyl on DNA of PCEs compared to control.

Statistical analysis of these results reveals very high significant increase in the perthousand of MN in test groups in comparison to control (P<0.001).

Time relation :

There is statistically insignificant decrease in the perthousand of MN in subgroup IIIa if compared to subgroup IIIb (P > 0.05). However, there is very high significant decrease in the perthousand of MN in subgroup IVa when compared to subgroup IVb (P< 0.001).

Table (8): It shows the results of increased methomyl dose on the perthousand of MNT in test groups.

Dose-response relationship:

It can be seen that, there are statistically insignificant changes in the perthousand of MNT in the form of insignificant increase in MN after 6h and insignificant decrease in MN after 24h (P> 0.05).

V Apoptosis (giemsa stain):

Table (9): It shows the effects of methomyl on the percentage of apoptotic peripheral lymphocytes stained with giemsa stain in the test groups compared to control.

Statistical analysis of these results reveals very high significant increase in the percentage of apoptotic lymphocytes in all test groups compared

to the control (P< 0.001).

Time relation:

It is observed that, after 24h, there is high significant increase in the percentage of apoptotic lymphocytes when subgroup Ib is compared to subgroup Ia and when subgroup IIb is compared to subgroup IIa (P< 0.01).

Table (10): It shows the effects of increasing the methomyl dose on the percentage of apoptotic lymphocytes in test groups.

Dose response relationship:

It can be observed that after 4h, there is statistically insignificant increase in the percentage of apoptotic lymphocytes (P> 0.05). However after 24h there is statistically significant decrease in the percentage of apoptotic lymphocytes (P< 0.05).

VI Apoptosis (acridine orange stain):

Table (11): It shows the effects of methomyl on the percentage of apoptotic peripheral lymphocytes (acridine orange stain) in test groups compared to control.

Statistical analysis of these data shows, high significant increase in the percentage of apoptotic lymphocytes when subgroup Ia is compared to the control (P< 0.01). However, there is very high significant increase in the percentage of apoptotic lymphocytes when subgroup Ib is compared to control (P< 0.001).

There is high significant increase in the percentage of apoptotic lymphocytes in subgroup IIa compared to control (P< 0.05). Meanwhile, there is very high significant increase in the percentage of apoptotic lymphocytes in subgroup IIb compared to control (P< 0.001).

Time relation :

There is statistically high significant increase in the percentage of apoptotic lymphocytes when subgroup Ib is compared to subgroup Ia and when subgroup IIb is compared to subgroup IIa (P< 0.01).

Table (12): It shows the effects of increased methomyl dose on the percentage of apoptotic lymphocytes in test groups.

Dose - response relationship:

It can be seen that there are statistically insignificant changes in the percentage of apoptotic lymphocytes. There is insignificant increase in the percentage of apoptotic lymphocytes, if subgroup IIa is compared to subgroup Ia (P>0.05). Meanwhile, there is insignificant decrease in the percentage of apoptotic lymphocytes if subgroup Ib is compared to subgroup Ib (P> 0.05).

DISCUSSION

In recent years, the use of pesticides in

agriculture has been increasing steadily. At present there are more than 1000 pesticides which carries more exposure of agricultural and industrial workers as well as more contamination of food with pesticide residues (Zeljezic and Garaj- Vrhovac, 2001). Egypt seems to consume 1-2% of the worldwide production of pesticides (Amr, 1999). As many reports about pesticides have shown that some of them have genotoxic properties, therefore evaluation of their genotoxicity should be extended using newer assays now available (Zeljezic and Garaj-Vrhovac, 2001).

Methomyl is a methyl carbamate formulation widely used in the control of insects. Occupational and accidental human exposure carries the risk of genetic damage (WHO, 1996). Earlier studies by Bonatti et al. (1994), indicated that methyl carbamates could be considered weak mutagens. Meanwhile methomyl induced numerical and structural chromosomal aberrations (Bolognesi et al., 1994).

The present study investigates the genotoxic effects of methomyl in vivo by 4 different methods; the comet and the alkaline elution assays, the micronucleus test and apoptosis. Peripheral lymphocytes, livers, spleens and bone marrow of mice are used to evaluate the genotoxic effects of methomyl on these tissues.

*** The comet assay:**

The present study shows that compared to control, methomyl induced very high significant increases in the percentage of lymphocytes exhibiting this comet formation in all test groups. However the results obtained after 24h from methomyl administration are insignificantly increased when compared to the results obtained after 4h. On the other hand, when groups I and II are compared, there are very high significant increases in the percentage of the comet assay. This indicates that increasing the methomyl dose is associated with very high significant increase in the comet assay and hence DNA damage is dose dependant.

