

Experimental evaluation of certain herbs against chlorpyrifos-induced oxidative stress and toxicity in poultry

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Received: 15 April 2009; Accepted: 23 August 2009

ABSTRACT

Male broiler chicks (1 225), day-old, were randomly divided into 15 groups consisting of 15 chicks in each group. Group 1 was maintained as basal diet control and group 2 on chlorpyrifos (CPS) @ 100 ppm in feed throughout 6 weeks as iron toxic control without any treatment. Groups 3 to 15 were maintained on CPS @ 100 ppm in feed for the 4 weeks (28 days) of study and thereafter, administered with different herbs and their combinations for remaining 2 weeks. The blood samples were drawn from wing vein on day 28 and day 42 from the birds in each group for the assay of erythrocytic superoxide dismutase (SOD) and catalase. Sera samples were separated from the blood for the estimation of alanine transaminase (ALT) and serum creatinine. The birds were sacrificed at the end of 6th week and tissues were collected for the assay of GSH and TBARS in liver and brain homogenates. The activity of SOD, catalase and ALT, and the concentration of TBARS and serum creatinine were increased significantly, while the concentration of total proteins and tissue GSH decreased significantly in all the groups as compared to basal diet control and the values showed significant improvement in groups 3–15 that were treated during the last 2 weeks. It is concluded that chlorpyrifos induces toxicity by generating reactive oxygen species and antioxidant herbs are useful in treating the chlorpyrifos-induced toxicity.

Key words: Broilers, Chlorpyrifos, Herbs, Oxidative stress

Goel *et al.* (2005) reported that chlorpyrifos induces oxidative stress by generating free radicals and inducing lipid peroxidation, and by altering the levels of enzymatic and non-enzymatic antioxidant defenses. Slotkin *et al.* (2005) reported that besides acetyl cholinesterase inhibition, there were changes characteristic of oxidative stress in the fish treated with organophosphates. Alvarez *et al.* (2008) studied the potential effects of chlorpyrifos (CPS) on oxidative stress and reported significant increases in malondialdehyde (MDA) levels in hepatic and aorta, kidney and plasma samples following an acute exposure with CPS. Free radicals generated by organophosphorous compounds can be counteracted by antioxidants (Pena-Llopis and Samuell 2005). Several herbs, spices and many Indian medicinal plants are considered potential sources of antioxidants (Farrukh *et al.* 2006). Therefore, present study was planned on broilers using certain herbs and herbal combinations against chlorpyrifos-induced toxicity.

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MATERIALS AND METHODS

Sexed male broiler chicks (*Cobb* strain; 225), day-old, were randomly divided into 15 groups consisting of 15 chicks in each group. All the birds were provided with feed and water *ad lib.* throughout the experiment. All the groups were maintained as per the treatment schedule given here for 6 weeks:

- Group 1: basal diet control (1–42 days).
- Group 2: chlorpyrifos (100 ppm) toxic control (1–42 days).
- Group 3: chlorpyrifos (1–28 days) + *Withania somnifera* @ 0.1% of feed (29–42 days).
- Group 4: chlorpyrifos (1–28 days) + *Ocimum sanctum* @ 0.1% of feed (29–42 days).
- Group 5: chlorpyrifos (1–28 days) + *Asparagus racemosus* @ 0.1% of feed (29–42 days).
- Group 6: chlorpyrifos (1–28 days) + *Andrographis paniculata* @ 0.1% of feed (29–42 days).
- Group 7: chlorpyrifos (1–28 days) + *Murraya koenigii* @ 0.1% of feed (29–42 days).
- Group 8: chlorpyrifos (1–28 days) + *shilajit* @ 0.1% of feed (29–42 days).
- Group 9: chlorpyrifos (1–28 days) + *Gymnema sylvestre* @ 0.1% of feed (29–42 days).

Group10: chlorpyrifos (1–28days) + *Allium sativum* @ 0.1% of feed (29–42 days).

Group 11: chlorpyrifos (1–28days) + *Spirulina* @ 0.1% of feed (29–42 days).

Group12: chlorpyrifos (1–28 days) + *Ginseng* @ 0.1% of feed (29–42 days).

Group13: chlorpyrifos (1–28 days) + *Withania somnifera*+ *Asparagus racemosus* +*Andrographis paniculata* @ 0.05% of each in feed (29–42 days).

Group14: chlorpyrifos (1–28 days) + *Withania somnifera*+ *Murraya koenigii* +*Allium sativum* @ 0.05% of each in feed (29–42 days).

