Combined therapy for Methyl Parathion toxicity in male albino mice: with special reference to Pathological, Biochemical and Cytogenetical changes

By

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SUMMARY

Methyl parathion (M.P.) is a pesticide widely used not only to protect crops but also illegally used in many countries for spraying homes and businesses to control insects.

The present study was planned to investigate the effects of M.P. on body organs of male albino mice. Mice were treated with 3.5 mg/kg b.wt. M.P. orally for 25 days. After that the animals were divided into 6 subgroups (A, B, C, D, E, and F). The first 5 subgroups were treated i.p. with atropine sulphate and/or sodium bicarbonate in different concentrations for 20 days as antidotes for M.P., while subgroup F continued to receive M.P. Animals treated with M.P. showed marked depression, body weight loss and arched back. At the end of the experimental period, all animals were sacrificed and blood was collected for serum separation. Acetylcholinesterase (AchE) was measured in serum and brain tissue. Results revealed a highly significant inhibition of AchE in serum and brain of subgroup F (271.5 ±14.0 and 132.6± 12.8u/l) respectively, when compared with the control sets (2808.3 ±116.2 and 1154.4 ± 49.3u/l respectively). Significant increase of AchE in serum and brain of subgroup C (treated with 75% atropine sulphate and 25% sodium bicarbonate). Total protein and nucleic acids concentrations were significantly decreased in subgroups F followed by B, E, and D respectively in liver as well as brain of treated mice in comparison to control.

Remarkable histopathological changes were observed, as degenerative and necrotic lesions in the parenchymatous organs with neuronal cells changes as well as demyelination in the sciatic nerve. Ultrastructural examination of the later nerve revealed partially destroyed myelin sheath and reduced size axoplasm of some neurofibers that were displaced by degenerated myelin sheath.
Cytogenetic analysis revealed marked chromosomal aberrations as well as reduced mitotic index in treated groups in comparison with control sets. We concluded that the use of atropine sulphate (0.75mg/kg b.wt.) in combination with sodium bicarbonate 2% (5mg/kg b.wt.) may be considered the most effective antidote in treatment of methyl parathion toxicity in mice.

**Key words:** Parathion, pathology, acetylcholinesterase, sciatic nerve E. M., chromosomal aberrations.

**INTRODUCTION**

One of the aims of the veterinary medicine is to successfully treat diseased animals and is usually specific for different kinds of explicit diagnosed diseases. This is not easily performed with regard to toxicosis, as the dosage and kind of a substance determines its toxicity. Of these thousands of substances with potential toxicity to domestic and wild animals, very few in fact have a specific antidote treatment (*Osweiler et al.*, 1985). However, it is fortunate that probably the most potentially hazards group of compounds, the anticholinesterase organophosphate has specific antidote.

Organophosphorus (OP) insecticides are of specific importance because of their extensive use combating the disease vectors and agriculture pests, especially after the problems of organochlorine residues in the environment.

Signs of poisoning due to OP insecticides develop very rapidly and treatment should be carried out within minutes of exposure if mortality is to be minimized (*Hansen, 1995*).

Methyl parathion (MP) (o, odimethyl-o, 4-nitrophenyl phosphorothionate) is a well known OP pesticide used in agricultural, although quite often illegally used indoors to overcome insects (*Narayana et al.*, 2006). MP, a toxic organophosphate insecticides approved for out door use only, is classified by the World Health Organization (WHO) as a category Ia (extremely toxic) and by the United States Environmental Protection Agency as a toxicity category I (most toxic) insecticide.

Human are exposed to MP in fruits and vegetables as residues of 0-2mg/kg, children being at higher risk of exposure (*Narayana et al.*, 2006). As the health effects of MP
exposure became evident with increasing public awareness. Exposure to OP insecticides induces undesirable behavioural changes in human and animals, including; anxiety, irritability, depression, congestive disturbances and sleep disorders (Zhu et al., 2004).

