The Protective Effect of Quercetin on Diazinon-Induced Oxidative Stress in Rats

Mohammed Talat Abbas

Department of Clinical Laboratory Science, College of Pharmacy, Kerbala

(NJC)

(Received on 10/11/2013) (Accepted for publication11/3/2014)

Abstract:

The aim of this study is to evaluate the protective effect of quercetin on diazinon-induced oxidative stress in the liver, kidney and heart tissues of male albino rats. The current study included 24 male rats, which were divided into 4 groups, each group included 6 rats: group 1 as a control group, Group 2 was given diazinon at 10 mg/kg/day, orally via gavage, Group 3 was given quercetin as intraperitoneal (IP) at 25 mg/kg/day and diazinon + quercetin group were given diazinon at 10 mg/kg/day/orally via gavage and quercetin at 25 mg/kg/day, IP for 4 weeks. The results show significantly increase in serum (AST and ALT activity), (urea and creatinine levels) and (Creatine kinase activity) which is associated with histopathological damage of the liver, kidney and heart tissue's architecture respectively due to the administration of the diazinon and this increase or damage is positively correlate with concentrations of the liver, kidney and heart MDA and negative correlation with the activity of catalase, superoxide dismutase and glutathione peroxidase on the liver, kidney and heart tissue homogenate, this modulation of the biological parameter histological damage is significantly neutralized by the administration of the quercetin. Our data suggest that supplementation of quercetin may be useful in reducing diazinon hepatotoxicity, nephrotoxicity and cardiotoxicity in rats which has the potential protective effect of quercetin and can be said.

Key words: Diazinon, Quercetin, Oxidative stress, Rats

الخلاصة

الهدف من الدراسة هي تقييم استخدام مادة الكوارستين لمعالجة جهد الاكسدة الناتج من استخدام diazinon. في هذه التجربة تم استخدام 24 جرذا ، قسمت إلى اربع مجامع كل مجموعه تضم 6 جرذا. المجموعة الأولى كانت المجموعة الضابطة. المجموعة الثانية كانت مجموعة diazinon في هذه المجموعة اعطيت للجردان
Mixture of diazinon and parathion was administered by the oral route at concentrations of 10/10 mg/kg daily in addition to diazinon and parathion mixture at concentrations of 25/10 mg/kg for a period of four weeks.

Materials and Methods: The results showed an increase in the activity of liver transaminases (ALT, AST, ALP) and malondialdehyde (MDA) with a simultaneous increase in the injury marker in the liver and heart tissues. The increase in these enzymes correlated with the decrease in the activity of catalase and superoxide dismutase in the liver, heart, and brain tissues. It can be concluded that parathion is a highly effective protective compound.

Keywords: Diazinon, Parathion, Oxidative Stress, Organophosphorus.
DZN inhibits acetyl cholinesterase activity and other organic functions. Mohammad et al. reported that toxic manifestations induced by OP may be related with the enhanced production of reactive oxygen species (ROS). In the body, these pesticides can disturb the balance of antioxidants as well as lipid peroxidation (LPO). Some reports have been published with respect to DZN and its effects on haematological and biochemical parameters of rat, rabbits and mice. Toxicities of OP insecticide DZN cause adverse effects on many organs. Other systems that could be affected are immune system, urinary system, reproductive system, pancreas, liver, kidney and heart.

Flavonoids or bioflavonoids (from the Latin word flavus meaning yellow), also collectively known as vitamin P and citrin, are a class of plant secondary metabolites or yellow pigments having a structure similar to that of flavones. Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. Flavonoids are constituents of fruits, vegetables, and plant-derived beverages, as well as components in herbal-containing dietary supplements. Among flavonoids, the flavonol quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the most widely distributed in human dietary sources. It is found in onions, apple, legumes, green tea, red grape wines, brassica green vegetables, citrus fruits and so on. This molecule has been reported to have potent antioxidant property and was found to be effective in various models of oxidative stress. It is prevents oxidative injury and cell death by several mechanisms, including scavenging oxygen radicals, inhibiting xanthine oxidase, lipid peroxidation, and chelating metal ions. In addition to that Quercetin have cardioprotective, anti-inflammatory, anti-cancer, anti-ulcer, anti-inflammatory, anti-allergic, antiviral and antibacterial effects. The aim of this study is to evaluate the protective effect of Quercetin on Diazinon-induced oxidative stress in male albino rats.
Materials and Methods

Chemicals:

All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade or the highest grade available. Diazinon 60 EC was applied as a commercial emulsifiable concentrate formulation containing 60% active ingredient, then, it was further diluted in distilled water to obtain the desired concentration. Rats were treated with Diazinon at a dose of 10 mg/kg/day, given orally via gavage for 4 weeks. Quercetin was given intraperitoneal (IP) at a dose of 25 mg/kg/day as described.