Garaj- Vrhovac and Zeljezic (2000), used the comet assay to evaluate the extent of DNA damage in peripheral blood lymphocytes of workers occupationally exposed to pesticides. There were statistically significant increases in tail length after long period of exposure. These effects decreased significantly after 6 months from being out of exposure although still above control figures.

An Egyptian study was performed on workers occupationally exposed to different types of pesticides. Lymphocytes of these workers showed a significant increase in chromosomal aberrations. This increase was irrespective of

their ages, duration of exposure or smoking habit (Amr, 1999).

Bonatti et al. (1994) and Bolognesi et al. (1994) also reported the DNA oxidative damage induced by methomyl. They suggested that methomyl may act indirectly through the formation of hydroxyl radicals. This hydroxyl radical reacts with guanine and forms 8- hydroxy deoxy guanosine (OH^8 dG). Hydroxylation of guanine at the C^8 position is considered to be a relevant factor for DNA damage and potential genotoxic and carcinogenic effects due to reactive oxygen species (Ames and Gold, 1991). Frage et al. (1990), suggested that the formation of OH^8 dG as a DNA oxidative damage could account for the induction of single strand breaks (SSB). They suggested that this hypothesis could be tested by administration of free radical scavengers prior to its use. The hydroxyl radical could damage the sugar- phosphate backbone and generate DNA - SSB (Leanderson et al., 1994).

This is supported by the fact that enzymes involved in the defense against harmful oxygen species such as super oxide dismutase, catalase and glutathion transferase are inhibited by different carbamate pesticides (Rannug and Rannug, 1984).

This means that methomyl causes oxidative DNA damage in peripheral lymphocytes that is dose related. This effect progresses after 24h but insignificantly increases over its effect after 4h.

*** The alkaline elution assay:**

a- Alkaline elution for splenic cells:

In the present work the alkaline elution rate constant for the splenic cells following methomyl injection shows a very high significant increase in all test groups when compared to control. The results obtained after 24h from methomyl administration are significantly increased if compared to those obtained after 4h. This means that there is still a significant increase in DNA damage in splenic cells 24h after exposure to methomyl, which may be due to deficient repair mechanisms. However there is insignificant increase in the elution rate constant when group II is compared to group I, i.e. DNA damage induced by methomyl in splenic cells is not dose dependant.

In a study by Lohitnavy and Sinhaseni (1998), the splenic weight and splenocyte viability decreased significantly after methomyl administration to rats. They explained this splenotoxicity by a direct oxidative cell injury, which was prevented by pretreatment with N- acetyl cysteine. In another study by Suramana et al. (2001), methomyl induced dose dependant microtubular disruption to rat splenic cells in vivo.

b- Alkaline elution for liver cells:

In the liver, methomyl induces very high significant increase in the elution rate constant in all test groups when compared to control. The results obtained after 24h from methomyl administration are insignificantly increased if compared to those obtained after 4h. This may be due to insufficient repair mechanisms, which fails to improve the damage and could not return to control values after 24h from injection.

In the present study, DNA damage induced by methomyl in liver cells is dose dependant after 4h from its administration. However after 24h, this dose dependant effect becomes insignificant. This may be due to induction of apoptosis by increased methomyl dose which causes more DNA damage that the cell can't withstand (Bishay et al., 2001).

These results are supported by Bolognesi et al. (1994). They found that methomyl increased the elution rate constant in the liver and the kidney of mice after 4h from injection. However this effect returned to control values after 24h. This was attributed to rapid metabolism and / or repair of DNA damage. In vitro, methomyl increased the elution rate constant in human lymphocytes insignificantly, but the commercial product Lannate 25 significantly increased this elution rate constant (Bonatti et al., 1994).

From the previous results we can conclude that methomyl induces a non- dose dependant DNA damage in splenic cells that increased significantly after 24h. On the other hand, on the liver, methomyl induces a dose- dependant DNA damage after 4h from its administration. After 24h, this dose- dependant DNA damage induced by methomyl on liver cells decreased which may be due to induction of apoptosis (Shou et al., 2002). This means that liver and splenic cells react differently to the genotoxicity of methomyl which may be due to difference in repair efficiency, metabolic activity and /or cell division.

*** The micronucleus test (MNT):**

In the present study, the micronucleus test studies the genotoxicity of methomyl on polychromatic erythroblasts (PCEs) in bone marrow of mice. The results of this test show very high significant increase in the perthousand of micronucleated PCEs induced by methomyl in all test groups when compared to control. If the results obtained after 24h from methomyl administration (as 2.5 mg/kg), compared to those obtained after 6h, there is insignificant decrease in the perthousand of micronucleated PCEs. This may be explained by metabolism of the insecticide (Bolognesi et al., 1994) and / or DNA repair mechanisms (Price, 1993; Hulla et al., 1999).

