The Influence of Vitamin C or Selenium on Paraquat-induced Toxicity in Guinea Pigs

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Abstract: Paraquat, PO (1,1’-dimethyl-4,4’-bipyridylium dichloride) is widely used herbicide known to cause fatal intoxication in both human and animals. This study was carried out to investigate additional information about the deleterious haematological, hepatic, renal and neurotoxicities of this herbicide on male guinea pigs. Animals were injected intraperitoneally with sub lethal dose (1 mg kg⁻¹ b.wt.). The tested parameters were determined after 7 days of PG injections. The data presented in this work showed that PO administration induced elevations in haematocrit values, while the number of blood platelets were decreased. The number of red blood cells, haemoglobin contents and total bilirubin concentrations showed no significant changes. It provoked sever perturbations in liver functions as indicated by inhibition of aspartate and alanine aminotransferase (AST), (ALT) activities. Paraquat administration resulted in marked impairment in the renal function as indicated by elevations in serum urea levels. The hepatic and renal dysfunctions lead to increases in serum total lipid and triglyceride concentrations. On the other hand, hypoglycaemia as well as hypoglycaemia were prominent. Also, significant decreases were demonstrated in serum total protein and albumin concentrations, while the serum globulins were significantly increased with unique declines in the measured serum cation concentrations (Fe³⁺, Na⁺ and K⁺) and the water content of blood, liver, kidney and lung. Serum uric acid concentrations and the water content of muscle are still at their normal levels. Paraquat administration revealed conspicuous disturbances in acetyl cholinesterase (Ach El and creatin kinase (CK) activities. Also, this work was planned to evaluate the ability of either vitamin C (vit. C) or selenium (Se) to prevent or reduce its toxic effects. The results show that i.p. injection of vit. C (100 mg kg⁻¹ b.wt.) was highly effective than Se (20 µ mol/kg b.wt.) in the protection against paraquat induced toxicity. The mode of toxic action of PG and the effect of either vit. C or Se in preventing or reducing these toxic effects were discussed in details. It is concluded that vit. C might be an important participant in the treatment of PO toxicity. Also, the treatment with vit. C should include a suitable hyperglycaemic drug.

Key words: Paraquat-induced toxicity, vitamin C, selenium, Guinea pigs

Introduction
Paraquat (1,1’-dimethyl-4,4’-bipyridylium dichloride; PQ) is a member of bipyridylium herbicides which was discovered in 1955 and placed on market in 1962 (Sagar, 1989). Its toxic effects in the rat were first described by Clark et al. (1966). This relatively non selective foliage-applied contact herbicide is widely used in over 130 countries (Sagar, 1989). PQ is toxic to various mammalian species and its toxicity affects lungs, liver, brain, kidneys, and other organs (Smith and Health, 1976; Corasaniti et al., 1992). Also, PQ has been reported to be a neurotoxicant in humans (Grant et al., 1988; Hughes, 1988), rat (De Gori et al., 1988; Iannone et al., 1988; Dey et al., 1990) and mouse (Wooley et al., 1989).

The herbicide PQ is an example of a compound that can raise the level of reactive oxygen species in that it can undergo redox cycling and produce reactive oxygen species such as hydroxyl radical and superoxide anion (Sies, 1988). Conditions of oxidative stress have been associated with major clinical conditions like rheumatoid arthritis, myocardial infarction, emphysema, and parkinson’s disease (Halliwell, 1987).

There have been numerous fatalities caused by accidental or deliberate ingestion throughout the world. However, its fatal dose is not yet established in man (Ameno et al., 1994). After the initial acute symptoms the patients is characterized free from symptoms for first two or three days and this period of well-being is misleading. Symptoms then appear and rapidly progress to death at a time when the amount of PQ in the body should be negligible. It is reasonable to presume that the damage occurs during this period of apparent well-being. Treatment to be effective would be applied during this latent period. As there is no known antidote to PQ (Soyannwo et al., 1968).

Many different therapeutic measures to treat PQ intoxication have been investigated, including the administration of antioxidants, such as reduced glutathione, vitamin E (Shahar et al., 1980), superoxide dismutase (Patterson and Rhodes, 1982), catalase and metal chelators such as deferoxamine (Van der Wal et al., 1990). The results, however, have not been satisfactory. Non of these agents was able to suppress or interfere with the formation of the PC radical and more effective therapy for PQ intoxication is needed.

It is assumed that reduction of oxidative damage is possible by increasing the antioxidant capacity of tissues and cells. There is increasing evidence that vitamin C (vit. C) and selenium (Se) have antioxidant properties (Barja et al., 1994; Stajn, 1997, respectively).