O.P have many structural similarities with naturally occurring compounds and their primary target of action in insects is the nervous system; they inhibit the release of the enzyme acetylcholinesterase (AchE) at the synaptic junction (Cabello et al., 2001). Eserine, parathion and malathion are cholinesterase inhibitors responsible for the hydrolysis of body cholinesterase, including acetylcholine at cholinergic synapses. The cytogenetic and cytotoxic effects of the pesticides methyl parathion were evaluated in mammalian test systems. The frequency of chromosome aberrations tends to show the genotoxicity of organophosphorus and organochlorine pesticides in a single-exposure response study. Methyl parathion was the most hazardous among the three (Vijayaraghavan and Nagarajan, 1994).

Aim of work, the present study was planned to investigate:
1- The toxic effects of methyl parathion regarding, AchE activity in blood and in brain tissues, total protein and nucleic acids contents in both liver and brain, its pathology on different body organs of albino mice and finally its cytogenicity.

2- The role of Atropine sulphate and/or sodium bicarbonate as antidotes to methyl parathion to ameliorate all the last mentioned toxicological parameters.

MATERIALS AND METHODS
Experimental design:
105 male albino mice about three months old were obtained from animal research unit in faculty of Medicine, Zagazig University.

They were adapted for lab conditions for two weeks before beginning of the experiment. Pellets food and tap water were left for the animals ad libitum. Animals were divided into two groups; G1 (15 mice), control group (that received no treatment) and G2 (90 mice), (treated group in which, mice received 3.5 mg/kg b.wt. methyl parathion (MP) by gavage once daily for 25 days, (Prashanthi et al., 2006).

Then G2 was divided into 6 subgroups, 15 mice each (A, B, C, D,E and F). These subgroups were treated either by atropine sulphate and/or sodium bicarbonate i.p. once daily for 20 days. The type of treatment for each subgroup was summarized in table (1).

Body weight was recorded weekly. At the end of the experi-
ment, all animals were exan-
guinted and blood samples were
collected and centrifuged at 3000
rpm for 15 minutes for serum
separation. Tissue specimens were
collected for histopathological ex-
amination.

Biochemical examination:

Acetylcholinesterase (AchE)
was measured in serum and in su-
pernatant of homogenized brain
tissue using spectrophotometer
(Milton Roy, Spectronic 1201) and
commercial kits (MPZ,124117,
Boehringer Manheim, Germany)
according to Ellman et al. (1961).
Total protein was measured ac-
cording to Doumas (1975).

Extraction and determination of
nucleic acids (DNA and RNA): samples of liver and brain tissues
were used for extraction of nucleic
acids (Melmed et al., 1976). DNA
was determined according to the
method of Dische and Schowerz,
(1937); its optical density was de-
termined by spectrophotometer at
600 nm. DNA content (mg/gm tis-
tue) was calculated by: multiply
the reading by 40.39. RNA was
determined according to Mejbaum
(1939), its optical density was
measured at 660 nm RNA content
(mg/gm tissue) was calculated by:
multiply the reading by 17.8.

Histopathological examination:

Specimens from liver, kidney,
heart, spleen, lungs, testis, brain
and sciatic nerve were obtained
from necropsed and freshly dead
animals of all experimental and
control groups and fixed in neu-
tral-buffered formalin. Specimens
then were routinely processed and
sectioned at 4-5um thickness. The
obtained sections were stained
with H & E according to Bancroft
et al. (1996). Special stains were
used on need as Cresyl violet and
Toluidine blue according to Ban-
croft et al. (1996).

Transmission Electron Micro-
scopic examination:

Specimens from sciatic nerves
were fixed in 5% cold Gluteralde-
hyde then post fixed in Osmium
tetraoxide. Tissue specimens were
then dehydrated in ascending
grades of ethyl alcohol. Then
specimens were immersed in pro-
pylene oxide and Epon and finally
in pure Epon. Semithin sections
were prepared and stained with 1%
Toluidine blue and examined by
light microscope. Blocks of inter-
esting specimens were ultra thin
sectioned and examined by JEM
CX11 Transmission Unit at Assiut
University Electron Microscopic
unit (Louis and Williams, 1995).