Meanwhile, when the results obtained,

after 24h from methomyl administration (as 5 mg/kg), compared to those obtained after 6h, they show a very high significant decrease in the perthousand of micronucleated cells. This can be explained by apoptosis. As increasing methomyl dose is associated with more DNA damage that the cell can not coapte with and reacts by apoptosis (Shou et al., 2002).

DNA damage induced by methomyl in PCEs as detected by MNT is not dose dependant. This is evidenced by the fact that, the results obtained from group IV are insignificantly changed when compared to those obtained from group III.

Bolognesi et al., (1994), showed a significant increase in micronuclei induced by methomyl in mice bone marrow. This is supported by the results of Bonatti et al. (1994). They observed a dose-dependent increase in micronuclei in human lymphocytes in vitro. This is in agreement with Wei et al., (1997). They showed an increase in micronuclei in blood cells of mice exposed to methomyl by different routes. On the other hand, Venegas et al. (1998), could not prove this effect in human lymphocytes of sprayers occupationally exposed to methomyl. This may be explained by the use of protective measures as masks and gloves and / or the use of lower dosage of the insecticide.

*** Apoptosis:**

Cell death is a consequence of irreversible and therefore lethal cell injury. The significance of cell death at tissue or organ level varies with the number of cells affected, the tissue functional reserve, the ability of remaining cells to proliferate and repair the injury and whether inflammation is initiated or not. Cell death may occur through necrosis or apoptosis. Apoptosis is a cell suicide that acts as a protective mechanism against carcinogenesis following DNA damage by toxicants (Haschek and Rousseaux, 1998).

The present study shows that methomyl induces very high significant increases in the percentage of apoptotic lymphocytes in all test groups when compared to control group. At the same time, when the results obtained after 24h from methomyl administration, are compared to those obtained after 4h, a high significant increase in the percentage of apoptotic lymphocytes is found. However, increasing the methomyl dose is associated with insignificant decrease in the percentage of apoptotic cells. This may be explained by occurrence of necrosis.

As necrosis is always associated with major disturbances in the cell while apoptosis is associated with factors that are not directly lethal. Chemical substances may give rise to necrotic cellular degeneration when present in sufficiently high concen-

trations. These concentrations vary greatly depending on the nature of the substance. The major disturbances that initiate toxic cell injury are those affecting the transport system of membranes, mitochondrial oxidative phosphorylation and direct damage to the organelle systems involved in protein synthesis (Verheyen, 1996). That is why the cell reacts by necrosis if the damage overwhelms the cell. But if the dying cell is capable of carrying its own orderly demise, the cell die by apoptosis (Thompson, 1999).

From the previous results (comet and alkaline elution assays and MNT), methomyl is found to induce very high significant increase in DNA damage in lymphocytes, PCEs, liver and splenic cells. When DNA damage exceeds the capacity of repair, p53 can initiate apoptosis (Kinzler and Voglestein, 1996). When repair is decreased, apoptosis increases (Hong et al., 1999). DNA damage induces p53 and cytochrome c / Apaf- 1 / caspase - 9 pathway (Su et al., 2002). Alkylating agents induce apoptosis by virtue of their ability to modify DNA bases. More specifically O_6 alkyl guanine lesions can trigger such programmed cell death (Meikrantz et al., 1998).

Hong et al. (1999), studied the relationship between DNA adduct levels, repair enzyme and apoptosis induced by a methylating agent on colonic crypts. They found that apoptosis was maximum at stem cells where DNA repair enzyme expression is low. They concluded that increased apoptosis in stem cells was due to decreased rate of repair rather than increased DNA damage.

In addition, Buschfort et al. (2002), stated that DNA repair processes can modulate the cytotoxic effects of alkylating agents. The response of blood cells to alkylating agent differed with higher frequencies of apoptosis in CD 34^+ cells. These cells showed a generalized reduction in DNA repair capacity.

Wang et al. (1998), proposed another mechanism for induction of apoptosis by N- methyl carbamate esters that is inhibition of the gap junctional intercellular communications. Methomyl was found to decrease membrane permeability due to increased ATP utilization and decrease in its level in diaphragm muscle (Gupta et al., 1994).

Cells vary in their susceptibility to toxicants and other stresses based on their type or differentiation, metabolic activity, mitotic phase and the point in their life span (Haschek and Rousseax, 1998). This explains why methomyl increased dosage give different responses in different tissues. In the present study methomyl induces very high significant DNA damage

in PCEs, peripheral lymphocytes, spleen and liver cells. Whereas increasing the methomyl dose produces insignificant changes in PCEs, spleen and liver (after 24h). Meanwhile, increasing methomyl dose is associated with very high significant increase in DNA damage in peripheral lymphocytes and a significant decrease in apoptotic lymphocytes after 24h. In the liver, increasing the methomyl dose significantly increases the rate of DNA damage after 4h then decreases after 24h. So, peripheral lymphocytes and liver are more affected than other tissues by increasing the dose.