Vitamin C is a water soluble vitamin. It is one of the biologic parameters involved in cell defence against oxygen free radicals. It is an ideal antioxidant to increase tissue protection from oxidative stress in human due to its easy, effective and safe dietary administration in large range of concentrations without harmful side effects. Altered antioxidant/prooxidant balance (Ogilvie et al., 1991) and strong plasma ascorbate depletion (Galley et al., 1996) have been recently observed in sepsis patients whereas therapy with antioxidants in animals (Goode and Webster, 1993) or with vitamin C in humans (Sawyer et al., 1989) increased survival during sepsis (Goode and Webster, 1993) or respiratory distress syndrome by as much as 50% (Sawyer et al., 1989). Selenium is an essential element and its role in normal metabolism has been well established. It exists in biological fluids bound to specific Se-binding proteins and largely as a constituent of various selenoproteins (Bansal et al., 1990; Bur and Hill, 1993).

It has been proved, scientifically, that selenium is a constituent of the human enzymes. It acts as a prosthetic group in the enzyme glutathione peroxidase. This enzyme is a natural antioxidant found in many tissues dependent upon a supply of NADPH₂.

Reduced activity of glutathione peroxidase contributes greatly to peroxidative damage (Zachara et al., 1990; Wang et al., 1994). It is reported that its protective effect is related to improvement of antioxidant defence system (Lane et al., 1991).

Paraquat toxicity was significantly enhanced in mice deficient in Se (Bus et al., 1975). Similarly, Omaye et al. (1976) reported the phenomenon of increased lethality of PQ in rats fed a selenium-deficient diet. Also, Glass et al. (1989)
demonstrated that enhanced lipid peroxidation is a major mechanism of lung injury in selenium-deficient rat lungs. The present study was planned to evaluate additional information about deleterious haematological, hepatic, renal and neuro-toxicities of PQ on guinea pigs as well as the protective effect of either vit. C or Se administration against its toxicities. Guinea pigs are ideal laboratory animals for this kind of studies since they simultaneously lack, like humans, the capacity to endogenously synthesize vit. C due to lack of expression of the L. gulono-gamma-lactone oxidase gene (Yagi, 1996).

Materials and Methods

Experimental animals and treatment: Male adult guinea pigs, weighing about (600 to 700 g) were used in this present experiments. They were purchased from Abees farm and were acclimated under standard conditions. Animals were housed in groups in stainless steel cages in a room at a temperature of 22-24°C and with a 12 h light/12 h dark cycle. Commercial diet and water were given ad libitum, twice daily. Paraquat (1,1'-dimethyl-4,4'-dipiridylidinium dichloride) was purchased from Sigma Chemical Co. (St. Louis, Mo, Sigma No. D-3506). Preliminary experiments were conducted to determine an intraperitoneally sublethal dose. It was found to be 1 mg kg

**superscript**1 b.wt. Vitamin C (L-ascorbic acid), was obtained as cebion ampoules from Merk, Germany. It was given to guinea pigs with a concentration of 100 mg kg

**superscript**1 b.wt. intraperitoneally as it was described by (Spittle, 1971). My preliminary experiments proved that this dose was more effective on preventing adverse side effects of PQ when divided into five consecutive days. Selenium as sodium selenite (Na2SeO3) was obtained from BDH England and was given intraperitoneally at a dose of 20 µ mol/kg b.wt. It was proved to be non toxic and more effective antioxidant when this dose was divided into 5 consecutive days (Araya et al., 1990; El-Soian, 1998; Mohamed, 1998).

Animal Groups: The animals were divided into six groups, ten animals each as follows:

**Group (1)** (Normal control group): The animals did not receive any treatment.

**Group (2)** (Vitamin C-treated group): The animals received 100 mg of vit. C/Kg b.wt. divided into 5 consecutive days.

**Group (3)** (Selenium-treated group): The animals received 20 µ mol of Se/Kg b.wt. divided into 5 consecutive days.

**Group (4)** (Paraquat-vit. C-treated group): The animals were given a single intraperitoneal sublethal dose of PQ (1 mg kg

**superscript**1 b.wt.).

**Group (5)** (Paraquat-vit. C-treated group): The animals were given PG (1 mg kg

**superscript**1 b.wt.) and vit. C (20 mg kg

**superscript**1 b.wt.) simultaneously followed by additional 4 doses of vit. C (20 mg kg

**superscript**1 b.wt.) for 4 days.

**Group (6)** (Paraquat-Se-treated group): The animals were treated similarly as group 4 using Se (4 µ mol/Kg b.wt.) instead of vit. C. Animals were killed by sudden decapitation at day 7 post paraquat dosing. Blood was collected in heparinized tubes for cellular determinations and non-heparinized tubes for serum preparation.

