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COMBINED THERAPY FOR AN ORGANOPHOSPHORUS INSECTICIDE (AZODRIN) TOXICITY IN MALE ALBINO MICE

(With 3 Tables & 16 Figures)

By

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استخدام علاج مزدوج للتسمم بالمبيد الحشري الفوسفوري (الأزودرين) في ذكور الجرزان البيضاء

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أجريت هذه الدراسة لبيان دور كل من الأتروبين وبيكربونات الصوديوم في علاج حالات التسمم بالمبيد الحشرى الفوسفورى (الأزودرين) تم استخدام ٨٤ جرزا أبيضا قسمت إلى مجموعتين، الأولى منها (١٢ جرزا واستخدمت كضابط أول للتجربة دون أن تعامل بأي شئ) والثانية (٧٢ جرزا أعطيت ٠,١ مجم الأزودرين / لتر عن طريق ماء الشرب لمدة ٢٥ يوماً متصلا). بعد انقضاء المدة السابقة ودون توقف إعطاء الأزودرين تم إعادة تقسيم المجموعة الثانية إلى ستة مجموعات أخرى فرعية (ا، ب، ج، د، هـ ، و) محتويه كل منها على ١٢ جرزاً ، حيث عولجت الخمس مجموعات الأولى منها عن طريق الحقن في البريتونى لمدة ٢٠ يوما متصلا على النحو التالي: مجموعة ا: أعطيت ٠,١ مجم من الأتروبين/ كجم من وزن الحيوان، مجموعة ب: أعطيت ٢٠ مجم بيكربونات الصوديوم ٢% / كجم من وزن الحيوان، مجموعة ج: أعطيت خليط من المركبين بنسبة ٢٥:٧٥ % (٠,٠٧٥ مجم أتروبين + ٥ مجم بيكربونات الصوديوم ٢% لكل كجم من وزن الحيوان)، مجموعة د: أعطيت خليط من المركبين بنسبة ٥٠:٥٠ % (٠,٠٥٠ مجم أتروبين + ١٠ مجم بيكربونات الصوديوم ٢% لكل كجم من وزن الحيوان، مجموعة هـ: أعطيت خليط من المركبين بنسبة ٧٥:٢٥% (٠,٠٢٥ مجم أتروبين + ١٥ مجم بيكربونات الصوديوم ٢% لكل كجم من وزن الحيوان) أما المجموعة الفرعية و: فواصلت تعاطى الأزودرين فقط دون علاج وأستخدمت كضابط ثاني للتجربة. في نهاية التجربة تم ذبح جميع الجرزان وأخذ منها الدم. تم قياس مستوى خميرة الأسيتيل كولين أستيريز فى كل من مصل الدم ونسيج المخ. كذلك قدرت كمية الفوسفور في نسيج كل من الكبد والكلى. ولدراسة التغيرات الباثولوجية تم أخذ عينات من كل من الكبد والكلى والغدة جار الكلوية والقلب والطحال والرئتين والمخ

level in liver tissues were observed in subgroup C (180.7 ± 14.9 ppm) when compared with subgroup A (209.3 ± 12.4 ppm), while there was increase in the level of P with no significance in case of kidney tissues. The most common symptoms observed in exposed mice were depression and decrease in the body weight with arched back. The most pronounced histopathological changes were observed in subgroup F while the mildest ones were those seen in subgroup C. Remarkable histopathological changes were detected in the parenchymatous organs in the form of degenerative and necrotic lesions. Also, neuronal changes and edema in the brain as well as demyclination in sciatic nerve were noticed. From our results we can conclude that the use of atropine sulfate (0.075 mg/kg b.w.) in combination with sodium bicarbonate 2 % (5 mg/kg b.w.) may consider the most effective antidote in treatment of azodrin toxicity in mice.

Key words: *Insecticide-azodrin-acetylcholinesterase-phosphorous- sciatic nerve.*

INTRODUCTION

One of the aims of veterinary medicine is to successfully treat diseased animals and treatment is usually specific for different kinds of an explicit diagnosed cause. This is not easily performed with regard to toxicosis, as the dosage 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 antidotal treatment (Osweiler et al., 1985). However, it is fortunate that probably the most potentially hazardous group of compounds, the anticholinesterase organophosphate has specific antidote. Organophosphorous (OP) insecticides are of general importance because of their extensive use in combating the disease vectors and agriculture pests 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 (Osweiler et al., 1985; Hungerford, 1990; Radostitis et al., 1994; Hansen, 1995).