The study of Osterod et al. (2001), can explain these results. They found that mutations increased significantly in hepatocytes but not in splenocytes, spermatocytes and kidney cells when DNA repair enzyme (glycosylase) was deficient. They explained this difference by high oxygen metabolism in the liver and that the liver is a slowly proliferating tissue. Accordingly, accumulation of oxidative DNA modifications is tissue-specific with difference in relevant cellular defense mechanisms.

This coincides with Suh et al. (2000), who stated that tissue responses to carcinogens differ due to different tissue- specific signaling pathways leading to distinct physiological responses. They found that methyl methansulfonate produced brain but not liver cancers in rats. This was attributed to significant increase in apoptotic cell death in the liver but not in the brain.

CONCLUSIONS

In the present study methomyl induces very high significant DNA damage in PCEs, peripheral lymphocytes, spleen and liver cells. Whereas increasing the methomyl dose produces insignificant changes in PCEs, spleen and liver (after 24h). Meanwhile, increasing methomyl dose is associated with very high significant increase in DNA damage in peripheral lymphocytes and a significant decrease in apoptotic lymphocytes after 24h. In the liver, increasing the methomyl dose significantly increases the rate of DNA damage after 4h then decreases after 24h. So, peripheral lymphocytes and liver are more affected than other tissues by increasing the dose.

Table (1): The percentage of the comet assay for control group and subgroups Ia, Ib, IIa and IIb.

	Control	Group I (2.5 mg/kg)		Group II (5 mg/kg)	
		Ia (4h)	Ib (24h)	IIa (4h)	IIb (24h)
Mean	11.2	17.86	19.46	24.73	26.73
S. D. ±	4.96	3.99	4.58	3.73	3.88
(Range)	(2-20)	(12-26)	(11-30)	(15-30)	(20-35)
t_1		5.6	12.5	43.15	45.3
P_1		<0.001	<0.001	<0.001	<0.001
24h versus 4h					
t_2		1.5		1.61	
P_2		>0.05		>0.05	

Table (2): The percentage of the comet assay for subgroups IIa and Ia and subgroups IIb and Ib.

	Ia	IIa	Ib	IIb
Mean	17.86	24.73	19.46	26.73
S. D. ±	3.99	3.73	4.58	3.88
(Range)	(12-26)	(15-30)	(11-30)	(20-35)
t		4.89		4.69
p		<0.001		<0.001

Table (3): The alkaline elution rate constant for the splenic cells of control group and subgroups Ia, Ib, IIa and IIb.

	Control	Group I (2.5 mg/kg)		Group II (5 mg/kg)	
		Ia (4h)	Ib (24h)	IIa (4h)	IIb (24h)
Mean	22.6	57.5	63.53	61.5	66.53
S. D. ±	5.04	8.13	9.21	9.7	8.59
(Range)	(15-31)	(44- 74)	(46-78)	(52-82)	(59-85)
t_1		5.01	8.9	6.91	8.53
P_1		<0.001	<0.001	<0.001	<0.001
24h versus 4h					
t_2		1.97		1.99	
P_2		<0.05		<0.05	

Table (4): The alkaline elution rate constant for subgroups Ia and IIa and subgroups Ib and IIb.

	Ia	IIa	Ib	IIb
Mean **S. D. ±** **(Range)** **t** **p**	57.5 8.13 (44-74)	61.5 9.7 (52-82) 1.22 > 0.05	63.53 9.21 (46-78)	66.53 8.59 (59-85) 0.92 >0.05

Table (5): The alkaline elution rate constant for the liver of control group and subgroups Ia, Ib, IIa and IIb.

	Control	Group I (2.5 mg/kg)		Group II (5 mg/kg)	
		Ia (4h)	Ib (24h)	IIa (4h)	IIb (24h)
Mean **S. D. ±** **(Range)** **t_1** **P_1**	22.6 5.04 (15-31)	57.5 8.69 (44- 74) 5.6 <0.001	62.5 9.31 (52-82) 7.2 < 0.001	64.33 9.16 (46.79) 6.11 <0.001	67.46 8.05 (59.85) 8.2 <0.001
24h versus 4h **t_2** **P_2**		1.2 >0.05		0.99 >0.05	

Table (6): The alkaline elution rate constant for the liver of subgroups Ia and IIa and subgroups Ib and IIb.

	Ia	IIa	Ib	IIb
Mean **S. D. ±** **(Range)** **t** **p**	57.73 8.69 (44-76)	64.33 9.16 (46-79) 2.02 < 0.05	62.5 9.31 (52-82)	67.46 8.05 (59-85) 1.56 >0.05

Table (7): The perthousand of the MNT of control group and subgroups IIIa, IIIb, IVa and IVb.