Physiological and biochemical determinations: Erythrocytic counts (ABCs), haemoglobin contents (Hb), haematocrit values (Hct), blood platelets counts (PLt) were determined by using the Coulter Courier Plus II. Serum was prepared by centrifugation of the blood at 8000 r.p.m. for 15 minutes. The activities of transaminases [aspartate aminotransferase (AST or GOT) and alanine aminotransferase (ALT or OPT)], acetylicolinesterase (AchE) and creatin kinase (CK) in the serum, as well as total lipids, triglycerides, cholesterol, blood sugar, total bilirubin, urea, total protein and albumin concentrations in the serum were estimated by using BM/Hitachi system 717 Automatic Analyzer.

Levels of iron in the serum were measured by atomic absorption spectrophotometer equipped with an HGA-400 graphite furnace (Beckman Labyte 800).

For the determination of water contents in organs (blood, liver, muscles, kidney and lung), the samples were preweighed in glass bottles. Tissue water contents were measured as weight loss on drying to constant weight at 105°C.

Statistical analysis: Measured values are presented as the arithmetic of ten experiments ± the standard error (S.E.). Results were analyzed using student’s t-test. Differences were considered significant at p<0.05.

The test significant of differences were calculated between:

a. Means of control group and the means of either of these treated groups: PQ group, vit. C group, Se group, PC) + vit. C group or PQ + Se group and they were refferred by letter (a).

B. Means of PQ group and either of PG + vit. C-treated group or PQ + Se-treated group. They were refferred by letter (b).

Results

Intraperitoneal administration of sublethal dose of PQ (1 mg kg

**superscript**1 b.wt.) produced significant changes in some haematological parameters of guinea pigs after 7 days. Table 1 depicts significant increases in haematocrit values, while numbers of blood platelets were significantly decreased. At the same time red cell counts as well as haemoglobin concentrations showed unsignificant alterations. Table 1 also shows that the simultaneous i.p. injection of a single dose of PQ and 20 mg vit. C/kg b.wt. followed by additional 4 doses of vit. C (20 mg kg

**superscript**1 b.wt.) for 4 days partially reduced the increases in haematocrit values and succeeded completely in preventing the decreases in the number of blood platelets that were induced by PQ.

The results depicated in Table 2 show that PQ administration resulted in marked decreases in liver transaminases (AST and ALT) and AchE activities, while CK activities were increased. The activities of both AST and ALT did not show any significant changes in the group injected with both PQ plus vit. C, while the activities of both AchE and CK were partially improved, since there still significant differences between their values (group 5) and those of both control and PQ-treated groups.

The data in Table 3 indicate that blood sugar levels and serum cholesterol concentrations were decreased, while serum total lipid and serum triglyceride concentrations were increase. The total bilirubin concentrations did not show significant changes as compared to control.

Table 3 also indicates that administration of PQ caused increases in serum urea levels. The serum total protein and albumin concentrations were significantly decreased, while serum globulins concentrations were increased. There were no significant alterations in serum uric acid levels. The total lipid, triglyceride and cholesterol concentrations were unchanged in the group treated with both PQ and vit. C, while the blood
Table 1: The effect of i.p. administration of a sublethal dose of PQ (1 mg kg⁻¹ b.wt.) with or without the i.p. injection of the antidote (100 mg of vit. C/kg b.wt. or 20 µ mol of sod.selenite/kg b.wt.) as well as the antidote alone on some haematological parameters

<table>
<thead>
<tr>
<th>Group number</th>
<th>Studied groups</th>
<th>R.B.C’s (x 10¹²/µl)</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>Pt (m/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>5.16 ± 0.020</td>
<td>13.92 ± 0.020</td>
<td>43.80 ± 0.002</td>
<td>273.0 ± 2.30</td>
</tr>
<tr>
<td>2</td>
<td>Vit. C</td>
<td>5.14 ± 0.060</td>
<td>13.91 ± 0.040</td>
<td>43.94 ± 0.013</td>
<td>269.0 ± 1.50</td>
</tr>
<tr>
<td>3</td>
<td>Se</td>
<td>5.15 ± 0.050</td>
<td>13.90 ± 0.090</td>
<td>43.97 ± 0.180</td>
<td>274.0 ± 2.70</td>
</tr>
<tr>
<td>4</td>
<td>PQ</td>
<td>5.17 ± 0.090</td>
<td>13.89 ± 0.102</td>
<td>57.37 ± 0.080*</td>
<td>153.0 ± 3.09*</td>
</tr>
<tr>
<td>5</td>
<td>PQ + vit. C</td>
<td>5.18 ± 0.080</td>
<td>13.94 ± 0.081</td>
<td>47.03 ± 0.193*</td>
<td>265.0 ± 4.82*</td>
</tr>
<tr>
<td>6</td>
<td>PQ + Se</td>
<td>5.15 ± 0.070</td>
<td>13.96 ± 0.108</td>
<td>57.01 ± 0.102*</td>
<td>220.0 ± 3.20*</td>
</tr>
</tbody>
</table>

Each value represents the mean of 10 experiments ± S. E.