Azodrin (an OP insecticide with trade name Monocrotophos) is the E-isomer of O,O-dimethyl-O-(1-methyl-3-methyl amino-3- oxo-1-propenyl) phosphate, with commercial name Azodrin . It is a water-soluble OP insecticide with high oral and moderate dermal toxicity. It is used for control of a broad spectrum pests which cause their serious effect on animal health (Lein et al., 1982, Sanders et al., 1985). Azodrin is classified by WHO as highly hazardous poison (Worthing and Hance,

1991). The toxicologically relevant mode of action is the inhibition of AchE activities (Skripsky and Loosli, 1994). Beside inhibition of AchE activity azodrin as organophosphorous compound caused some pathological changes as haemorrhagic gastroentritis, pulmonary oedema, degenerative changes in liver and kidneys and demyelinating lesions in the spinal cord and in the peripheral nerves in man and experimental animals (Clark and Clark, 1975). The LD_{50} of monocrotophos, is 17-18 mg/kg for male rats and 20 mg/kg for female rats. The LD_{50} for dermal exposure is 126 mg/kg for male rats, 112 mg/kg for female rats, and 354 mg/kg for rabbits (Budavari, 1989). Symptoms of monocrotophos poisoning are similar to those of other organophosphate compounds. (Gallo and Lawryk, 1991).

Sodium bicarbonate is an extremely well-known agent that historically has been used for a variety of medical conditions. Despite the widespread use of oral bicarbonate, little documented toxicity has occurred, and the emergency medicine literature contains no reports of toxicity caused by the ingestion of baking soda (Thomas and Stone, 1994). There is both a rationale and experimental evidence for giving sodium bicarbonate to prolong survival during hypoxia (Halperin, et al., 1996). Baroni, et al., (1999) reported that treatment with sodium bicarbonate reduces structural renal damage, albumin reabsorption, and renal Transforming growth factor-beta (TGF-beta) production in rats with doxorubicin-induced nephropathy. Levy, (1998) mentioned that administration of sodium bicarbonate during cardiac arrest and hypoxic acidosis has become increasingly controversial.

Atropine is a competitive antagonist of acetylcholine at the muscarinic receptors (Randy et al., 1999). Overatropinization may cause heat stress, constipation, bladder dysfunction, ileus, and worsen the outcome, as observed in severe insecticide poisoning (Ladislaus and Steven 1999). So, due to the toxic effect of large dose of atropine, the aim of our work, to evaluate the role of sodium bicarbonate alone and/or in combination with atropine in treatment of azodrin toxicity as a method to minimize atropine dose.

MATERIALS and METHODS

Experimental design:

Eighty-four male albino mice about three months old (Animal House, Faculty of Medicine, Assiut University) were used. They were

housed two weeks before starting the experiment for adaptation under standard laboratory conditions (12 h light, 12 h darkness, feed pellets and tap water ad libitum). Animals were divided into, control group (G1, containing twelve mice and received no treatment) and exposed group (G2, containing seventy two mice and received 0.1 mg azodrin/L of drinking water for 25 days). Then, G2 was divided into six subgroups (A, B, C, D, E, & F) containing twelve mice each. First 5 subgroups were treated intra-peritonealy (i.p.) for 20 days as follows: A (0.1 mg atropine/kg b.w.), B (20 mg $NaHCO_3$ 2%/kg b.w.), C (0.075 mg/kg b.w. atropine + 5 mg $NaHCO_3$ 2%/kg b.w.) as 75:25%, D (0.050 mg/kg b.w. atropine + 10 mg $NaHCO_3$ 2%/kg b.w.) as 50:50%, and E (0.025 mg/kg b.w. + 15 mg $NaHCO_3$ 2%/kg b.w.) as 25:75%, while F continued in receiving azodrin only. Body weight gain was calculated weekly.

At the end of the experiment, all animals were sacrificed and serum were collected. Acetylcholinesterase (AchE) was measured in serum and brain tissues, while phosphorous level (P) was estimated in liver and kidney tissues. Tissue samples were taken from liver, kidney, heart, spleen, lungs, brain, testes, and sciatic nerve for histopathological examination. As well as other samples of sciatic nerves were collected for electron microscope examination.

Determination of Acetylcholinesterase:

Acetylcholinesterase was measured in serum and supernatant of homogenized brain tissues spectrophotometrically (Milton Roy, spectronic 1201) by using commercial kites (MP2, 124117, Boehringer Mannheim, Germany) according to the method described by Ellman et. al. (1961).

Determination of phosphorus levels:

Phosphorus content was measured in liver and kidney tissues after wet digestion Spectrophotometerically by using commercially available diagnostic kits (Biomerieux Sa 45.903 000 F/Rcs Toile/France) according to Gomory, (1942).

Histopathology:

Representative samples from liver, heart, kidneys, lungs, spleen, testes, brain and sciatic nerve were obtained from necropsed and freshly dead animals of all experimental groups and fixed in neutral-buffered formalin. Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 3 and stained with

hematoxylin and eosin (HE). Additional brain sections were stained with cresyl violet according to Bancroft and Stevens (1982).

Transmission Electron microscope:

For electron microscopy, samples from sciatic nerves were obtained and fixed by immersion in 3 % buffered glutaraldehyde and then post-fixed in osmium tetroxide. Tissue samples were then dehydrated in ascending grades of ethanol and embedded in Epon-812. Semi-thin sections were made and stained with 1% toluidine blue. Ultra-thin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined under transmission electron microscope (JEOL, 100 CXII) operated at 80 Kv.