Cytogenetic studies;

All animals were injected
i.m with 0.25ml/100gm b. wt. col-
chicines solution (0.5%) ninety
minutes before exanguination and
both femurs were dissected for cul-
turing the bone marrow according
to Yoshida and Amano, (1965).
The mitotic index was calculated by
counting 500 cells for each animal and scoring the number of cells in mitosis per 500 cells by the following formula:

\[
M.I. = \frac{\text{No of metaphase cells}}{\text{total No of cells}} \times 100 \quad \text{(Brusick, 1980)}.
\]

**Statistical analysis:**

The obtained data were statistically analysed using student-t test according to Snedecor and Cochran, (1980).

**RESULTS**

Treatment of mice with MP at a dose level of 3.5mg/kg b. wt. orally for 25 days revealed marked symptoms of toxicity as general depression, weight loss, sometimes arched back and some other times nervous manifestations.

**Biochemical analysis:**

A significant inhibition of AchE was noticed in the treated groups in serum as well as in brain as compared with control sets (table 2). The most pronounced serum AchE inhibition was recorded in subgroup F followed by E, B, D, A and C respectively. While the markedly recognized brain AchE inhibition was in subgroup F followed by B, E, D, C and A respectively. Comparing with subgroup F, all subgroups showed significant increase in serum AchE level except subgroup B and E. While, comparing with subgroup A all subgroups showed significant decrease in the AchE level except in subgroup C.

Results concerning total protein, DNA and RNA concentrations in liver and brain of treated mice, are summarized in table (3).

**Gross pathology:**

Marked pathological changes were observed in mice received MP as; generalized organ congestion including the meningeal one with sometimes petechial haemorrhages. Most livers were mottled and hearts were pale and flaccid with excess catarrh on the GIT mucosa. Much less severe changes were observed in animals of subgroup A followed by C.

**Histopathology:**

All the treated subgroups showed different degrees of histological alterations. The most pronounced changes were noticed in MP treated subgroup (F) while the mildest changes were in subgroup C (which was treated with 75% atropine and 25% NaHCO₃), followed by A (which was treated with 100% atropine).

MP caused severe toxic changes, the most conspicuous of which were in liver which included diffuse hepatocellular granular degeneration (Fig. 1) and dissociation, together with focal parenchymal necrosis, mostly centrilobular (Fig. 2). Single cell necrosis (apoptosis) was also obvious along the parenchymal cells which appeared as scattered shrunken, deeply eosinophilic irregular hepatocytes along the hepatic parenchyma (Fig. 3). The later cells stained densely with Toluidine
blue (Fig. 4). Frequent haemorrhages were noticed.

The other organ changes included myocardial degeneration in the form of multiple homogenous eosinophilic and wavy appearance of the muscle fibers with frequent intermuscular haemorrhages were observed (Fig. 5). Marked renal tubular degenerative changes as swollen tubular epithelial cells and necrosis with frequent desquamation (Fig. 6), together with marked lymphoecytic depletion of the splenic follicles (Fig. 7) and the appearance of the underlying reticular fibers with an obvious hemosiderosis.

Picture of catarrhal gastroenteritis with oedematous submucosa was noticed.

In addition, testicular changes in the form of defective spermatogenesis with increased number of abnormal spermatid forms with multiple spermatid giant cells as well as disorganization in the spermatogonial cells layers were observed (Fig. 8 and 9).

Brain lesions included meningeal congestion and oedema with neuronal cell changes as some of which appeared shrunken darkly stained with small pyknotic nuclei or without any nuclear structures and pericellular oedema (Fig. 10). Other neurons were swollen with central chromatolysis and dispersion of the nuclear chromatin (Fig. 11). Marked focal aggregations of glia cells were observed in cerebral cortex and demyelination was also noticed. The examined sciatic nerves showed variation in the density of neurofibers, myelin sheaths and interfibers oedema that separating the nerve fibers in the endoneurium with demyelination of the nerve fibers (Fig.12).

Electron Microscopic examination:

Electron microscopical examination of the previously mentioned nerves revealed partially destroyed sheath of the neurofibers which appeared lamellated instead of the normal electron dense appearance. The axoplasm of these neurofibers contained disoriented neurofilaments. The axoplasm of some neurofibers was reduced and displaced by expanded degenerated myelin sheaths (Fig. 13 and 14).

No comparable microscopic or ultra microscopic changes were detected in control animals.