Table 2: The effect of i.p. administration of a sublethal dose of PQ (1 mg kg⁻¹ b.wt.) with or without the i.p. injection of vit. C/kg b.wt. or 20 µ mol of sod.selenite/kg b.wt.) as well as the antidote alone on some serum enzymes activities

<table>
<thead>
<tr>
<th>Group number</th>
<th>Studied groups</th>
<th>AST (GOT) (µmol of sod.selenite/kg b.wt.)</th>
<th>ALT (GPT) (µmol of sod.selenite/kg b.wt.)</th>
<th>AchE (µmol of sod.selenite/kg b.wt.)</th>
<th>CK (µmol of sod.selenite/kg b.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>85.06 ± 2.030</td>
<td>55.03 ± 0.901</td>
<td>5057 ± 20.50</td>
<td>76.60 ± 1.801</td>
</tr>
<tr>
<td>2</td>
<td>Vit. C</td>
<td>84.01 ± 1.986</td>
<td>54.09 ± 0.821</td>
<td>5060 ± 18.90</td>
<td>75.91 ± 1.302</td>
</tr>
<tr>
<td>3</td>
<td>Se</td>
<td>86.02 ± 1.899</td>
<td>55.07 ± 0.392</td>
<td>5050 ± 15.60</td>
<td>76.80 ± 1.200</td>
</tr>
<tr>
<td>4</td>
<td>PQ</td>
<td>53.62 ± 0.908a</td>
<td>40.53 ± 0.123a</td>
<td>2647 ± 19.90a</td>
<td>132.10 ± 1.93a</td>
</tr>
<tr>
<td>5</td>
<td>PO + vit. C</td>
<td>86.01 ± 2.201b</td>
<td>56.03 ± 1.981b</td>
<td>3651 ± 16.20ab</td>
<td>88.40 ± 1.895a, b</td>
</tr>
<tr>
<td>6</td>
<td>PO + Se</td>
<td>59.58 ± 2.703a,b</td>
<td>61.90 ± 0.806a,b</td>
<td>2602 ± 20.90a</td>
<td>126.10 ± 1.086a</td>
</tr>
</tbody>
</table>

Each value represents the mean of 10 experiments ± S. E.

Discussion

It has been observed that the intraperitoneal administration of a sublethal dose of PQ (1 mg kg⁻¹ b.wt.) evoked marked changes in some haematological parameters of guinea pigs after 7 days of injection. Significant increases were observed in the haematocrit values while numbers of blood platelets were significantly decreased. On the other hand, red cell counts and haemoglobin concentrations remained in the normal range. Also, no significant changes were reported for the total bilirubin concentrations.

The present results confirm the previous observations of other investigators on man as well as on experimental animals. For example, Soyanwao et al. (1968) measured the haemoglobin concentration of a farmer (patient) after 4 days of ingestion of a mouthful of gramaxone (paraquat). He found that it was 14.1 g/100 ml. Increased haematocrit values have been observed after dosing rats with PQ (Fisher et al., 1975).

Lock (1979) found that the great vessel haematocrit in rats was significantly increased after oral PQ administration. On the other hand, he observed no significant differences in total red cell volumes.

Paraquat produced haemoconcentration in the rat (Fisher et al., 1975; Crabtree et al., 1977; Lock, 1979), the increase in haematocrit being due to a reduced plasma volume. The reduction of plasma volume is a consequence of a large fluid shift from the capillaries into the lumen of the gastrointestinal tract seen after oral administration of PQ and diuresis following PQ s.c. or p.o. Thrombocytopenia can result from decreased platelet production, increased platelet destruction or sequestration of platelets in the spleen. Decreased platelet production can result from a reduced number of megakaryocytes in the bone marrow and/or the lungs or from ineffective platelet production from normal numbers of megakaryocytes (Bell et al., 1976; Campbell et al., 1984). Paraquat is known as a pulmonary toxicant. Regardless of the route of administration, it produces lung injury due to its selective accumulation. Therefore, the observed decreases in the number of blood platelets may be due to PQ-induced lung damage.