Statistical analysis:

Student's t-test was used to determine the statistical significance of the obtained results (Gad and Weil, 1986), where the propability values were 0.05 and 0.001.

RESULTS

Symptoms of toxicity was severely observed in subgroup F (which received azodrin only) in the form of depression, body weight loss and arched back.

The levels of AchE in serum and brain were summarized in Table and Figure, 1. All exposed and treated subgroups showed significant inhibition of AchE either in serum or brain tissues when compared with control group G1. The most pronounced AchE inhibition in serum was recorded in subgroup E followed by subgroup B, F, D, A and C respectively. In the brain tissue, the severely recognized inhibition was revealed in subgroup F followed by E, A, D, B and C respectively. In comparison with subgroup F, all subgroups showed significant increase in serum AchE level except subgroup B & E which showed significant decrease. In comparison with subgroup A, all subgroups showed significant decrease in the amount of AchE levels in serum except in subgroup C, which showed significant increase. In case of brain AchE levels, significant decrease was recorded in subgroup E and F, while in subgroup C there is increase with no significant.

Phosphorous levels were summarized in Table and Figure, 2. In liver, phosphorous levels were increased in all treated groups but a significant increases were showen only in subgroups B, E and F when

compared with control group. As compared with subgroup F, a significant increases were showen in subgroup B and E, while a significant decrease was showen in subgroup C. But in kidneys, a significant increases in phosphorous content were showen in all subgroups when compared with control one. As compared with subgroup F, a significant increases were showen in all subgroups.

Gross pathology:

Necropsed animals from azodrin subgroup showed significant gross pathological changes which included diffuse organ congestion and petechial hemorrhages. Livers were mottled (pale foci on background of congested parenchyma). Lungs were congested and edematous. Heart was pale and flaccid. Excess catarrh was noticed on the gastro-intestinal mucosa. Except for meningeal congestion, no gross lesions were seen in the brain. Much less severe changes were observed in the animals which received azodrin and atropine (subgroup A) No comparable gross changes were detected in other treated or control groups.

Histopathology:

The most pronounced histological changes were noticed in animals which received azodrin only (subgroup F), while the mildest microscopic changes were observed in subgroup C which treated with 75% atropine and 25% $NaHCO_3$. The histological changes observed in subgroup A (received 100% atropine) were of moderate degree. Animals of other groups had histological changes of varying severity. Table (3) shows histological scoring of changes observed in the various subgroups. Although microscopic changes were seen in every organ and tissue examined in animals of subgroup F, the most remarkable changes were in the hepatic ones. These included diffuse hepatocellular degeneration. Hepatocytes were swollen and had cytoplasm of granular ground-glass appearance (Fig. 3). Discrete areas of hepatic parenchymal necrosis were detected. Centrolobular necrosis was frequent (Fig. 4). Shrunken hepatic cells having irregular outline, deeply eosinophilic cytoplasm and condensed nuclei were seen scattered in the hepatic parenchyma (Fig. 5). In toluidine blue-stained sections, the irregular shrunken hepatocytes were densely stained (Fig. 6). Edema of hepatic tissue accentuated the dissociation of hepatocytes. Haemorrhages were frequent in the hepatic tissue. Cholangioles were occasionaly visible and distended. Focal aggregates of lymphoid cells were also found in the hepatic tissue. In addition to the hepatic changes, other organs and tissues examined of mice in subgroup F showed significant

histopathological changes. Lungs showed congestive edema, emphysema and haemorrhages. Hearts had degenerated pale myofibers which frequently had wavy appearance. Haemorrhages were frequently seen in the cardiac tissue (Fig. 7). Tubular degenerative changes involving convoluted and collecting tubules were a common finding in the kidneys (Fig. 8). The degenerated tubular epithelial cells were swollen and detached. Haemosiderosis was frequently observed in the examined spleens (Fig. 9). The splenic follicles were noticeably depleted from their lymphoid elements. Gastro-intestinal tract disclosed haemorrhages and edematous changes in submucosa and necrotic desquamative changes of mucosal epithelium. Adrenal cortical cells were disrupted and haemorrhages were scattered in the adrenal cortex. In the testicles, stages of spermatogenesis in the seminiferous tubules were incomplete and number of abnormal spermatid forms was increased (Fig. 10). Spermatogonia cells and Sertoli cells were disorganized. In the examined brain tissues, meninges were congested, edematous and had tiny haemorrhages. Demyelination (reticular formation) was noticed in the white matter (Fig. 11). Considerable number of neuronal cells in gray matter were shrunken, leaving empty spaces around them (Fig. 12). Other neurons revealed dispersion of nuclear chromatin and decreased density of Nissl's substance (Fig. 13). Pericellular edema was frequent. The examined sciatic nerves showed edema separating the nerve fibers in the endoneurium (Fig. 14). There were variations in the density of myelin sheaths of neurofibers.

Electron microscopic examination of sciatic nerves of mice in subgroup F revealed neurofibers with partially destroyed myelin sheaths which appeared as laminated material instead of the normal electron-dense appearance (Figs. 15 & 16). The axoplasm of these neurofibers contained disoriented neurofilaments. Some neurofibers had reduced sized axoplasm which displaced by the expanded degenerated myelin sheaths. No comparable microscopic or ultra-microscopic changes were detected in the control animals.