	Control	Group III (2.5 mg/kg)		Group IV (5 mg/kg)	
		IIIa (6h)	IIIb (24h)	IVa (6h)	IVb (24h)
Mean	134.66	229.73	222.6	248.33	216.86
S. D. ±	19.01	47.27	37.37	33.75	13.76
(Range)	(94-167)	(143-370)	(143-300)	(180-333)	(200-250)
t_1		32.71	28.6	61.3	50.66
P_1		<0.001	<0.001	<0.001	<0.001
24h versus 4h					
t_2		1.2		3.9	
P_2		>0.05		<0.001	

Table (8): The perthousand of MNT for subgroups IIIa and IVa and subgroups IIIb and IVb.

	IIIa	IVa	IIIb	IVb
Mean	229.73	248.33	222.6	216.86
S. D. ±	47.27	33.75	37.37	13.76
(Range)	(143-370)	(180-333)	(143-300)	(200-250)
t		1.24		0.56
p		> 0.05		> 0.05

Table (9); The percentage of apoptotic lymphocytes (giemsa stain) in control group and subgroups Ia, Ib, IIa and IIb.

	Control	Group I (2.5 mg/kg)		Group II (5 mg/kg)	
		Ia (4h)	Ib (24h)	IIa (4h)	IIb (24h)
Mean	18.66	47.66	62.33	45.66	55.64
S.D. +	7.18	11.47	11.15	12.47	7.71
(Range)	(10-30)	(30-70)	(40-90)	(30-70)	(45-75)
t		8.2	15.41	7.81	12.3
P		< 0.001	< 0.001	< 0.001	< 0.001
24h versus 4h					
t		3.5		3.31	
P		<0.01		< 0.01	

Table (10): The percentage of apoptotic lymphocytes of subgroups Ia and IIa and subgroups Ib and IIb.

	Ia	IIa	Ib	IIb
Mean	47.66	45.66	62.33	55.64
S.D. +	11.47	12.47	11.15	7.71
(Range)	(30-70)	(30-70)	(40-90)	(45-75)
t		1.98		2.4
p		> 0.05		<0.05

Table (11): The percentage of apoptotic lymphocytes (acridine orange stain) in control and subgroups Ia, Ib, IIa and IIb.

	Control	Group I (2.5 mg/kg)		Group II (5 mg/kg)	
		Ia (4h)	Ib (24h)	IIa (4h)	IIb (24h)
Mean	38	43.66	58.66	46	55.73
S.D. +	9.22	13.81	14.81	9.48	5.62
(Range)	(25-50)	(25-70)	(40- 90)	(30-70)	(50-70)
t		2.4	10.3	2.6	16.21
P		< 0.01	< 0.001	< 0.01	< 0.001
24h versus 4h					
t		3.5		3.9	
P		< 0.01		< 0.01	

Table (12): The percentage of apoptotic lymphocytes of subgroups Ia, Ib, IIa and IIb.

	Ia	IIa	Ib	IIb
Mean	43.66	46	58.66	55.73
S.D. ±	13.81	9.48	14.81	5.62
(Range)	(20-70)	(30-70)	(40-90)	(50-70)
t		0.54		0.72
p		> 0.05		> 0.05