Group15: chlorpyrifos (1–28 days) + *Spirulina* + *Shilajit* + *Gymnema sylvestre* @ 0.05% of each in feed (29–42 days).

Group 1 was negative control and group 2 was toxic control throughout the study. Groups 3 to 15 were toxic controls up to the end of fourth week (1–28 days) and subsequently these were kept on treatment with herbs and herbal combinations for the next two weeks (29–42 days). The blood samples were drawn from wing vein on 28th and 42nd day for the assay of superoxide dismutase (SOD) (Marklund and Marklund 1974) and catalase (Caliborne 1985). Sera samples were separated for the estimation of concentration of total proteins and serum creatinine, and activity of alanine transaminase (ALT) by using diagnostic kits. The birds were sacrificed at the end of sixth week and tissues were collected for the assay of GSH (Moron *et al.* 1979) and TBARS (Subramanian *et al.* 1988) in liver and

brain homogenates. The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) 10th version. Differences between means tested using Duncan's multiple comparison test and significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The activity of SOD (U/g protein) was significantly ($P < 0.05$) increased in the CPS toxic controls (groups 2 to 15). However, following supplementation of herbs and their combinations in test, at the end of sixth week there was significant ($P < 0.05$) decrease in the activity of SOD in groups 3 to 15 as compared to their corresponding fourth week values and the activity in toxic control group 2 (Table 1). The activity of catalase (m mol/min) in the basal diet control (group 1) at the end of fourth week was significantly ($P < 0.05$) increased in the CPS toxic controls (groups 2 to 15). However, following supplementation of herbs and their combinations in test at the end of sixth week, there was a significant ($P < 0.05$) decrease in the activity of catalase in groups 3 to 15 as compared to their corresponding fourth week values and the activity in the toxic control group 2 (Table 1).

The concentration of TBARS (nmoles MDA/g protein) in liver recorded at the end of sixth week was significantly ($P < 0.05$) increased in the CPS toxic control (group 2). Treatment with herbs and herbal combinations in the last 2 weeks resulted in a significant ($P < 0.05$) decrease in the TBARS concentrations in groups 3 to 15 as compared to CPS treatment group. The concentration of GSH (mg/g

Table 1. Activity of super oxide dismutase (SOD) and catalase in different groups

Group	SOD (U/g protein)		Catalase (mmol/min)	
	4th week	6th week	4th week	6th week
Basal diet control (1–42 d)	42.09±0.42 ^{aA}	45.31±0.73 ^{aB}	2.43±0.025 ^{aA}	3.05±0.018 ^{aB}
CPS Control (1–42 d)	82.03±0.58 ^{bA}	90.78±0.65 ^{jB}	5.77±0.016 ^{bA}	6.51±0.165 ^{iB}
CPS (28 d) + <i>W. somnifera</i> (29–42 d)	81.90±0.61 ^{bA}	55.91±0.30 ^{dB}	5.78±0.023 ^{bA}	3.91±0.014 ^{dB}
CPS (28 d) + <i>O. sanctum</i> (29–42 d)	82.05±0.56 ^{bA}	65.44±0.39 ^{gB}	5.80±0.074 ^{bA}	4.37±0.005 ^{fB}
CPS (28 d) + <i>A. racemosus</i> (29–42 d)	82.09±0.53 ^{bA}	68.52±0.38 ^{hB}	5.79±0.023 ^{bA}	4.54±0.018 ^{gB}
CPS (28 d) + <i>A. paniculata</i> (29–42 d)	82.10±0.59 ^{bA}	68.98±0.48 ^{hB}	5.78±0.009 ^{bA}	4.56±0.018 ^{gB}
CPS (28 d) + <i>M. koenigii</i> (29–42 d)	82.06±0.62 ^{bA}	60.79±0.55 ^{fB}	5.80±0.027 ^{bA}	4.18±0.003 ^{eB}
CPS (28 d) + <i>shilajit</i> (29–42 d)	81.91±0.53 ^{bA}	72.82±0.34 ^{iB}	5.83±0.022 ^{bA}	4.72±0.013 ^{hB}
CPS (28 d) + <i>G. sylvestre</i> (29–42 d)	82.01±0.74 ^{bA}	59.14±0.60 ^{eB}	5.80±0.018 ^{bA}	4.11±0.008 ^{eB}
CPS (28 d) + <i>A. sativum</i> (29–42 d)	82.07±0.54 ^{bA}	59.56±0.48 ^{efB}	5.80±0.021 ^{bA}	4.16±0.004 ^{eB}
CPS (28 d) + <i>Spirulina</i> (29–42 d)	82.11±0.53 ^{bA}	64.74±0.43 ^{gB}	5.81±0.021 ^{bA}	4.31±0.007 ^{fB}
CPS (28 d) + <i>Ginseng</i> (29–42 d)	81.99±0.68 ^{bA}	55.29±0.29 ^{dB}	5.80±0.020 ^{bA}	3.86±0.018 ^{dB}
CPS (28 d) + <i>W. somnifera</i> + <i>A. racemosus</i> + <i>A. paniculata</i> (29–42 d)	82.13±0.67 ^{bA}	48.91±0.36 ^{bB}	5.80±0.026 ^{bA}	3.53±0.014 ^{bB}
CPS (28 d) + <i>W. somnifera</i> + <i>M. koenigii</i> + <i>A. sativum</i> (29–42 d)	82.30±0.64 ^{bA}	51.62±0.33 ^{cB}	5.77±0.033 ^{bA}	3.58±0.007 ^{bcB}
CPS (28 d) + <i>Spirulina</i> + <i>Shilajit</i> + <i>G. sylvestre</i> (29–42 d)	82.24±0.47 ^{bA}	52.62±0.34 ^{cB}	5.77±0.035 ^{bA}	3.66±0.015 ^{cB}