DISCUSSION

Organophosphates inhibit AchE lead to increasing concentrations of acetylcholine in the synaptic gap. In the gastrointestinal tract, muscarinic receptors mediate an increased intestinal motility and tone, which leads to spastic contraction, abdominal cramps, and diarrhea. In addition, acetylcholine impairs sodium and water absorption and

increases chloride secretion, which aggravates diarrhea (Klaus et al., 1999). He also mentioned that the specific antidotes to organophosphates are atropine and obidoxime. Atropine is a cholinergic blocking agent that effectively competes with acetylcholine at the binding site, alleviating many poison symptoms, the most important being respiratory tract secretions (Randy et al., 1999).

Azodrin caused a significant inhibition of the brain AchE activity. In vitro studies with partially purified preparations of rat brain AchE revealed that monochrotophos was potent anticholinesterase agent (Siddiqui, et. al., 1991). Our results revealed that all azodrin exposed and treated subgroups were showed significant inhibition of AchE either in serum or brain tissue when compared with control group. Chicken brain AchE was found to be most sensitive to inhibition by monochrotophos followed by that of rat whereas mice and pigeon were almost equally sensitive to acetylcholine inhibition (Siddiqui, et. al., 1988). Sandhu and Malik (1990) mentioned that repeated oral administration of monochrotophos in buffalo calves in doses of 0.5 and 2.0 mg/kg b.w./day produced significant inactivation of plasma cholinesterase (24 - 31%). Swamy et. al. (1992), stated that during the development of behavioral tolerance to the organophosphate azodrin, the inhibition of acetylcholinesterase and BuChE in albino rats and the elevation of its content were progressive till 7 days and were followed by a recovery trend towards normally. The changes correlated with the appearance of signs and symptoms of toxicity, which were mainly cholinergic. A 15 to 25 percent depression in cholinesterase means that slight poisoning has taken place. A 25 to 35 percent drop signals moderate poisoning, and a 35 to 50 percent decline in the cholinesterase readings indicates severe poisoning (Paul, 1987). The most pronounced AchE inhibition in serum of the azodrin treated subgroups were recorded in 25:75% atropine: $NaHCO_3$ followed by 100% $NaHCO_3$, only, 50:50% atropine: $NaHCO_3$, 100% atropine and 75:25% atropine: $NaHCO_3$. Also the most pronounced AchE inhibition in the brain tissue of the azodrin treated subgroups were recorded in 25:75% atropine: $NaHCO_3$ followed by 100% atropine, 50:50% atropine: $NaHCO_3$, 100% $NaHCO_3$, only, and 75:25% atropine: $NaHCO_3$. Oral administration of monocrotophos to rats and goats, is rapidly almost completely absorbed from the intestinal tract. Monocrotophos and its metabolites are distributed between the organs and usually the highest concentrations are found in the organs involved in the elimination process, i.e. liver and kidney (Muche, 1994).

In mammals, 60-65% is excreted within 24 hours, predominantly in the urine (Gallo and Lawryk, 1991). Halperin, et al., (1996). Found that, there is both a rationale and experimental evidence for giving sodium bicarbonate to prolong survival during hypoxia. Baroni, et al., (1999) reported that treatment with sodium bicarbonate reduces structural renal damage, albumin reabsorption, and renal TGF-beta production in rats with doxorubicin-induced nephropathy. Donovan, et al., (1999), mentioned that sodium bicarbonate is used to reverse the cardiotoxic effects of some drugs with membrane stabilizing activity. Hypertonic sodium bicarbonate partially reversed sodium channel blocked (Curry, et al., 1996). Cordoba, et. al., (1983) found that the intravenous injection of sodium bicarbonate produced recovery to dogs injected intraperitonial with 30 mg/kg O, O- dimethyl-O-2,2-dichlorovinyle phosphate (DDVP), improvement in the poisoned state and survival of 84.6% of the patient. They also recorded that use of difenhydramine and sodium bicarbonate reduce the quantity of atropine required and the use of reactivators produces rapid improvement and diminished mortality. He also mentioned that intravenous injection of sodium bicarbonate produced recovery of the acid-base balance, where acidosis was observed after organophosphorous poisoning.

In liver, phosphorous levels were increased in all treated groups but a significant increases were showen only in subgroups B, E and F when compared with control group. As compared with subgroup F, a significant increase was showen in subgroup B and E, while a significant decrease was showen in subgroup C. These indicate the lowest consentration of phosphorous in subgroup C. But in kidneys, a significant increases in phosphorous content were showen in all subgroups when compared with control one. As compared with subgroup F, the highest significant increases were showen in subgroups D, and C. Lloyd, et al., (1996) mentioned that sodium bicarbonate produced a significant metabolic alkalosis and an increase in urine pH.