Animals:

Twenty four male albino rats weighing about 200-220 g were used in this study. The animals were kept under good ventilation and received a balanced diet and water ad libitum throughout the experimental period. The experimental was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments of animals, and approved by the animals ethical committee of kerbala University. Rats were divided randomly into four main groups (n= 6) as follow:

1) Control group received, standard diet without any treatment;
2) Diazinon-treated group, received standard diet supplemented orally with diazinon at dose of 10 mg/kg/day for a period of 4 weeks;
3) Quercetin-treated group, received standard diet, which was injected IP with Quercetin at a dose of 25mg /kg/day for a period of 4 weeks and
4) Diazinon+Quercetin-treated group,rats were treated with diazinon(10 mg/kg) and quercetin (25 mg/kg) daily for 4 weeks.

After 4 weeks, overnight fasted animals were sacrificed by cervical dislocation, and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at 860 X g for 20 min, and then quickly frozen at -20°C for biochemical analysis (AST, ALT, urea, creatinine and CK). Small pieces of liver, kidney and heart tissues were separately weighed and homogenized in 10 volumes of cold 0.01 M Tris-HCL buffer (pH 7.4), using an automatic homogenizer. The homogenates were then centrifuged at
15,000 rpm for 15 min at 4 °C. Clear supernatants were used for the Malondialdehyde (MDA), Catalase (CAT), Superoxide Dismutase (SOD), and glutathione peroxidase (GSH$_{PX}$) assays.

**Biochemical analysis:**
Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel methods. Serum creatinine concentration was measured by Jaffé reaction. Where as serum urea concentration was measured by enzymatic colorimetric method. Creatine kinase (CK) was measured by Rosalki methods. Tissue protein levels were measured according to the method used by Lowry et al. Tissue MDA assays were performed according to the guidelines of Ohkawa et al. MDA is a product of lipid peroxidation that reacts with Thiobarbituric acid (TBA) under acidic conditions at 95 °C, forming a pink complex that absorbs at 532 nm. 1,1,3,3 Tetraethoxypropane was used as the standard. The results are expressed as nmol/g tissue. SOD activity was measured according to the method of Winterbourn. It is based on the ability of superoxide dismutase to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide, absorbances were monitored at wave length 560 nm. Tissue catalase was assayed according to the method of Beers and Sizer. Catalase catalyses the decomposition of hydrogen peroxide ($H_2O_2$) to water and oxygen, The enzyme activity was followed by the decreasing in absorbance at 240 nm at 15 second intervals. Tissue glutathione peroxidase was assayed according to the method of Leopold Flone et al. Oxidized glutathione formed during glutathione peroxidase reaction is instantly and continuously reduced by an excess of glutathione reductase activity for a constant level of glutathione. The concomitant oxidation of NADPH is monitored spectrophotometrically at 340 nm.

**Histopathological studies:**
The kidney, heart and liver tissues were excised and fixed in 10% formalin and stained with hematoxylin and eosin and then observed under microscope for histopathological changes.

**Statistical analysis:**
The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey’s test. The results were expressed as means ± MSE and P < 0.05 was considered to be statistically significant.

**Results:**
The administration of diazinon to the rats, cause the significant increase in blood urea, serum creatinine levels, AST, ALT and CK activity when we compared with control group. Co-administration of quercetin will decrease the rise in blood urea, serum creatinine levels, AST, ALT and CK activity.(figure 3, 4, 5, 6 and 7 respectively).
Figure (3): The effect of the treatments of the groups in blood urea concentration, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Figure (4): The effect of the treatments of the groups in serum creatinine concentration, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05
Figure (5): The effect of the treatments of the groups in serum AST activity, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05.

Figure (6): The effect of the treatments of the groups in serum ALT activity, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05.
Figure (7): The effect of the treatments of the groups in serum CK activity, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05.