The present results show that the administration of PQ decreased the activities of serum transaminases significantly. Transaminases (aminotransferases) can function both in amino acid catabolism and biosynthesis. Pyridoxal phosphate resides at the catalytic site of all transaminases. Increased serum transaminase activity in liver disease is assumed to reflect leaking from injured cells. Conversely, initially elevated serum transaminase activities may fall as the clinical course of massive hepatic necrosis deteriorates, suggesting that the liver is so severely damaged that little enzyme activity remains (Zakim and Boyer, 1982).
Wershana: The influence of vitamin C or selenium on paraquat-induced administration of a sublethal dose of PQ caused deleterious effects on the haemopoietic system as well as the hepatic and renal organs. It may also cause perturbations in the functions of other tissues or glands.

The present work demonstrated that PQ administration resulted in significant hyperlipidaemia, hypertriglyceridaemia and hypercholesterolaemia. Hyperlipidaemia has long been recognized as a complication of some forms of parenchymal liver disease. Also, hyperlipidaemia is common in the nephrotic syndrome. The primary mechanism appears to be increased hepatic synthesis, but reduced catabolism of lipids (Wyngaarden and Smith, 1985).

Triglycerides concentration is elevated in cases of liver dysfunction, chronic uremia and dialysis and nephrotic syndrome. Also, diuretic agents may associated with small increases in triglyceride levels (Wyngaarden and Smith, 1985).

Cholesterol concentrations are determined by metabolic functions which are influenced by integrity of vital organs such as liver and kidney (Marcus and Milton, 1982). Excessive insulin secretion, excessive removal, by inadequate production of glucose or by a combination of both mechanisms. Excessive insulin secretion results were also seen in rabbits (Ryhanen et al., 1984). Also, Shakoori et al. (1988) and Saleh (1990) have shown that different insecticides and herbicides produce decreases in cholesterol content.

Thus, it appears that PQ administration caused disturbances in lipid metabolism as a result of hepatic and renal dysfunction. Hypoglycaemia has been reported in a wide variety of acquired hepatic diseases. It may be caused by excessive removal, by inadequate production of glucose or by a combination of both mechanisms. Excessive insulin secretion results in hypoglycaemia (Campbell et al., 1984). The hypoglycaemia of congestive heart failure, sepsis and Reye’s syndrome is considered to be due to hepatic mechanism (Wyngaarden and Smith, 1985).

The liver cell, which is freely permeable to glucose, is a principal mean of regulating the blood glucose concentration. Defective enzymes of gluconeogenesis lead to hypoglycaemia. Because gluconeogenesis is dependent upon fatty acid oxidation, any impairment in fatty acid oxidation leads also to hypoglycaemia.

The data presented demonstrated that PQ induced significant decreases in serum total protein and albumin concentrations, while the serum globulins were significantly increased. Concentration of protein determines colloidal osmotic pressure of plasma. The concentration of protein in plasma is influenced by hepatic failure and renal function. It decreased in acute or chronic glomerulonephritis, nephrosis and acute or chronic hepatic insufficiency (Marcus and Milton, 1982). Albumin is synthesized exclusively in the liver. The synthetic rate is influenced by systemic or liver disease, thyroid

<p>| Table 3: The effect of i.p. administration of a sublethal dose of PQ (1 mg kg	extsuperscript{-1} b.w.t.) with or without the i.p. injection of the antidote (100 mg of vit. C/kg b.w.t. or 20 µmol of sod. selenite/kg b.w.t.) as well as the antidote alone on some biochemical indices |</p>
<table>
<thead>
<tr>
<th>Studied Groups</th>
<th>Normal control</th>
<th>PQ</th>
<th>PQ + Se</th>
<th>PQ + vit. C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (µg/dL)</td>
<td>59.2±0.4</td>
<td>57.5±0.3</td>
<td>55.4±0.2</td>
<td>57.2±0.3</td>
</tr>
<tr>
<td>Serum sodium (m mol/L)</td>
<td>146.2±0.4</td>
<td>146.3±0.3</td>
<td>146.1±0.2</td>
<td>146.4±0.3</td>
</tr>
<tr>
<td>Serum potassium (m mol/L)</td>
<td>3.76±0.1</td>
<td>3.76±0.1</td>
<td>3.76±0.1</td>
<td>3.76±0.1</td>
</tr>
</tbody>
</table>

Each value represents the mean of 10 experiments±S.E.

Thus, it appears that PQ administration depressed transaminases activities as a result of sever liver dysfunction.

The data presented demonstrated that the administration of PQ caused impairment in the renal function as indicated by elevations in the serum urea concentrations. Uric acid concentrations remained at the normal levels. Soyannwo et al. (1968) described a case of a farmer after taking a mouthful of gramaxone (paraquat), his blood urea levels were 178 mg/100 ml and 310 mg/100 ml after 4 and 6 days of PQ ingestion, respectively. Similarly, Shahar et al. (1980) found that the plasma urea concentration of a 3-year-old boy increased to 60 mg/100 ml after 4 days of swallowing of a mouthful of 20 % PQ solution, his initial plasma urea concentration was 12 mg/100 ml after 24 h of ingestion. In experimental animals, PQ induced similar effect, a reduction in renal function has been reported following ingestion. In experimental animals, PQ induced similar effect, a reduction in renal function has been reported following ingestion.