The more severe symptoms of toxicity in the form of depression, lossing of the body weight and arched back were observed in mice exposed to azodrin only (subgroup F), same symptom were observed in a mild degree in subgroup B, D, E. and not observed in other groups. Necropsied animals from azodrin exposed group showed significant gross pathological lesions. These included diffuse organ congestion and petechial haemorrhages. Livers were mottled (pale foci on background of congested parenchyma). Lungs were congested and edematous. Heart was pale and flaccid. Excess catarrh was noticed on the gastro-intestinal

mucosa. Except for meningeal congestion, no gross lesions were seen in the brain. Other subgroups showed similar pathological changes in variable degree with mild degree in subgroup treated by 75: 25% atropin: $NaHCO_3$. No comparable gross changes were detected in control group.

The most pronounced pathological changes were those of animals of subgroup F (Azodrin only). The mildest ones were in subgroup C (75% atropine: 25% NaHCO3). The other groups showed less severe histopathological changes compared with subgroup F. This variation in severity may point to the ability of the added compounds to ameriolate the damaging effect of azodrin toxicity. The hepatic response of animals in subgroup F was mainly a necrotizing one. Some organophosphorous insecticides also have necrotizing hepatic effect (Kovalenko et al., 1975). This indicates the severity of azodrin-induced hepatic toxicosis which exceeded the classical toxin-induced hepatic degenerative changes (MacLachlan and Cullen, 1995). Scattered cell necrosis was seen frequently in the examined hepatic tissues. The necrosed hepatic cells were shrunken and had densely eosinophilic cytoplasm and condensed nucleus. This histological change was previously described as single cell necrosis in the liver but recently is reported as hepatic apoptosis (Pritchard and Buttler, 1989, Sharma, et.al., 1997). As observed in the present cases, the apoptotic cells are usually shrunken and characterized histologically as densely eosinophilic structures. Apoptosis is known to be a special form of individual hepatocellular death (Popp and Cattley, 1991), i.e. the apoptotic changes involve scattered hepatic cells but not encompass large parenchymal areas. The observed hepatic apoptosis was not associated with any inflammatory reaction. Absence of released cellular contents (harmful intracellular molecules) from apoptotic cells explains the lack of inflammatory cell reaction (Bursch, et al., 1988). Enhancement of apoptosis was reported in some hepatotoxicities caused by chemicals which induce peroxisome proliferation in rodents (Benedetti, et. al., 1988).

Spleens of subgroup F were depleted of their follicular lymphoid elements. Depletion of splenic lymphocytes was reported in organophosphate toxicity by Sastry (1999). Undoubtedly, this effect has a negative impact on the animal immune system. The observed testicular changes are in consistence with the known degenerative effect of organophosphorus insecticides on the testicles (Hashimoto et al., 1968; Steinburger and Sud, 1970; Egyed, 1973). The more affected cells in the seminiferous tubules were the more mature cells of the germinal epithelium (spermatid stages). It was found that some organophosphorus

insecticides acts first on mature germinal epithelium and not on cells where the mitotic process is active (Mazzanti et al., 1964). In general, the parenchymatous pathological changes observed in our cases are in line with those reported on the toxicity of organophophorus insecticides in various animal species (Namba et al., 1971; Maddy and Ridelle, 1977; Brar and Sandhu, 1991). Pathological changes of brain and sciatic nerve observed in the presently treated animals conform with those reported by Abou-Donia and Lapadula (1990). The observed neuronal changes appear to be one of the effects of azodrin toxicity on the central nervous system. Neurotoxicants may act directly on cell components or indirectly by altering the cell metabolism or oxygen supply (Koestner and Norton, 1991). Many organophosphate insecticides act directly by combining with membrance receptors or with intracellular components (Davis and Richardson, 1980). The present neuronal changes includes chromatolysis (decreased density of Nissl's substance) and dispersion of the nuclear chromatin. These morphologic changes usually occur when the neuronal cell oxidative metabolism is interferred by the neurotoxicant with loss of energy needed for cellular functions (Spencer and Schumburg, 1980 and Duchen, 1984). The encountered nerve changes, which included axonal degeneration and myelin disintegration, represent an example of the distal axonopathies induced by neurotoxic organophosphates (Koestner and Norton, 1991). These axonopathic changes involve primarily the large diameter axons of peripheral nerves. According to the latter authors, neurotoxic organophosphates can inhibit the neurotoxic esterase and the inhibition process of this enzyme through unknown mechanism leads to distal axonopathies. The demonstrated myelin changes (myelinopathy) was probably caused by direct damage evoked by azodrin similar to the effect of other toxins such as triethylin, hexachlorophane and cyanate (Cammer, 1980). The process starts by interlammelar vacuolization of myelin and finally rupture and disintegration of the distended myelin followed by axonal degeneration. Myelin loss was proposed to be due to greater exposure of the lamellar surfaces to catabolic enzymes such as phospholipases ans neutral proteinase (Sabri and Spencer, 1980).

From the results we obtained, we can conclude that usage of atropine sulfate in combination with $NaHCO_3$ (75:25) is the mostly effective antidote in treatment of azodrin toxicity in mice.rather than atropine sulfate alone. This conclusion is based upon the performed biochemical and pathological examinations.

