Original Paper

Toxicological Effects of Quinalphos and its Subsequent Reversal by Using Root Extract of *Withania somnifera* and Leaf Pulp of *Aloe barbadensis*

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ABSTRACT

The present study was designed to investigate the protective effect of *Withania somnifera* and *Aloe barbadensis* in pesticide-induced toxicity. Quinalphos (QP) at a dose of 14mg/kg body weight in male wistar rats for 15 days produced a reversible type of liver and kidney necrosis characterized by altered levels of various biochemical enzymes and endogenous antioxidants. Treatment with *W. somnifera* and *A. barbadensis* extracts resulted in a significant protective effect in QP-intoxicated hepatic and renal damage, as evidenced by diminished levels of SGOT, SGPT, ALP, ACP, LDH, total bilirubin, direct bilirubin and creatinine, and enhanced levels of total protein and albumin which were affected by quinalphos intoxication. Elevated levels of malondialdehyde (MDA) and lipid peroxidation in liver and kidney also significantly declined after the treatment with the extracts. Further, the reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity also returned to normal. This clearly indicates that the ethanolic extract of *W. somnifera* and aqueous extract of *Aloe barbadensis* can afford protection from QP-induced toxicity.

Key Words: Quinalphos, Oxidative stress, Lipid peroxidation, Superoxide dismutase, Glutathione, *Withania somnifera, Aloe barbadensis*

Introduction

The widespread use of pesticides in agriculture to control pests and enhance crop production has magnified the adverse health effects of these agricultural chemicals in non-target animals and humans.[1] It has been estimated that 3000,000 cases of severe poisoning, and 220,000 deaths are caused globally every year due to pesticides.[2] Due to their lower persistence in the environment, organophosphorus (OP) compounds are widely used in agriculture, medicine, and industry. However, they are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase (AChE), leading to accumulation of acetylcholine and subsequent activation of cholinergic, muscarinic, and nicotinic receptors.[3]

The Phase I enzyme system, comprising cytochrome P450 and flavin monooxygenases, that metabolize pesticides and other xenobiotics can also be either induced or inhibited by the pesticides or their metabolites. The induction and increased activity of Phase I enzymes leads to the accumulation of reactive oxygen species (ROS) and to the development of oxidative stress.[4,5]. It has been reported that OPs may induce oxidative stress on acute exposure in humans[6,7] and animals.[8,9] QP [O,O-diethyl-O-quinoxalinyl-phosphorothidate] is one such organophosphorus pesticide with tremendous utility in mixed pest control due to its insecticidal and acaricidal properties, and is used extensively in India and exported to many countries.[10,11]

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Exposure to QP can differentially modify endogenous antioxidants such as malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD), which can lead to the development of oxidative stress in some tissues. QP intoxication significantly changes the SGOT, SGPT, ALP, ACP and LDH activity[12]. Plant extracts with antioxidant activity are traditionally used to strengthen the natural immune defenses. Several antioxidant dietary compound classes have been suggested to have health benefits. Evidence shows consumption of these products leads to a decrease in various pro-inflammatory and / or oxidative stress biomarkers[13, 14] *W. somnifera* and *A. barbadensis* are used for the treatment of several disorders around the world[15]. As these two plants are having antioxidant properties and therefore it is important to elucidate their role for the treatment of pesticide poisoning.

Materials and Methods

1. *Chemicals and plant extract*
   Quinalphos (QP, O,O-diethyl O-quinoxalin-2-yl phosphorothioate), an organophosphate insecticide widely used in India, manufactured by Gharda Chemicals Ltd., Mumbai was procured from a local supplier. All other chemicals were of analytical grade. Dried roots of *W. somnifera* were purchased from a local dealer. The roots were washed, air dried, and powdered. 60% ethanolic extract was prepared, and stored at room temperature. Aloe gel was prepared by peeling off the green layer of the fresh, fleshy leaves; the transparent pulp was then homogenized and passed through cheesecloth. The resulting pulp extract was used for treating animals, and was prepared fresh each time.