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decreases in the serum Na2+ and K+ concentrations.

The present study indicated that PG induced significant deficiency without anaemia (Campbell et al., 1982). Also, it relatively common for the plasma iron level to fall as soon as iron stores are depleted but before the haemoglobin concentration has fallen producing a state of iron deficiency without anaemia (Campbell et al., 1982). This is particularly common in cows, where the iron stores are depleted but before the haemoglobin concentration has fallen producing a state of iron deficiency without anaemia (Campbell et al., 1982).

Iron concentration in the plasma is determined by several factors including: absorption from the intestine, storage in the liver, spleen and marrow, break down or loss of iron from the liver, spleen and intestine, as well as the destruction of red blood cells (Milton, 1982).

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The present study indicated that PG induced significant decreases in the serum Fe3+ as a result of PQ injection. Iron concentration in the plasma is determined by several factors including: absorption from the intestine, storage in the liver, spleen and marrow, break down or loss of iron from the liver, spleen and intestine, as well as the destruction of red blood cells (Milton, 1982). The mechanism for the increased serum iron concentration in iron deficiency is not known. Thus, the reduction in renal excretory function produced by bipyridyls is probably not direct effect of these chemicals on the kidney but is secondary to a reduction in plasma volume which alters renal haemodynamics.

Wershana: The influence of vitamin C or selenium on paraquat-induced

Table 5: The effect of i.p. administration of a sublethal dose of PQ (1 mg kg−1 b.wt.) with or without the i.p. injection of the antidote (100 mg of vit C/kg b.wt. or 20 μmol of sod. selena/kg b.wt.) as well as the antidote alone on the water content on some guinea pig organs (g H2O/g dry wt.)

<table>
<thead>
<tr>
<th>Studied Tissue</th>
<th>Blood</th>
<th>Liver</th>
<th>Muscle</th>
<th>Kidney</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.12 ±0.001</td>
<td>2.754 ±0.001</td>
<td>2.754 ±0.001</td>
<td>2.571 ±0.001</td>
<td>3.224 ±0.002</td>
</tr>
<tr>
<td>Vit. C</td>
<td>5.11 ±0.002</td>
<td>2.756 ±0.002</td>
<td>2.753 ±0.001</td>
<td>2.572 ±0.002</td>
<td>3.225 ±0.001</td>
</tr>
<tr>
<td>Se</td>
<td>5.12 ±0.002</td>
<td>2.755 ±0.002</td>
<td>2.752 ±0.001</td>
<td>2.572 ±0.002</td>
<td>3.226 ±0.002</td>
</tr>
<tr>
<td>PQ</td>
<td>3.60 ±0.001x</td>
<td>1.545 ±0.001x</td>
<td>2.756 ±0.003</td>
<td>2.091 ±0.001x</td>
<td>3.688 ±0.001x</td>
</tr>
<tr>
<td>PQ + vit. C</td>
<td>4.090 ±0.622x</td>
<td>2.151 ±0.001x</td>
<td>2.752 ±0.002</td>
<td>2.097 ±0.003x</td>
<td>3.688 ±0.001x</td>
</tr>
<tr>
<td>PQ + Se</td>
<td>3.10 ±0.009x</td>
<td>1.545 ±0.001x</td>
<td>2.777 ±0.004</td>
<td>1.661 ±0.001x</td>
<td>2.019 ±0.004x</td>
</tr>
</tbody>
</table>

Each value represents the mean of 10 experiments ± S.E.

a, b = Statistically significant (p<0.05) when compared with values of the control group or PQ - treated group, respectively.

renal insufficiency, especially with inadequate sodium intake; renal tubular acidosis and unusual losses via the gastrointestinal tract, as in a acute or chronic diarrhea.

The potassium depletion has profound effects on neuromuscular, cardiac and renal function and acid-base status (Marcus and Milton, 1982). It is obvious from Table 5 that PG injection resulted in significant dehydration in the blood, liver, kidney and lung, while the plasma iron level did not affected. Lock (1979) demonstrated similar dehydration in the blood and lung following s.c. administration of PQ to rats. He stated that the reduction in the plasma volume is a consequence of diuresis. He noticed that water loading bipyridyl-treated rats either p.o. or i.p. does not rehydrate them. The mechanism whereby bipyridyls (paraquat or diquat) alter water redistribution, causing fluid loss into the lumen of the gastrointestinal tract, is not known. Thus, the reduction in renal excretory function produced by bipyridyls is probably not direct effect of these chemicals on the kidney but is secondary to a reduction in plasma volume which alters renal haemodynamics.