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LEGENDS OF FIGURES

Fig. 3: Degenerated hepatocytes in the liver of mouse from subgroup F. The degenerated cells have cytoplasm of ground-glass appearance and nuclei with much less dense chromatin. H & E. X 280.

Fig. 4: Centrolobular necrosis (*) in the liver of a mouse from subgroup F. H & E. X125.

Fig. 5: Shrunken irregular hepatocytes (arrows) scattered in the liver parenchyma of a mouse from subgroup F. The shrunken cells have hypereosinophilic cytoplasm. H & E. X 125.

Fig. 6: Irregular densely stained shrunken hepatocytes (arrow). The nuclei of the shrunken cells are condensed and hyperchromatic. Mouse from subgroup F. Toluidine blue. X 125.

Fig. 7: Degenerated myocardial fibers having wavy appearance. Extravasated erythrocytes between myofibers are also observed. Heart of a mouse from subgroup F. H & E. X 250.

Fig. 8: Degenerated convoluted tubules in the kidney of a mouse from subgroup F. The degenerated tubular epithelial cells are swollen and detached. H & E. X 280.

Fig. 9: Haemosiderosis in the spleen of a mouse from subgroup F. The splenic follicle (F) is depleted of lymphocytes. H & E. X 125.

Fig. 10: Incomplete stages of spermatogenesis in a seminiferous tubule (ST). The number of spermatid forms is much lower. Abnormal Spermatid forms (arrow) are also seen. Testicle of a mouse from subgroup F. Toluidine blue X 280.

Fig. 11: Reticular formation (arrow) due to demyelination of nerve fibers in the brain of a mouse from subgroup F. H & E. X 125.

Fig. 12: Shrunken neuronal cells in the gray matter of brain of a mouse from subgroup F. The shrunken cells are irregular and have densely stained cytoplasm. H & E. X 125.

Fig. 13: Neuronal cells showing pale cytoplasm (chromatolysis, arrow). The density of Nissl's substance in these neurons is much lower. Cresyl violet stain. X 125.

Fig. 14: Sciatic nerve showing endoneurial edema separating the neurofibers. The density of myelin sheaths of neurofibers varies greatly. A mouse from subgroup F. Toluidine blue X 125.

Fig. 15: Neurofibers (NF) showing destroyed myelin sheaths (arrows) which have laminated appearance and interlammelar vacuolization. The axoplasm (*) is irregular and reduced in size. Sciatic nerve from a mouse in subgroup F. Transmission electron micrograph. X 4320.

Fig. 16: Infolded myelin sheath (arrow) of a neurofiber in sciatic nerve. The myelin sheath is partially destroyed to give the laminated appearance. Axoplasm (*) is reduced in size and contains more neurofilaments. A mouse in subgroup F. Transmission electron micrograph. X 10720.

Table 1. Acetylcholinesterase level (U/l) in control, animals exposed to azodrin and animals treated with either atropine and / or sodium bicarbonate.

Animals	Treatment	AchE in serum	% of serum AchE in relation to control	AchE in brain tissue	% of brain AchE in relation to control
G1	Control	2808.3 ± 116	100 ± 4.13	1154.4 ± 49.0	100 ± 4.24
Subgroup A	Atropine (100%)	402.4±18.8 $^{a, c}$	14.33 ± 0.66	280.4 ± 12.4 $^{a, c}$	24.29 ± 1.07
Subgroup B	$NaHCO_3$ (100%)	241. ± 14.5 ^{a,c,f}	8.59 ± 0.51	317.6 ± 11.2 $^{a, c}$	27.52 ± 0.97
Subgroup C	Atropine + $NaHCO_3$ (75 : 25%)	635.5±18.8 ^{a,c,f}	22.63 ± 0.66	325.0±10.1 ^{a,c,e}	28.16 ± 0.87
Subgroup D	Atropine + $NaHCO_3$ (50 : 50%)	329.1±14.2 ^{a,b,c}	11.72 ± 0.51	304.5 ± 11.4 $^{a, c}$	26.38 ± 0.98
Subgroup E	Atropine + $NaHCO_3$ (25 : 75%)	235.8±18.9 ^{a,c,f}	8.39 ± 0.67	182.4±07.8 ^{a,b,f}	15.80 ± 0.67
Subgroup F	Azodrin only	271.5 ± 14.0 ^{b,f}	9.66 ± 0.49	132.6 ± 12.8 ^{a,f}	11.49 ± 1.10

a: Significantly different from control at $p < 0.001$.
b,c : 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 2. Phosphorous level (ppm) in control, animals exposed to azodrin and animals treated with either atropine and / or sodium bicarbonate.