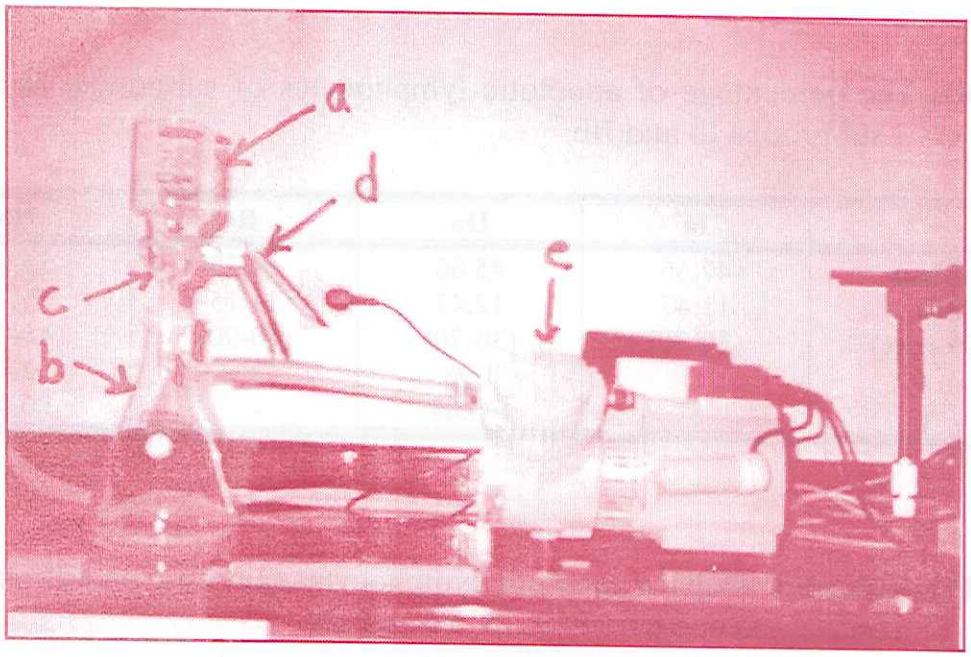

Fig. (1) : The solvent vacuum filtration apparatus:
a- Glass funnel b- Flask
c- Filter glass support d- Clamp
e- Vacuum

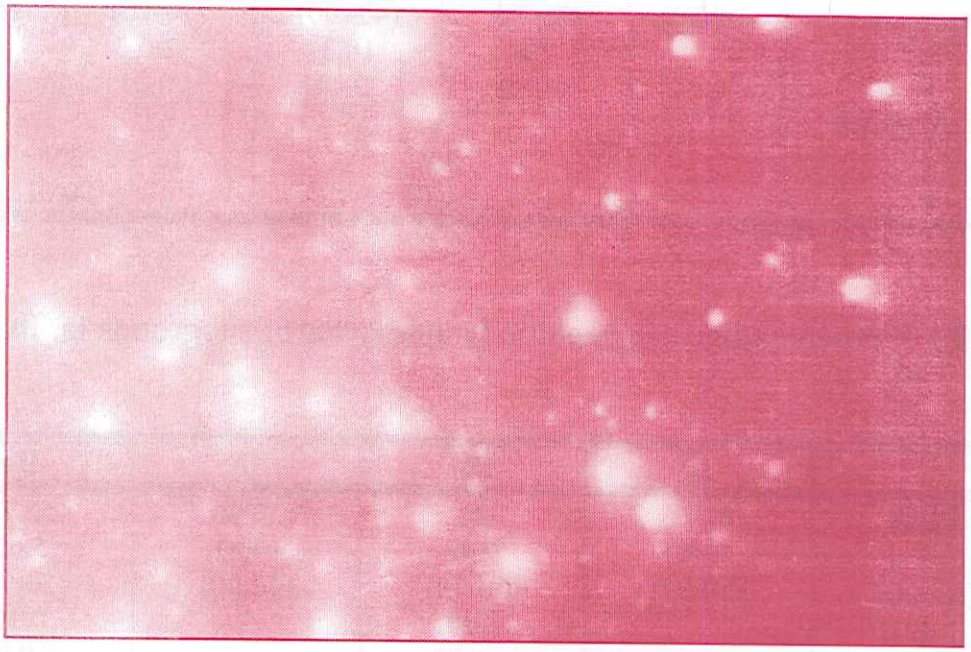

Fig. (2) : The comet assay for lymphocytes of subgroup IIa. Damaged DNA spot: migration towards the anode (arrow).

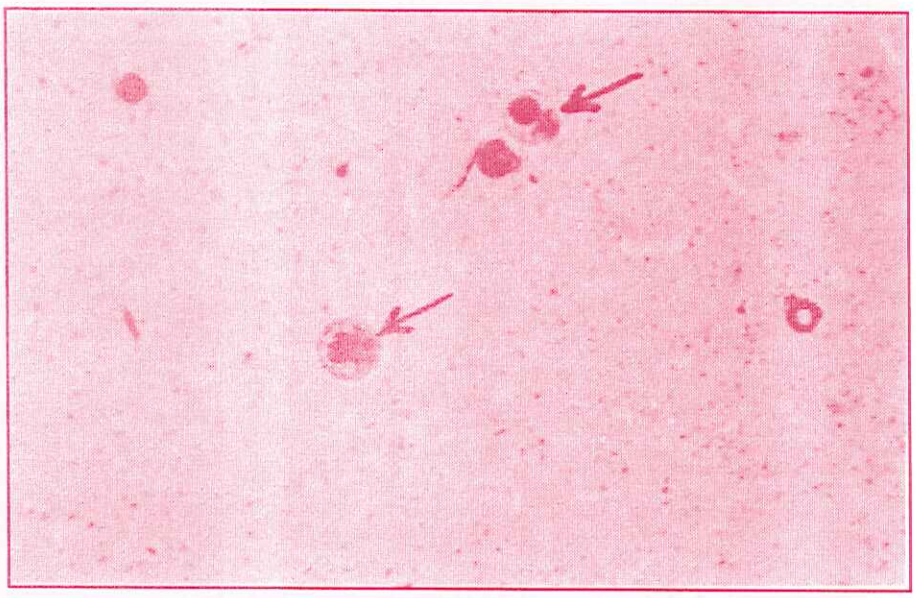

Fig. (3) : Micronucleated polychromatic erythrocytes of mice (subgroup IIa).