The present study also indicated that PQ is a potent inhibitor of acetyl cholinesterase activity. Similar results were obtained by Di Marzio and Tortorelli (1994).

The normal function of acetyl cholinesterase is to terminate neurotransmission due to acetylcholine that has been liberated at cholinergic nerve endings in response to nervous stimuli. The enzyme activity in serum is reduced in poisoning by organophosphorous compounds (used as insecticides), ishock, anemia, cancer and malnutrition (Campbell et al., 1984).

The potency of PQ as a neurotoxic agent has been investigated to a limited extent only and the reports give ambiguous information. De Gori et al. (1988) reported that systemic and intracerebral administration of PQ to adult rat causes both behavioural and neurological disorders, including decreased locomotor activity. Tanner (1989) indicated that the link between PQ and a possible induction of parkenson’s diseases is constituted by paraquat’s structural similarity to the 1-methyl-4-phenyldium ion, a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

The present results showed that PQ administration induced marked increase in creatine kinase activity.

The mitochondrial isoenzyme of creatin kinase functions in muscle cells to catalyze the transfer of a high-energy phosphate bond from ATP to creatin to form creatin phosphate. Creatin kinase activity increases in myocardial infarction, muscular dystrophy and in the disease of the central nervous system (Campbell et al., 1984). Since PQ induced disturbances in the nervous system as indicated by inhibition of acetyl cholinesterase activity, thus increased activity of creatin kinase may be due to neural toxicity. Also, it may be due to cardiac infarction.

Soyannwo et al. (1968) demonstrated the development of...
cardiac arrest (ventricular asystole) of a farmer after eight days of ingestion of mouthful of gramoxone (paraquat). Some investigators tried to interpret the biochemical mechanisms by which PQ causes cell damage. The damage may be related, in part, to biological reduction oxidation cycles of PQ which involves molecular oxygen and reduced flavoprotein. As a result of the reduction-oxidation cycles of PQ, oxygen radical generation, free radical-catalyzed lipid peroxidation, and membrane lysis presumably occur (Farrington et al., 1973; Winterbourne, 1981; Youngman and Elstner, 1981). In vitro experiments have demonstrated an enhancement of lipid peroxidation in lung, liver and brain microsomes following PQ administration (Talcott et al., 1979; Peter et al., 1996).

Another hypothesis concerning PQ toxicity assumes that herbicide-induced tissue damage may be not necessarily or totally dependent on peroxidation of membrane phospholipids in cells. It has been suggested that NADPH is oxidized by oxygen free radicals generated in the cyclic reduction and re-oxidation of PQ and that the resulted NADPH depletion may account for cell death by disturbing vital physiological and biochemical functions (Rose et al., 1976a; Breglius et al., 1982). Because most oxidized glutathione is reduced rapidly by oxidized glutathione reductase with NADPH as a reductant, oxidized glutathione increases when the NADPH supply becomes rate-limiting. In the case of a direct induction of lipid peroxidation or after an indirect impairment of cellular oxidative defenses by reduced glutathione depletion, an involvement of oxygen free radical reactions due to PQ toxicity is very probable (Melchiorri et al., 1996).

The result depicted in the present investigation clearly show that the simultaneous administration of a sublethal dose of PQ (1 mg kg^{-1} b.wt.) and 20 mg kg^{-1} b.wt. of vit. C followed by additional 4 doses of vit. C (20 mg kg^{-1} b.wt.) for 4 days reduced the serious toxic effects of PQ. It is evident from these results that the co-administration of vit. C with PQ succeeded in preventing the decreases in the number of blood platelets. I mentioned before that thrombocytopenia that was caused by PQ administration may be due to lung damage. Thus, the protective effect of vit. C is through the prevention of PQ mediated lung damage.

The co-administration of vit. C plus PQ also succeeded in returning the activities of AST and ALT back to their normal conditions since there were no significant difference between the control and group (5). In addition, the co-administration of vit. C with PQ succeeded in preventing the perturbations in the total lipids, triglycerides, cholesterol and Fe^{3+} concentrations which were caused by PQ alone. Similar modulatory effect of vit. C on the genotoxicity of pesticides in Swiss albino mice was investigated (Khan and Sinha, 1994). Also, the protection afforded by vit. C against oxidative stress has been well documented in many studies. Sharaf et al. (1978) observed a decreased level of glutathione in ascorbic acid deficient animals. Vitamin C was singled out by Frei et al. (1988, 1989), Nandi et al. (1991), Ogilvie et al. (1991), Galley et al. (1996) and Regoli and Winstone (1999) in its effectiveness to protect lipid from peroxidative damage and have shown that it is a potential scavenger of reactive oxygen species.