Cytogenetic results:

Our results revealed that MP administration to albino mice evoked a highly significant increase in the mean values of chromosomal aberrations which also was noticed in groups B, E and D. Results were shown in table (4). Types of chromosomal aberrations were ring, fragmented, deletion, chromatid break, sticky, centromeric, end to end, stretching and polyploidy chromosomes (Fig. 15, 16, 17 and 18).
Legends Of Figures:

Fig. 1: Liver of mouse from subgroup F showing ground-glass appearance of degenerated swollen hepatocytes. H&E. X 400.

Fig. 2: Liver of mouse from subgroup F showing centrilobular necrosis. H&E. X 200.

Fig. 3: Hepatic parenchyma of mouse from subgroup F revealing irregularly shrunken hepatocytes with hypereosinophilic cytoplasm scattered along the hepatic parenchyma. H&E. X 400.

Fig. 4: The previously mentioned shrunken hepatocytes showing hyperchromatic and condensed nuclei. Toluidine blue. X 400.

Fig. 5: Myocardium of mouse from subgroup F showing extravasated erythrocytes between the degenerated myocardial muscle fibers. H&E. X 100.

Fig. 6: Kidney of mouse from subgroup F revealing marked tubular degeneration with desquamation of the renal tubular epithelial cells. H&E. X 200.

Fig. 7: Spleen of mouse from subgroup F showing severe lymphocytic depletion from the lymphoid follicles. H&E. X 400.

Fig. 8: Defective spermatogenesis in the testicular tissue of mouse from subgroup F. H&E. X 400.

Fig. 9: Incomplete stages of spermatogenesis with abnormal spermatid forms and spermatid giant cells in testis of mouse from subgroup F. Toluidine blue. X 200.

Fig. 10: Brain of mouse from subgroup F showing marked neuronal degeneration as shrunken with darkly stained cytoplasm. H&E. X 400.

Fig. 11: Neuronal cells showing decreased density of Nissl’s substance (chromatolysis). Cresyl violet stain. X 400.

Fig. 12: Sciatic nerve showing endoneurial oedema separating the neurofibers with variable density of myelin sheaths. Toluidine blue. X 400.

Fig. 13: Transmission electron micrograph of sciatic nerve of subgroup F mouse revealing destroyed myelin sheath which have lamellated appearance. X 4320.

Fig. 14: Transmission electron micrograph of sciatic nerve of subgroup F revealing partially destroyed myelin sheath, reduced axoplasm and contains more neurofilaments. X 10720.

Fig. 15, 16, 17 and 18: presenting the metaphase spread obtained from femur bone marrow culture of mice of different treated groups as: ring chromosome (r), deletion (d), chromatid breaks (b), stretching (s) and polyploidy (p).
Table (1): The type of treatment of each subgroup, the dose of the treated substance and its route of administration.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Type of treatment</th>
<th>Route of administration</th>
<th>Period of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control group</td>
<td>i.p.</td>
<td>20 days.</td>
</tr>
<tr>
<td>Subgroup A</td>
<td>0.1 mg atropine sulphate/kg b.wt.</td>
<td>i.p.</td>
<td>20 days.</td>
</tr>
<tr>
<td>Subgroup B</td>
<td>20mg NaHCO₃ 2%/kg b.wt.</td>
<td>i.p.</td>
<td>20 days.</td>
</tr>
<tr>
<td>Subgroup C</td>
<td>0.075mg/kg b.wt. + 5 mg NaHCO₃ 2% k b.wt. (75%:25%)</td>
<td>i.p.</td>
<td>20 days.</td>
</tr>
<tr>
<td>Subgroup D</td>
<td>0.50mg/kg b.wt atropine + 10mg NaHCO₃ 2% g b.wt. (50%:50%).</td>
<td>i.p.</td>
<td>20 days.</td>
</tr>
<tr>
<td>Subgroup E</td>
<td>0.025mg/kg b.wt. atropine +15gNaHCO₃ 2% kg b. wt. as (25%:75%).</td>
<td>i.p.</td>
<td>20 days.</td>
</tr>
<tr>
<td>Subgroup F</td>
<td>Continued to receive MP only.</td>
<td>p.o.</td>
<td>20 days.</td>
</tr>
</tbody>
</table>

Table (2): AchE levels (u/l) in control and exposed mice to MP and atropine sulphate and/or NaHCO₃.