2. *Experimental animals and treatment*
   Healthy adult male Wistar rats weighing 250–350 g were selected. The animals were housed five per cage, under controlled conditions of temperature (22 ± 1°C), humidity (60 ±5%), and light (12 h light: 12 h dark cycle), with unlimited access to food pellets and water. The animals were acclimatized for five days prior to the experiments. Group 1 animals, which served as control, were given basal diet only. Group 2, which comprised fifteen animals, were given 14 mg/ kg body weight of QP orally for 15 days. On the 15th day, five animals were sacrificed. Half of the remaining animals were given plant extract of *W. somnifera* at a dose of 200mg/kg body weight, while the rest were given *A. barbadensis* at a dose of 500 mg/kg body weight for the next 15 days. After the treatment period all animals were sacrificed. Blood was collected by cardiac puncture, and serum was separated and stored at 4-5°C for the biochemical assays. Liver and kidney tissues were dissected out, and rinsed with ice cold distilled water, chilled sucrose solution (20%), and distilled water. The tissues were immediately stored at 0°C till further biochemical analysis was carried out. One gm of liver tissue was homogenized in 10 mL ice cold Tris-hydrochloric buffer. The prepared liver tissue homogenates were centrifuged at 3500 rpm for 15 minutes, and the supernatant was used for the determination of various antioxidant parameters.

3. *Antioxidant parameters*
   Liver and kidney malondialdehyde (MDA) levels, reduced glutathione (GSH) levels, and superoxide dismutase activities of the control, QP-treated, *W. somnifera*- and *A. barbadensis*-treated animals were estimated by the methods of Ohkawa *et al,*[16] Beutler *et al,*[17] and Misra *et al*[18] respectively.

4. *Biochemical analysis of blood*
   Total protein, albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH), total bilirubin, direct bilirubin and creatinine levels of the control, QP-treated, and *W. somnifera*- and *A. barbadensis*-treated animals were estimated by using reagent kits (Qualigens Ltd. Mumbai, India).

5. *Statistical analysis*
   Statistical analysis was performed using Statfi statistical software. Results are presented as mean ± SEM of five animals in each group. Statistical difference between the means of the various groups was analyzed using one way analysis of variance (ANOVA). Data were considered statistically significant at $p = 0.05$.

Results

1. *Effect on organ weight*
   There was a significant reduction in the liver and kidney weight in the QP-treated animals as compared to that of control animals. Treatment with *W. somnifera* extract and *A. barbadensis* extract showed increase in the liver and kidney weight as compared to the QP-treated animals (**Table 1**).

Table 1 Weight (mg/gm body weight) of liver and kidney at the end of the experiment (mean ± SEM)

Organ	Control	QP Treatment	*W. somnifera* Treatment	*A. barbadensis* Treatment
Liver	36.963 ± 0.99	29.926 ± 0.62*a	35.904 ± 1.057*b	34.084 ± 0.803*b
Kidney	16.63 ± 0.41	14.45 ± 0.277*a	16.44 ± 0.909	15.37 ± 0.314*b
*a: P< 0.05 when compared with control		*b: P < 0.05 when compared with QP treated group		

2. *Effect on liver and kidney antioxidant parameters*
In QP-treated animals there was a significant elevation in the liver and kidney MDA levels when compared to the control group. The ethanolic extract of *W. somnifera* significantly reduced the levels of both liver and kidney MDA. Similar effects were also seen following treatment with *A. barbadensis* extract, which also reduced the liver and kidney MDA levels.

The present study showed that QP causes a significant increase in reduced glutathione contents in the liver and kidney as compared to the control. The plant extract treatment caused a decrease in the GSH levels of the liver and the kidney.

The results also showed a significant increase in the SOD activity in the liver and kidney of the QP-treated animals when compared to the control. Consistent with other results in this study, the plant extract treatment lowered the SOD activity (**Table 2** and **Table 3**).

3. *Biochemical analysis of blood*
A significant increase in the levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate

Table 2 Liver antioxidant levels of animal controls following chronic dose of QP, ethanolic extract of *W. somnifera* and *A. barbadensis* extracts (mean ± SEM)

Groups	MDA (nmol/mg of protein)	GSH (mg/mg of protein)	SOD (unit/ mg of protein)
Control	4.99 ± 0.38	17.36 ± 0.27	7.62 ± 1.67
QP	7.60 ± 0.47*a	26.83 ± 0.76*a	10.47 ± 0.5
W.somnifera treatment	5.57 ± 0.31*b	18.89 ± 0.48*b	9.44 ± 0.33
A.barbadensis treatment	6.02 ± 0.41*b	19.52 ± 1.75*b	9.47 ± 0.32
*a: P< 0.05 when compared with control *b: P < 0.05 when compared with QP treated group			