Most mammals synthesize ascorbic acid is apparently to protect the tissues from oxidant damage (Chatterjee et al., 1975; Chatterjee, 1978). However, humans, primated guinea pigs and flying mammals lack L-gulono-Lactone oxidase, the terminal enzyme in the pathway of ascorbic acid biosynthesis (Chatterjee, 1973). Accordingly these species are dependent on dietary sources of ascorbic acid and in case of ascorbic acid deficiency, these animals would be susceptible to lipid peroxidation (Othman and Moumena, 1998).

Recently, Cadenas et al. (1998) observed increases in superoxide dismutase and reduced glutathione in the vitamin C-supplemented guinea pigs. Also, Othman and Moumena (1998) demonstrated that the administration of vit. C caused marked stimulation of endogenous antioxidants (superoxide dismutase, glutathione reductase and glutathione) and reduction of lipid peroxidation in the liver of guinea pigs. Superoxide may act as an endogenous toxin. The occurrence of superoxide dismutases in nearly all aerobic creatures promoted the suggestion that O_2^- is an obligatory byproduct of a cellular metabolism in which oxygen serves as a recipient of electrons (Frank et al., 1980). According to this view superoxide dismutases have evolved to prevent the otherwise deleterious effects of O_2^- (Kroll et al., 1988).

The co-administration of PQ plus vit. C succeeded partially in preventing the impairment in the renal function. There are significant differences in the serum concentrations of urea between group (5) and both control and PQ-treated group. It also, succeeded partially in preventing the disturbances in the serum total proteins, albumin, globulins. Na^+ and K^+ concentrations, water contents of the selected organs (blood, liver, kidney and lung), haematocrit values and the activities of both AchE and CK that were caused by PQ alone. The kidney achieved high concentrations of PQ, greatly in excess of the concentration in plasma (Rose et al., 1976b). Paraquat is almost exclusively excreted by the kidney and it may suppress its own excretion causing its accumulation in this organ and may also in other organs (Ecker et al., 1975; Bismuth et al., 1987; Pond et al., 1993). Therefore, vit. C administration in this investigation provided partial protective effect in the previously mentioned parameters rather than complete protection against PQ-induced toxicities.

In this investigation, the effects of Se administration on PD-induced disturbances in some haematological, physiological and biochemical parameters were studied. Although, there is an increasing evidence that Se has antioxidant properties and can provide protection from free harmful radicals, in vivo (Young, 1981) and the Se deficiency results in liver necrosis in rats (Johnson et al., 1981). Also, superoxide dismutase showed a moderate enhancement upon applying Se to Wistar rats (Newsholme and Leach, 1985) and Se is an integral component of glutathione peroxidase, an enzyme with an intracellular antioxidant role (Stadtman, 1990). In addition, Othman and Moumena (1998) observed that the administration of Se to guinea pigs caused marked stimulation of endogenous antioxidants and reduction of lipid peroxidation in liver. The present data revealed that the co-administration of Se with PQ succeeded partially in preventing the disturbances in the number of blood platelets, transaminases activities, total lipids, triglycerides, cholesterol and Fe^{3+} concentrations, while it failed in preventing the disturbances in serum urea, total proteins, albumin, globulins, Na^+ and K^+ concentrations, water contents of the tested organs, haematocrit values and activities of both AchE and CK that were caused by PQ injection. The accumulation of PQ in the kidney and some other organs, as previously mentioned, may cause the lack of protective effect of Se against PQ-induced perturbations in these parameters.

The results of the present work show that the administration of either vit. C or Se into normal guinea pigs did not affect blood glucose level. Similar results were obtained by Othman and Moumena (1998). My results also revealed that the administration of either vit. C or Se with PQ induced hypoglycaemic effect than that caused by PQ alone. Othman and Moumena (1998) found that vit. C or Se administration to diazoxide hyperglycaemic guinea pigs resulted in lowering the
Wershana: The influence of vitamin C or selenium on paraquat-induced blood sugar level to normal values. The hypoglycaemic effect of vitamin C in that case was interpreted to be due to an increase in insulin action (Paolisso et al., 1995) or an enhancement of glucose uptake and utilization by peripheral tissues (Safinaz and Bakshwan, 1988). It is important to note that the negative control has no effect on all the studied parameters i.e. the dose of either vitamin C or selenium is safe.

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References


Wershana: The influence of vitamin C or selenium on paraquat-induced toxicity.


Mohamed, N., 1998. A comparative study between the effect of selenium and isax on side effects induced by the antioxidant drug cisplatin in adult male rabbits. Ph.D. Thesis, Department of Zoology, Faculty of Science, Alexandria University, ARE.