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Type of treatment</th>
<th>AchE in serum.</th>
<th>% of serum AchE in relation to control.</th>
<th>AchE in brain tissue.</th>
<th>% of brain AchE in relation to control.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control.</td>
<td>2808.3 ± 116</td>
<td>100 ± 4.13</td>
<td>1154.4 ± 49.0</td>
<td>100 ± 4.24</td>
</tr>
<tr>
<td>Subgroup A</td>
<td>Atropine sulphate (100%).</td>
<td>402.4± 18.8 ac</td>
<td>14.33 ± 0.66</td>
<td>533.4±12.4ac</td>
<td>46.2 ± 1.07</td>
</tr>
<tr>
<td>Subgroup B</td>
<td>NaHCO₃ (100%).</td>
<td>241± 14.5a,c,f</td>
<td>8.59 ± 0.51</td>
<td>173.6± 11.2a,c</td>
<td>14.9 ± 0.97</td>
</tr>
<tr>
<td>Subgroup C</td>
<td>Atropine + NaHCO₃(75:25%)</td>
<td>635.5±18.8a,c,f</td>
<td>22.63 ± 0.66</td>
<td>325.0±10.1a,c,e</td>
<td>28.16 ± 0.87</td>
</tr>
<tr>
<td>Subgroup D</td>
<td>Atropine + NaHCO₃ (50:50).</td>
<td>329.1±14.2a,b,e</td>
<td>11.72 ± 0.51</td>
<td>304.5± 11.4a,c</td>
<td>26.38 ± 0.98</td>
</tr>
<tr>
<td>Subgroup E</td>
<td>Atropine + NaHCO₃(25:75%)</td>
<td>235.8±18.9a,c,f</td>
<td>8.39±0.67</td>
<td>182.4±7.8a,b,f</td>
<td>15.8 ± 0.67</td>
</tr>
<tr>
<td>Subgroup F</td>
<td>Methyl parathion only.</td>
<td>231.5± 14a,f</td>
<td>8.2±0.49</td>
<td>132.6± 12.8a,f</td>
<td>11.49±1.10</td>
</tr>
</tbody>
</table>

a, b: significantly different from control at P<0.05 and P<0.001
c, d: significantly different from subgroup F at P<0.05 and P<0.001
e, f: significantly different from subgroup A at P<0.05 and P<0.001
Table (3): Total protein, DNA and RNA concentrations in liver and brain tissues in control and exposed mice to MP and atropine sulphate and / or NaHCO₃.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver.</th>
<th>Brain.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein (gm/dl).</td>
<td>DNA (mg/g tissue).</td>
</tr>
<tr>
<td>G1 (control)</td>
<td>6.7 ±0.3</td>
<td>8.8 ±0.4</td>
</tr>
<tr>
<td>Subgroup A</td>
<td>5.9± 0.4</td>
<td>8.2± 0.3</td>
</tr>
<tr>
<td>Subgroup B</td>
<td>4.3±0.4*</td>
<td>5.1±0.3*</td>
</tr>
<tr>
<td>Subgroup C</td>
<td>6.1 ±0.6</td>
<td>7.9 ±0.3</td>
</tr>
<tr>
<td>Subgroup D</td>
<td>5.8±0.2*</td>
<td>6.2±0.3*</td>
</tr>
<tr>
<td>Subgroup E</td>
<td>5.1± 0.3*</td>
<td>6.4 0.1*</td>
</tr>
<tr>
<td>Subgroup F</td>
<td>4.8±0.2*</td>
<td>4.1±0.3**</td>
</tr>
</tbody>
</table>

* P < 0.001
** P < 0.0001
Table (4): Cytogenetic analysis of control and exposed mice to MP and Atropine sulphate and/or NaHCO$_3$.