Animals	Treatment	P level in liver	% of P in liver in relation to control	P level in kidney tissue	% of P in kidney in relation to control
G1	Control	180.5 ± 16.1	100 ± 8.91	56.9 ± 1.7	100 ± 2.98
Subgroup A	Atropine (100%)	209.3 ± 12.4	115.9 ± 6.86	363.5±5.5[b,d]	638.8 ± 9.66
Subgroup B	$NaHCO_3$ (100%)	306.4± 22.1[b,c,e]	169.7 ± 12.24	267.1±5.7[b,d,f]	469.4 ± 10.01
Subgroup C	Atropine + $NaHCO_3$ (75 : 25%)	180.7 ± 14.9[c]	100.1 ± 8.25	368.6 ± 6.2[b,d]	647.8 ± 10.89
Subgroup D	Atropine + $NaHCO_3$ (50 : 50%)	224.9 ± 23.2	124.5 ± 12.84	448.9±6.0[b,d,f]	788.9 ± 10.54
Subgroup E	Atropine + $NaHCO_3$ (25 : 75%)	332.6± 17.6[b,c,f]	184.2 ± 9.74	192.5±4.5[b,d,f]	338.3 ± 7.90
Subgroup F	Azodrine only	242.3 ± 17.4[a]	134.2 ± 9.63	152.9 ± 3.6[e,f]	268.7 ± 6.32

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: Scoring of the histological changes observed in the various subgroups

Changes	Subgroup A Atropine 100%	Subgroup B $NaHCO_3$ 100%	Subgroup C Atropine: $NaHCO_3$ 75:25%	Subgroup D Atropine: $NaHCO_3$ 50:50%	Subgroup E Atropine: $NaHCO_3$ 25:75%	Subgroup C Azodrin only
Hepato-cellular degeneration and necrosis	++*	++	+	++	+	+++
Pulmonary congestive edema & haemorrhages	++	+++	+	++	++	+++
Myocardial degeneration and haemorrhages	++	++	+	+	+	+++
Renal tubular degeneration	++	++	+	++	++	+++
Splenic haemosiderosis and follicular depletion	+	+	+	++	++	+++
Incomplete spermatogenesis and abnormal spermatids	+	++	+	+	++	+++
Neuronal degeneration and chromatolysis	+	–	–	+	+	+++
Myelin disintegration (sciatic nerve)	–	+	–	–	+	+++

* +++, Severe; ++, Moderate; +, Mild.

Fig. 1: AChE (U/L) in control, azodrin exposed and treated animals with atropine and NaCO3

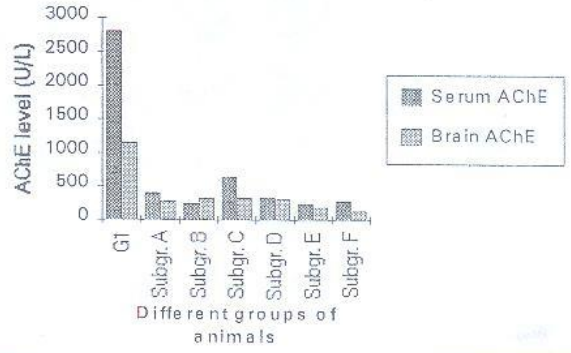
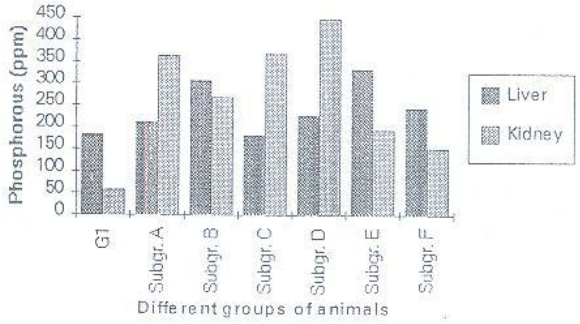
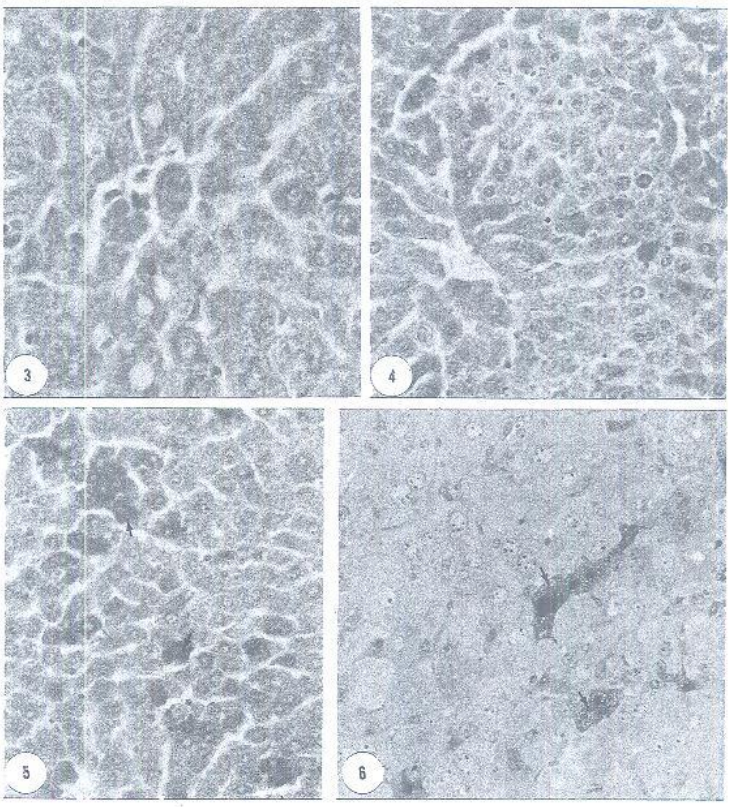


Fig. 2: Phosphorous levels (ppm) in control, azodrin exposed and treated animals with atropine and NaCO3.