W., *Withania*; *O.*, *Ocimum*; *A. racemosus*, *Asparagus racemosus*; *A. paniculata*, *Andrographis paniculata*; *M.*, *Murraya*; *G.*, *Gymnema*; *A. sativum*, *Allium sativum*. Values are mean ± S.E (n = 6); one-way ANOVA (SPSS); means with different alphabets as superscripts differ significantly ($P < 0.05$): capital alphabets (horizontal comparison); small alphabets (vertical comparison).

Table 2. Concentration of TBARS and GSH in liver and brain of different groups

Group	TBARS (nmoles of MDA/g protein)		GSH (mg/g protein)	
	Liver	Brain	Liver	Brain
Basal diet control (1–42 d)	92.45±0.93 ^a	507.70±0.65 ^a	27.85±0.43 ^k	37.41±0.20 ^h
CPS Control (1–42 d)	178.40±1.28 ^k	654.20±0.60 ⁱ	11.69±0.20 ^a	20.00±0.11 ^a
CPS (28 d) + <i>W. somnifera</i> (29–42 d)	118.34±0.33 ^e	530.07±0.62 ^e	19.95±0.13 ^g	29.73±0.31 ^e
CPS (28 d) + <i>O. sanctum</i> (29–42 d)	130.87±0.28 ^h	542.16±0.47 ^g	16.59±0.11 ^d	26.04±0.12 ^c
CPS (28 d) + <i>A. racemosus</i> (29–42 d)	134.08±0.36 ⁱ	551.37±0.35 ^h	15.83±0.24 ^c	24.78±0.10 ^b
CPS (28 d) + <i>A. paniculata</i> (29–42 d)	134.73±0.71 ^{ij}	552.09±0.60 ^h	15.52±0.22 ^{bc}	24.18±0.19 ^b
CPS (28 d) + <i>M. koenigii</i> (29–42 d)	122.98±0.77 ^f	536.62±0.41 ^f	18.06±0.20 ^f	27.53±0.32 ^d
CPS (28 d) + <i>shilajit</i> (29–42 d)	136.41±0.54 ^j	551.77±0.30 ^h	14.87±0.20 ^b	24.41±0.19 ^b
CPS (28 d) + <i>G. sylvestre</i> (29–42 d)	121.56±0.35 ^f	535.60±0.39 ^f	18.71±0.16 ^f	28.06±0.33 ^d
CPS (28 d) + <i>A. sativum</i> (29–42 d)	122.02±0.63 ^f	536.07±0.50 ^f	18.22±0.18 ^f	27.64±0.30 ^d
CPS (28 d) + <i>Spirulina</i> (29–42 d)	127.51±0.37 ^g	541.63±0.45 ^g	17.25±0.05 ^e	26.18±0.05 ^c
CPS (28 d) + <i>Ginseng</i> (29–42 d)	117.82±0.59 ^e	527.36±0.40 ^d	20.16±0.22 ^g	30.06±0.06 ^e
CPS (28 d) + <i>W. somnifera</i> + <i>A. racemosus</i> + <i>A. paniculata</i> (29–42 d)	110.70±0.37 ^b	515.12±0.43 ^b	26.41±0.29 ^j	33.83±0.15 ^g
CPS (28 d) + <i>W. somnifera</i> + <i>M. koenigii</i> + <i>A. sativum</i> (29–42 d)	112.73±0.60 ^c	516.32±0.75 ^b	24.09±0.26 ⁱ	33.25±0.24 ^g
CPS (28 d) + <i>Spirulina</i> + <i>shilajit</i> + <i>G. sylvestre</i> (29–42 d)	115.64±0.48 ^d	519.72±0.93 ^c	21.93±0.29 ^h	31.46±0.17 ^f