<table>
<thead>
<tr>
<th>Group</th>
<th>No of examined metaphase cells</th>
<th>Type of aberrations.</th>
<th>Total damage.</th>
<th>No. of cell counted</th>
<th>No of metaphasic cells</th>
<th>Mitotic index (MI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ring</td>
<td>Fragmented</td>
<td>Deletion</td>
<td>Chromatid break</td>
<td>Sticky</td>
</tr>
<tr>
<td>G1 (control).</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Subgroup A.</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Subgroup B.</td>
<td>500</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Subgroup C.</td>
<td>500</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Subgroup D.</td>
<td>500</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Subgroup E.</td>
<td>500</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Subgroup F.</td>
<td>500</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

*significantly differed from control P<0.05.

** Highly significant differed from control P<0.001
Methyl parathion (MP), a toxic organophosphate insecticide approved for outdoor use only, is classified by the World Health Organization (WHO) as a Category Ia (extremely toxic). The present study revealed that twenty five days exposure of male albino mice to MP resulted in marked general toxicological parameters. The later were corrected with variable degrees on the administration of atropine sulphate and/or sodium bicarbonate. Klaus et al. (1999), mentioned that the specific antidotes for organophosphates are atropine and abidoxime. Atropine is a cholinergic blocking agent that effectively competes with acetylcholine at the binding sites, alleviating many poisoning symptoms, the most important being respiratory tract secretions (Randy et al., 1999).

In our work, Mp caused a significant inhibition of both blood and brain AchE activity as compared with control group. Those results were in accordance with many authors as, Cabello et al., (2001) who found that AchE activity decreased in serum of rats treated with parathion. Also Veronesi and Pope, 1990, reported that AchE was depressed 73% in their study on rat pups dosed with parathion.

Moreover, Nagymajtenyi et al. (1988) in their work on acute and chronic exposure of rats to MP recorded substantial changes in the measured AchE activity in blood, brain and other organs. In our work, the most pronounced inhibition of AchE activity in serum was recorded in subgroup E (25:75% atropine and NaHco₃) followed by subgroup B (100% NaHco₃), subgroup D (50:50% atropine: NaHCO₃), subgroup A (100% atropine) and subgroup C (75:25% atropine: NaHco₃). Also the most pronounced AchE inhibition in the brain was recorded in subgroup E followed by A, D, B and C. It is well known that methyl parathion is converted to methyl paraoxon which is the active inhibitor of the acetylcholinesterase. This conversion occurs within minutes of administration, and both substances are mainly detoxified in the liver (IPCS, 1993).

Our results indicated that the decrease in AchE activity was corrected in mice treated with 75:25% atropine: NaHCO₃ and 100% atropine. A result which is correlated with others like Zhu et al. (2004) who recorded 50% decrease in blood AchE activity in MP treated rats; this decrease was reversed by the administration of atropine sulphate, a muscarinic receptor antagonist, indicating an involvement of muscarinic receptor. Hence, parathion is an organophosphorus insecticide that is potent AchE in-
hibitor being responsible for the hydrolysis of body choline esters, including acetyl choline at the cholinergic synapses. Atropine is a parasympatholytic alkaloid, is used as antidote to AchE inhibitors (Cabello et al., 2001).

Halperin et al. (1996), found that there are both a rational and experimental evidence for giving sodium bicarbonate to prolong the survival during hypoxia. While Cordoba et al. (1983) recorded that the use of NaHCO$_3$ reduces the quantity of atropine required and can produce recovery of the acid-base balance, where acidosis was observed after organophosphates poisoning. Also, Baroni et al. (1999) reported that treatment with NaHCO$_3$ reduces both structural and functional renal damage.

Severe symptoms of toxicity in the form of depression, body weight loss and arched back were observed in MP exposed mice. The same symptoms but of milder degree were noticed in subgroup B, D and E.

P.M. examination of MP exposed mice revealed diffuse organ congestion and petechiation including meningeal congestion, with excess catarrh on the GIT mucosa.

Marked pathological lesions were observed in MP treated group (F). Variable degrees of severity (but milder than subgroup F) were observed in the other subgroups. These variations in the severity may be pointed to the ability of the added compounds to ameliorate the damaging effect of MP toxicity.