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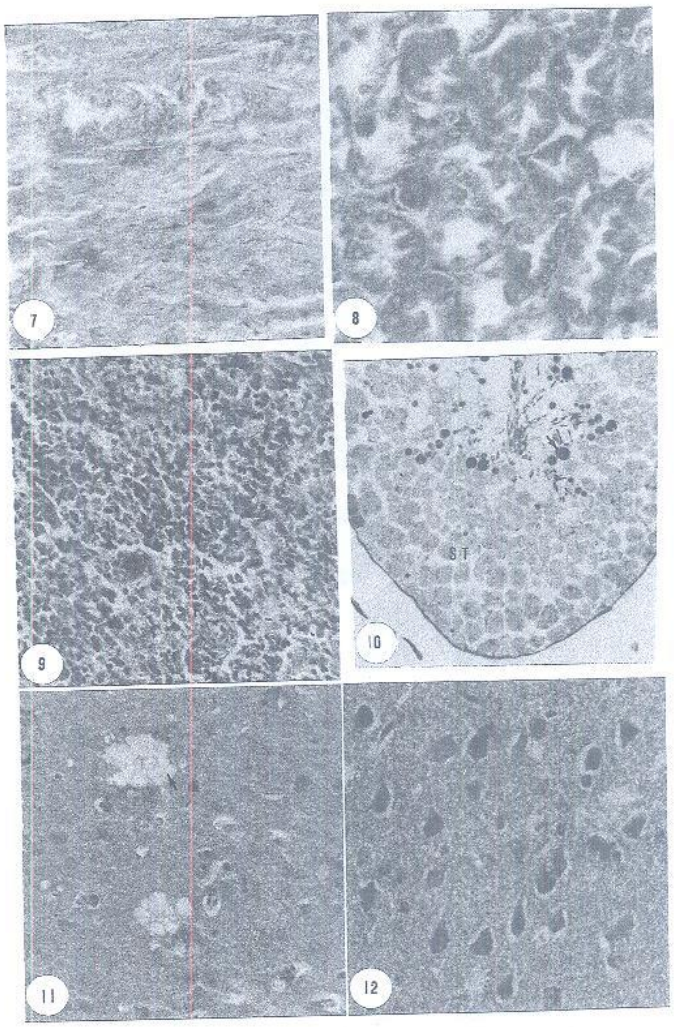
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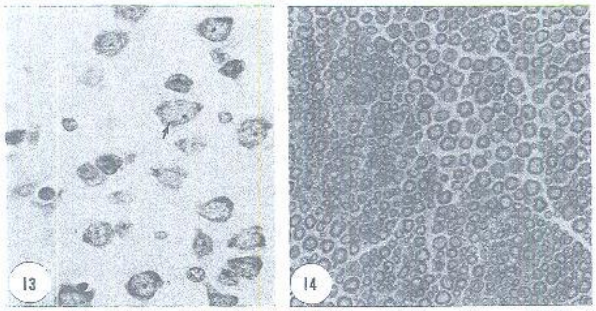
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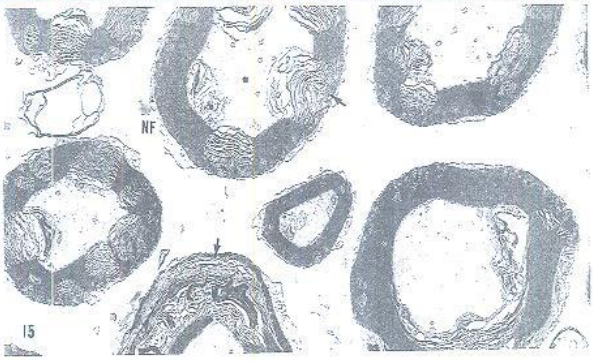
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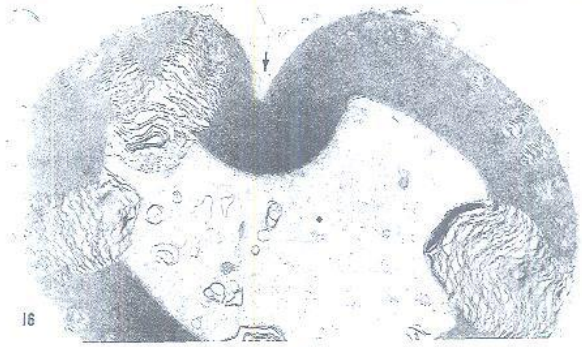
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