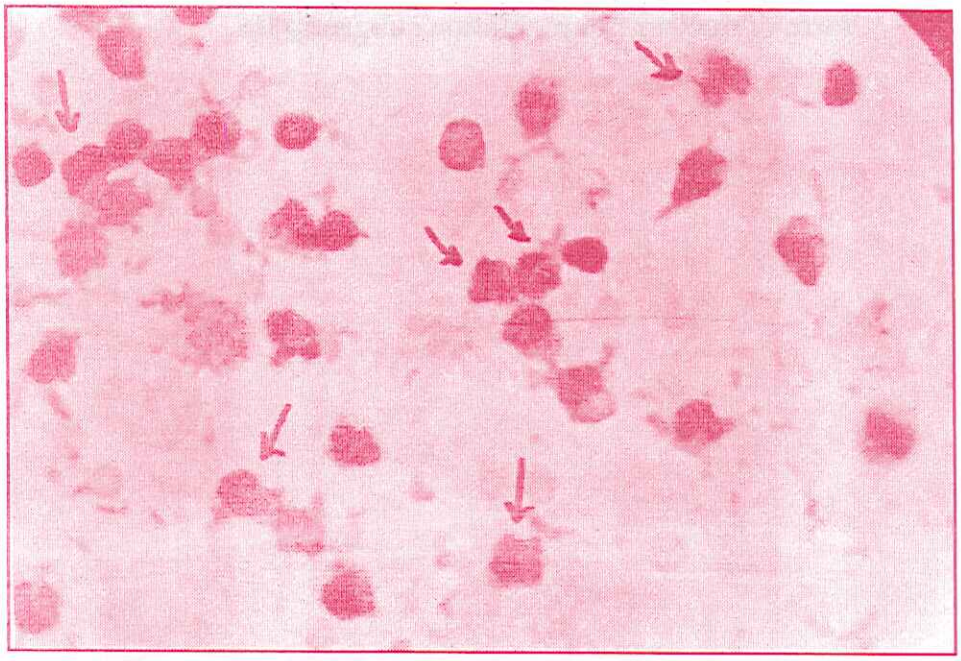

Fig (4) : Apoptotic lymphocytes stained by giemsa in the form of nuclear fragmentation (subgroup IIa).

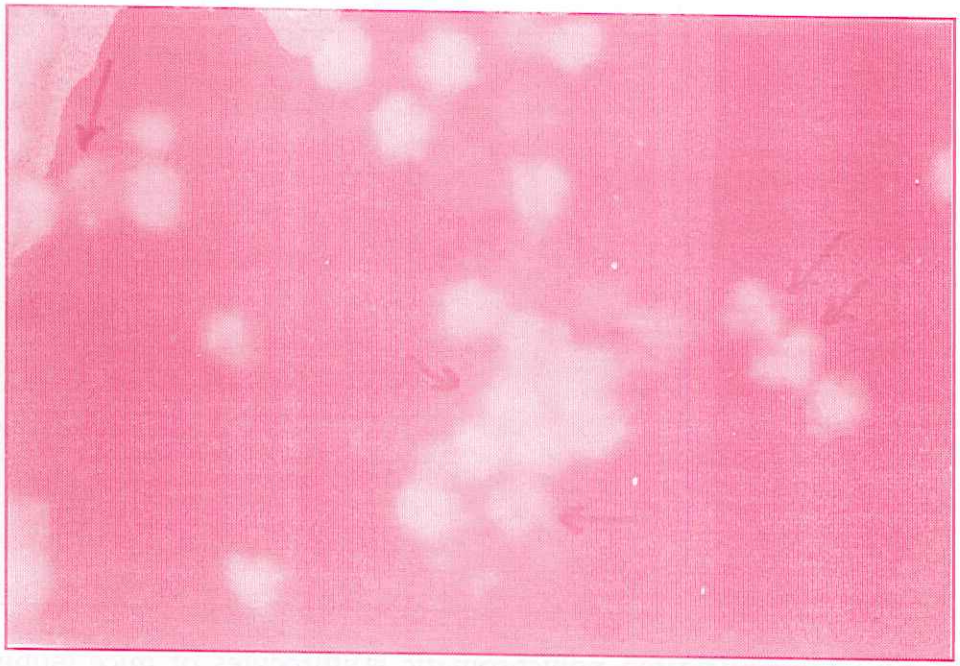

Fig. (5) : Apoptotic lymphocytes stained by acridine orange stain in the form of nuclear fragmentation subgroup IIa.