The observed pathological lesions included hepatic and renal toxicosis, as degenerative and necrotic changes with more pronounced hepatic single cell necrosis (apoptosis), as well as testicular pathology as defective spermatogenesis and abnormal spermatids. In addition to neuronal degeneration and splenolic lymphocytic depletion were observed.

This generalized organ toxicity of MP pointed to the wide distribution of MP and its metabolites in the body. Such opinion agreed with Gallo and Lawryk (1991), who stated that; Mp and its metabolites in the body are distributed between the body organs and usually the highest concentration is found in the organs involved in the elimination process as liver and kidney. In mammals, 60-65% is excreted within 2 hrs, predominantly in urine.

Pathological changes were identical with those previously described by other authors as Prashanthi et al. (2006) and Narayana et al. (2006) who reported testicular pathology, Zurich et al. (2004) and Guizzetti et al. (2005), who reported brain pathology. Remarkable apoptotic cells appeared
as shrunken, densely eosinophilic structures. The later is known to be a special form of individual scattered hepatocellular death that was not associated with any inflammatory reaction. The later lack of inflammatory reaction could be re-claimed to absence of released harmful intracellular molecules from apoptotic cells (Bursch et al., 1988). Enhancement of apoptosis was reported in some hepatotoxicities caused by chemicals which induce peroxisome proliferation in rodents (Benedetti et al., 1980). Apoptosis and other cellular degenerative and necrotic changes could be attributed to the cytotoxicity of MP and its metabolites during their metabolism. Such opinion is supported by that mentioned by Guizzetti et al. (2005), as organophosphorus insecticides have a cytotoxic effect which appears to account only for a small part of the effect on DNA synthesis. The aforementioned explanation supported our cytogenetic changes represented by of decreased DNA, RNA and protein concentrations.

The observed lymphocytic depletion in spleens of subgroup F was also reported in organophosphate toxicity by Sastry (1999). undoubtly, this effect has a negative impact on the animal immunity.

The obtained testicular pathology is in accordance with that given by many authors as Prashanthi et al. (2006) and Narayana et al. (2006) who reported that MP is a reproductive toxicant in male rat and causes deterioration in the structural integrity of the reproductive organs as well as in the biochemical parameters in the epididymis.

While, brain pathology in the present study was mimic that described by Zurich et al. (2004) and Guizzetti et al. (2005). Neurotoxicity may be related to direct action of M.P. on nerve cell membrane and cellular components or it may disrupts their metabolism. Such explanation is supported with that of many authors as Zhu et al. (2004) who claimed the neurotoxicity of parathion to causation of neuronal excitation which could contribute to the neuronal basis of organophosphate insecticides induced behavioural changes.

Generally, neurotoxicants may act directly on cell component or indirectly by altering the cell metabolism or oxygen supply (Koestner and Norton, 1991). Many organophosphate insecticides act directly by combining with membrane receptors or with intracellular components (Davis and Richardson, 1980). The later opinion explains the observed neuronal degenerative and necrotic changes. Zurich et al. (2004) attributed marked gliosis to that; glial cells provide neuroprotection.
against organophosphorus toxicity.

Concerning, the encountered sciatic nerve changes which included axonal degeneration and myelin disintegration which were supported by the transmission electron micrograph examination of those nerves. The last mention changes represent an example of the distal axonopathies induced by neurotoxic organophosphates. Such neurotoxicant compounds can inhibit the neurotoxic esterase, such enzymatic inhibition leads to distal axonopathies (Koestner and Norton, 1991).

Our results regarding total protein, DNA and RNA concentrations in mice livers showed that, subgroup F and subgroups (B, E, and D) rekindled a significant decrease in these concentrations. On the other hand, subgroups treated either with atropine alone or concurrently received NaHCO₃ and atropine (25:75%) were more or less similar to control. Brain contents of total protein and nucleic acids revealed significant low levels in subgroups B, F and E only with no significant changes in other groups.

These findings endorsed with those recorded by Bartoli et al. (1991), who reported that 14C-methyl parathion was covalently bound to DNA, RNA and proteins of various rat and mouse organs 22 hr after i.p. injection. They also added that, covalent binding index (CBI) to liver DNA was low in both species and typical of weak initiators. The labeling of RNA and proteins from different organs of both species was slightly higher than DNA binding. Although they observed that there was no interaction with brain nucleic acids.