Table 3 Kidney antioxidant levels of animal controls following chronic dose of QP, ethanolic extract of *W. somnifera* and *A. barbadensis* extracts (mean ± SEM)

Groups	MDA (nmol/mg of protein)	GSH (mg/mg of protein)	SOD (unit/ mg of protein)
Control	3.83 ± 0.19	16.01 ± 0.87	3.85 ± 0.20
QP	6.43 ± 0.74*a	31.02 ± 1.69*a	4.64 ± 0.18*a
W.somnifera treatment	4.39 ± 0.183*b	16.39 ± 0.48*b	4.03 ± 0.07*b
A. barbadenis treatment	5.53 ± 0.43*b	16.52 ± 1.03*b	4.32 ± 0.04
*a: P< 0.05 when compared with control *b: P < 0.05 when compared with QP treated group			

pyruvate transaminase (SGPT), alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydgenase (LDH), total bilirubin, direct bilirubin and creatinine was observed in the QP-treated animals as compared to the control. Total protein and albumin levels decreased on QP treatment. The levels came back to normal on *W.somnifera* and *A.barbadensis* treatment.

Discussion

Quinalphos (QP) induces toxicity not only through inhibition of acetylcholinesterase (AChE), but also through alteration in endogeneos antioxidants by causing oxidative stress. It has been suggested that a phytotherapeutic approach to modern drug development could provide invaluable drugs from medicinal plants. In the present study, QP treatment was found to produce toxic effects in both liver and kidney tissues, and QP-induced liver and kidney injury is both severe and rapid in onset.[19-22] The results of this study indicate an increase in SOD activity in the animals given QP, as compared to controls. The increase might be due to the fact that in response to increase in the concentration of free radicals, the biological system increases the activity of antioxidant enzymes in order to combat the increasing free radicals, The resultant superoxide radicals induces SOD expression. The SOD level after *W. somnifera* and *A. barbadensis* treatment was nearly restored to the control values.

This study showed increased MDA levels in the liver and kidney of pesticide-treated rats at both chronic and acute doses, which might arise mainly from damaged Kupffer cells. The increased MDA of liver indicates enhanced lipid peroxidation due to tissue injury, and failure of the antioxidant defence mechanism, which prevents the formation of excess free radicals. Plant extract treatment was found to significantly decrease LPO levels in these tissues, in both the chronic and acute dose treated groups. Thus, it appears that the orally administered ethanolic root extract of *W. somnifera* and leaf pulp of *A. barbadensis* protects against QP-induced toxicity, possibly through the inhibition of increased LPO.

In this study, the levels of reduced glutathione (GSH) were found to be increased significantly in both acute and chronic dose levels of pesticide-intoxicated rats as compared with the control. This can be explained by the fact that pesticide-induced overproduction of free radicals has an inhibitory effect on the enzymes responsible for removal of ROS such as GPx, as a result of which reduced glutathione is not converted to the oxidized form, and its level increases. Plant extract treatment restores GSH levels nearer the control values, probably because the antioxidants in the extracts reduces the oxidative stress, thereby restoring the GPx activity to normal.

Assessment of liver function can be made by estimating the activities of total protein, albumin, SGOT, SGPT, ALP, ACP, LDH, total and direct bilirubin, and creatinine. The results of this study show elevated levels of SGPT, SGOT, ALP, LDH, total and direct bilirubin, indicating extensive liver damage. A significant increase in creatinine levels suggests impairement of the glomerular function and tubular damage in the kidneys. These results are in agreement with other studies. The reduced concentration of SGPT, SGOT, ALP, LDH, total and direct bilirubin as a result of administration of ethanolic extract of *W. somnifera* and aqueous extract of *A. barbadensis* could probably be due to the presence of antioxidants in the extract. The tendency of these marker enzymes to return to near-normalcy in *W. somnifera*- and *A. barbadensis*-treated animals is a clear manifestation of anti-hepatic and anti-renal effects of these plant extracts. Thus, from the foregoing findings, it can be concluded that *W. somnifera* and *A. barbadensis* can prevent or slow down the oxidative damage induced by QP, due to the antioxidant chemicals present in them. Further studies on other models, and extensive clinical trials are needed to confirm these results. In addition, further work is required to clarify how these plant extracts increases antioxidant enzyme activity.

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