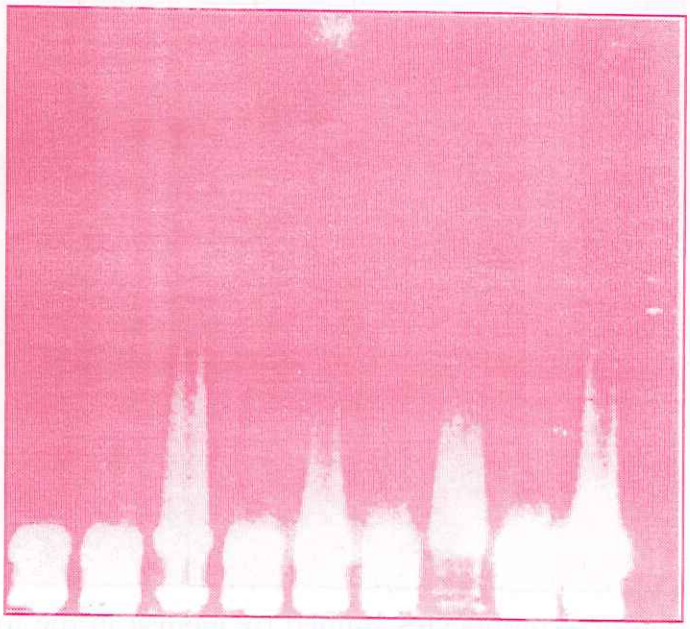

Fig. (6) : The ladder- like pattern with gel electrophoresis in apoptotic lymphocytes.

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تأثيرات الميثوميل على المادة الوراثية : دراسة تجريبية

المشتركون فى البحث

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من أقسام الطب الشرعى والسموم الإكلينيكية والميكروبيولوچى والمناعة الطبية* كلية الطب - جامعة المنصورة

إن الانتشار الواسع لاستخدام المبيدات الحشرية يحمل فى طياته التعرض الأكثر للفلاحين ولعمال صناعة المبيدات وكذلك تلوث الطعام ببقايا هذه المبيدات الحشرية لذلك كان من الواجب على الباحثين دراسة تأثير هذه المبيدات على الشريط الوراثى خصوصاً وأن هذا الشريط يحمل كل صفات الإنسان وهو أغلى الموروثات. وقد أختير الميثوميل كمثال لمجموعة الكاربامات وهو أحد المبيدات الحشرية الواسعة الانتشار حيث يستخدم لمقاومة الحشرات المنزلية وحشرات القطن والفواكه والخضروات وكذلك نباتات الزينة، هذا وقد يتعرض الإنسان للميثوميل أثناء تصنيعه أو استخدامه أو عن طريق أكل طعام ملوث ببقايا هذا المبيد.

ولقد أعطى الميثوميل للفئران بجرعات مختلفة وقد أختيرت خلايا الدم البيضاء وخلايا النخاع المتعددة الألوان المنتجة لكرات الدم الحمراء وخلايا الكبد والطحال لدراسة تأثير الميثوميل على المادة الوراثية لهذه الأنسجة وذلك عن طريق الفصل الكهربائى بالجيل وموت الخلايا المبرمج لخلايا الدم البيضاء واختبار الأنوية الصغيرة لخلايا النخاع المتعددة الألوان المنتجة لكرات الدم الحمراء والترشيح القلوى لخلايا الكبد والطحال.

وقد دلت النتائج على أن الميثوميل قد أحدث تأثيراً شديداً للمادة الوراثية فى الأنسجة تحت الاختبار ولم يتحسن هذا التأثر بعد ٢٤ ساعة من استخدامه إلا فى خلايا النخاع العظمى بالرغم من أنها لم تعد إلى الوضع الطبيعى.

وقد لوحظ أن الأنسجة تظهر تأثراً مختلفاً لزيادة جرعة الميثوميل وقد يرجع هذا إلى اختلاف سرعة العمليات الأيضية بكل منها وكذلك قدرة الخلايا على إصلاح التلف بالمادة الوراثية وكذلك سرعة انقسام الخلايا بكل منها وعليه فإننا نوصى بالآتى :

١- الدراسة المتأنية لكل المبيدات الحشرية قبل استعمالها ودراسة الآثار الجانبية لهذه المبيدات وعمل دراسة جدوى لاستنتاج مدى فائدة هذه المبيدات مقارنة بالآثار الجانبية لها.

٢- أخذ الاحتياطات اللازمة أثناء تصنيع واستخدام هذه المبيدات حتى لايتعرض الفلاحون أو العمال لجرعات عالية منها.

٣- الإقلال بقدر الإمكان من استخدام هذه المبيدات الحشرية وابدالها بالمقاومة البيولوجية.

٤- من الأفضل استخدام أكثر من طريقة لإثبات قدرة أى مادة على إحداث تلف بالمادة الوراثية لتفادى النتائج الخاطئة.