Values are Mean ± S.E (n = 6); one-way ANOVA (SPSS); means with different alphabets as superscripts differ significantly (P<0.05).

protein) in liver and brain recorded at the end of sixth week was significantly (P < 0.05) decreased in the CPS toxic control (group 2). In the groups that were treated with herbs and herbal combinations in the last 2 weeks (groups 3 to 15), there was a significant (P < 0.05) increase in GSH levels as compared to CPS toxic control (Table 2).

In this study, the concentration of GSH was assessed in liver as it is the major organ involved in xenobiotic metabolism and in brain as it is susceptible to the free radical damage being rich in peroxidizable fatty acids. TBARS concentration in these tissues was analyzed to determine the extent of peroxidative stress and the activity of catalase and SOD was assessed as they form the major components of enzymatic antioxidant defense system in the living system. In the present study, the concentration of GSH was decreased in liver and brain, while the activity of SOD and catalase in blood, and TBARS concentration in liver and brain were significantly increased in the CPS toxic control group, suggesting an ongoing oxidative stress. All the changes in the antioxidant defense profile were significantly reversed, when treated with herbs and herbal combinations. These results were attributed to the antioxidant properties of steroidal lactones such as withanolides, which are the antioxidant principles in the *Withania somnifera* (Jaiprakash *et al.* 2002), phenols, glycosides and saponins in leaves of *Ocimum sanctum* (Farrukh *et al.* 2006), racemofuran in *Asparagus racemosus* (Wiboonpun *et al.* 2004), flavanoids, diterpenoids and polyphenols in *Andrographis paniculata* (Reddy *et al.* 2003), carbazole alkaloids from the leaves of *Murraya koenigii* (Tachibana *et al.* 2003), fulvic acid of

shilajit (Ghoshal *et al.* 1988), gymnemic acids (Gholap and Kar 2005) and gymnemasins (Sahu *et al.* 1996) of *Gymnema sylvestre*, ascorbic acid, tocopherols, phenols and thiols (as sulphur compounds) in *Allium sativum* (Yang *et al.* 2004), β-carotene and tocopherols of spirulina (Challem *et al.* 1981) and ginsenosides Rg3, Rb1, Rg1, RC, Rb2 and Rd of ginseng (Ki Sung Kang *et al.* 2007).

The total protein concentration (g/dl) recorded at the end of fourth week was significantly (P < 0.05) decreased in the CPS toxic controls (groups 2 to 15). However, following supplementation with herbs and herbal combinations in test, there was a significant (P < 0.05) increase in the total protein concentration in groups 3 to 15 at the end of sixth week as compared to their corresponding fourth week values and toxic control group 2. The activity of ALT (IU/L) recorded at the end of fourth week was significantly (P < 0.05) increased in the CPS toxic controls (groups 2 to 15). Following treatment with herbs and herbal combinations in groups 3 to 15, there was significant (P < 0.05) decrease in ALT activity at the end of sixth week as compared to CPS control group 2 (Table 3). The activity of ALT was determined to assess the degree of damage to liver as the levels of certain enzymes like ALT, AST, GGT etc. are reported to be elevated following hepatocellular injury (Kaneko *et al.* 1997). In this study, the activity of ALT was significantly elevated in the CPS toxic control group, indicating the hepato-cellular insult following administration of CPS. The elevated levels of hepatic enzymes could be due to the oxidative damage by free radicals as evident from the results of antioxidant defense profile. Further, supplementation with herbs and herbal

Table 3. Activity of ALT and concentration of serum total proteins and creatinine in different groups