In the liver, kidney and heart tissues, MDA was significantly increased in diazinon treated group as compared to control group and this rise in MDA was decreased by quercetin. Antioxidant enzyme (SOD, CAT, GPx) activities were significantly different between the diazinon and the diazinon+quercetin groups. Compared to the control group, diazinon administration significantly decreased SOD, CAT, GPx activity in the liver, kidney and heart tissues, while quercetin administration increased them compared with that in the diazinon group (figure 8,9,10,11,12,13,14,15,16,17,18 and 19).
Figure (8): The effect of the treatments of the groups in MDA concentration in liver homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05.

Figure (9): The effect of the treatments of the groups in MDA concentration in kidney homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05.
Figure (10): The effect of the treatments of the groups in MDA concentration in heart homogenate, values are expressed as mean ± SME (n=6). P values were calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05.
Figure (11): The effect of the treatments of the groups in GPx activity in liver homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Figure (12): The effect of the treatments of the groups in GPx activity in kidney homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05
Figure (13): The effect of the treatments of the groups in GPx activity in heart homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Figure (14): The effect of the treatments of the groups in SOD activity in liver homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05
Figure (15): The effect of the treatments of the groups in SOD activity in kidney homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Figure (16): The effect of the treatments of the groups in SOD activity in heart homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05
Figure (17): The effect of the treatments of the groups in CAT activity in liver homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Figure (18): The effect of the treatments of the groups in CAT activity in kidney homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05
Figure (19): The effect of the treatments of the groups in CAT activity in kidney homogenate, values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Histopathological observation - The histology of the heart tissue from control and quercetin-treated animals showed normal morphological appearances, (Figure 20 and Figure 21) whereas in diazinon group, showed necrosis ,congestion ,degeneration ,disruptions of loss of myofibrils and vacuolization of the cytoplasm were observed.(Figure 22) The histology of heart tissues from Diazinon+ Quercetin group showed less necrosis, degeneration, loss of myofibrils and vacuolization of the cytoplasm.(Figure 23).

The histology of the liver tissue from control and quercetin-treated animals showed normal histological structure of hepatocytes , central vein and blood sinusoid , nucleus .(figure 24 and 25), whereas in diazinon group, showed cellular infiltrations ,degenerative changes of hepatic cells with cell necrosis and disarrangement of normal hepatic cells were observed.(figure 26) The histology of liver tissues from Diazinon+ Quercetin group showed less degeneration , necrosis and disarrangement of normal hepatic cells.(figure 27)

The histology of the kidney tissue from control and Quercetin-treated animals showed normal morphological appearances(figure 28 and 29), whereas in diazinon group showed congestion, necrosis , degeneration in the epithelial cells of renal tubules and swelling in the lining endothelium of the glomerulus . (figure 30) The histology of kidney tissues from Diazinon+ Quercetin group showed less congestion, necrosis , degeneration in the epithelial cells of renal tubules and swelling in the lining endothelium of the glomerulus. (figure 31).
Figure (20): heart section from a control rat showing normal morphology.

Figure (21): heart section from rat treated with Quercetin showing normal structure.

Figure (22): heart section from rat treated with diazinon showing necrosis, congestion, degeneration, disruptions of loss of myofibrils and vacuolization of the cytoplasm.
Figure (23): Heart section from rat treated with diazinon+Quercetin showing less necrosis, degeneration, loss of myofibrils and vacuolization of the cytoplasm when compared with diazinon group.

Figure (24): Liver section from a control rat showing normal histological structure of Hepatocytes, Central Vein and blood Sinusoid, Nucleus.

Figure (25): Liver section from rat treated with Quercetin showing normal morphology.
Figure (26): Liver section from rat treated with diazinon showing cellular infiltrations, degenerative changes of hepatic cells with cell necrosis and disarrangement of normal hepatic cells.

Figure (27): Liver section from rat treated with Quercetin showing less cellular infiltrations and degenerative changes of hepatic cells with cell necrosis.

Figure (28): Kidney section from a control rat showing normal structure of glomerulus and proximal tubule.
Figure (29): Kidney section from rat treated with Quercetin showing normal structure.

Figure (30): Kidney section from rat treated with diazinon showing congestion, necrosis, degeneration in the epithelial cells of renal tubules and swelling in the lining endothelium of the glomerulus.

Figure (31): Kidney section from rat treated with diazinon+ Quercetin showing less necrosis, degeneration in the epithelial cells of renal tubules and swelling in the lining endothelium of the glomerulus.