Nayeemunnisa and Begum (1992) evoked that sublethal doses of methyl parathion (O,O-dimethyl-O-nitrophenyl thiophosphate) injected intraperitoneally to 21 day old rat pups induced regional alterations in the central nervous system (CNS) regarding the levels of total RNA, total proteins, Levels of RNA and total proteins content exhibited remarkable decrease in 21 day old methyl parathion treated animals.

Interestingly enough, our findings revealed, a highly significant increase in the total chromosomal aberrations in subgroups F, B, D and E, (the most noticed aberrations were polyploidy, deletions, ring chromosomes, stretching and centromeric attenuations) and a highly significant decrease in mitotic index in the same groups. While subgroups received antidotes either atropine alone (A) and the co-treated subgroup with NaHCO₃ (75% atropin+25% NaHCO₃) showed only significant elevation in aberrations and dimensions in MI in comparison to control.

(MP) was investigated by
geno-toxic method in male Wister rats following a 28-day oral exposure to methylparathion (MP). Un- 
deger et al., 2000 observed that, MP increased the number of numerical but not the structural chromosome aberrations.

**Conclusion:**
The present results indicate that MP acts on multiple body organs and exposure to which remains a potential health risk. Our findings are important to general public. Also using of atropine sulphate in combination with NaHco₃ (75:25%) was the most effective antidote in the treatment of parathion toxicity in mice rather than atropine sulphate alone.

Generally preventive measures in reducing the use of toxic chemicals should be taken seriously to protect human health and the environment.

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علاج مزدوج للتسمم بالميثيل باراثيون في ذكور الجرذان مع الأشارة إلى التغييرات الباثولوجية والكيميائية الحيوية

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الملخص العربي

صممت هذه الدراسة لمعرفة التأثير الضار للميثيل باراثيون (مبيد حشرى شائع الاستخدام) على الأعضاء المختلفة لذكور الجرذان وكيفية معالجة هذا التأثير الضار باستعمال سلفات الأتروبين وبيكربونات الصوديوم.

تم تجريع الجرذان بالميثيل باراثيون 35 ملجم/كم وزن لمدة 20 يوم بعد ذلك قسمت هذه الجرذان إلى 6 مجموعات ثانية كل منها تم حقنها بسلفات الأتروبين بمفردها أو مع بيكربونات الصوديوم بتركيزات مختلفة لمدة 20 يوم. بينما استمرت المجموعة السادسة في أخذ الميثيل باراثيون بمفرده في نهاية التجربة تم تجميع الدم من جميع الحيوانات وفصل السيرام وأخذ عينات من الأعضاء الداخلية للفحص الميكروسكوب والكيميائي. وتم أيضاً أخذ عينات من النسيج المخ في المجموعة السادسة مقارنة بالمجموعة الضامنة بينما وضع هذا النقص في مستوى هذة المجموعة في المجموعة التي تم معالجتها بـ 25% سلفات الأتروبين + 25% بيكربونات الصوديوم. وأيضا لوحظ نقص في تركيز الحمض النووي والبروتين الكلي في نسيج الكبد والمخ في المجموعة السادسة مقارنة بباقي المجموعات. بينما لوحظ تصحيح هذا النقص في المجموعات التي عولجت بالأتروبين وبيكربونات الصوديوم.

وأشارت التغييرات الهيستوفاغزية للمجموعة السادسة إلى تغييرات تكنكية وتتكز في خلايا الأعضاء البنائية والخلايا الصمعية مع تأثر في وظائف البالونات وصغر في حجم الأكس فلاز لبعض الثديات الصمعية. وبالإضافة إلى أن الوراثة الجينية وجد خلل واضح في الكروموسومات من أنفاضة في معدل الانتشار المبتوًا في المجموعات المعالجة خاصة المجموعة السادسة بالنسبة المئوية ضامنة وأخيراً وجد أن 0.05 ملجم/كم سلفات الأتروبين مع 0.05 ملجم/كم بيكربونات الصوديوم كانت أفضل ترقياً لحالات التسمم بالميثيل باراثيون.

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