Group	ALT (IU/L)		Total proteins (mg/dl)		Creatinine (mg/dl)	
	4th week	6th week	4th week	6th week	4th week	6th week
Bsal diet control (1–42 d)	16.90±0.36 ^{aA}	25.06±0.97 ^{aB}	3.50±0.140 ^{bA}	4.95±0.030 ^{eB}	0.40±0.018 ^{aA}	0.50±0.012 ^{aB}
CPS control (1–42 d)	32.93±0.83 ^{bA}	70.25±1.22 ^{kB}	1.24±0.057 ^{aA}	1.94±0.015 ^{aB}	0.59±0.018 ^{bA}	1.18±0.018 ^{gB}
CPS (28 d) + <i>W. somnifera</i> (29–42 d)	32.64±1.07 ^{bA}	44.59±0.39 ^{eB}	1.25±0.050 ^{aA}	4.58±0.040 ^{dB}	0.59±0.018 ^{bA}	0.75±0.009 ^{bB}
CPS (28 d) + <i>O. sanctum</i> (29–42 d)	32.93±0.83 ^{bA}	53.05±0.36 ^{hB}	1.24±0.066 ^{aA}	4.17±0.032 ^{bB}	0.62±0.012 ^{bA}	0.84±0.012 ^{defB}
CPS (28 d) + <i>A. racemosus</i> (29–42 d)	32.06±0.97 ^{bA}	54.80±0.36 ^{iB}	1.28±0.045 ^{aA}	4.23±0.011 ^{bB}	0.58±0.021 ^{bA}	0.88±0.015 ^{fB}
CPS (28 d) + <i>A. paniculata</i> (29–42 d)	33.52±0.83 ^{bA}	58.59±0.39 ^{jB}	1.23±0.066 ^{aA}	4.37±0.016 ^{cB}	0.59±0.018 ^{bA}	0.87±0.046 ^{fB}
CPS (28 d) + <i>M. koenigii</i> (29–42 d)	32.64±0.58 ^{bA}	48.38±0.73 ^{fB}	1.26±0.045 ^{aA}	4.18±0.033 ^{bB}	0.59±0.018 ^{bA}	0.85±0.013 ^{efB}
CPS (28 d) + <i>shilajit</i> (29–42 d)	32.93±0.70 ^{bA}	58.59±0.39 ^{jB}	1.28±0.051 ^{aA}	4.43±0.046 ^{cB}	0.58±0.015 ^{bA}	0.89±0.009 ^{fB}
CPS (28 d) + <i>G. sylvestre</i> (29–42 d)	32.93±0.83 ^{bA}	46.64±0.36 ^{fB}	1.25±0.065 ^{aA}	4.46±0.056 ^{cB}	0.59±0.018 ^{bA}	0.81±0.009 ^{deB}
CPS (28 d) + <i>A. sativum</i> (29–42 d)	32.64±0.86 ^{bA}	47.22±0.63 ^{fB}	1.26±0.054 ^{aA}	4.16±0.011 ^{bB}	0.59±0.018 ^{bA}	0.79±0.013 ^{cdB}
CPS (28 d) + <i>Spirulina</i> (29–42 d)	32.06±0.58 ^{bA}	51.01±0.29 ^{gB}	1.21±0.089 ^{aA}	4.23±0.013 ^{bB}	0.59±0.018 ^{bA}	0.79±0.013 ^{cdB}
CPS (28 d) + <i>Ginseng</i> (29–42 d)	32.64±0.97 ^{bA}	41.97±0.63 ^{dB}	1.24±0.048 ^{aA}	4.60±0.056 ^{dB}	0.59±0.018 ^{bA}	0.72±0.012 ^{bB}
CPS (28 d) + <i>W. somnifera</i> + <i>A. racemosus</i> + <i>A. paniculata</i> (29–42 d)	32.64±0.97 ^{bA}	39.35±0.59 ^{bcB}	1.24±0.051 ^{aA}	4.70±0.026 ^{dB}	0.58±0.015 ^{bA}	0.73±0.013 ^{bB}
CPS (28 D) + <i>W. somnifera</i> + <i>M. koenigii</i> + <i>A. sativum</i> (29–42 d)	32.35±0.87 ^{bA}	38.18±0.29 ^{bB}	1.20±0.069 ^{aA}	4.65±0.062 ^{dB}	0.58±0.015 ^{bA}	0.72±0.012 ^{bB}
CPS (28 D) + <i>Spirulina</i> + <i>shilajit</i> + <i>G. sylvestre</i> (29–42d)	32.64±0.86 ^{bA}	40.81±0.36 ^{cdB}	1.19±0.084 ^{aA}	4.63±0.074 ^{dB}	0.58±0.015 ^{bA}	0.71±0.009 ^{bB}

Values are mean ± SE (n = 6); one-way ANOVA (SPSS); means with different alphabets as superscripts differ significantly (P<0.05); capital alphabets (horizontal comparison); small alphabets (vertical comparison).

combinations following CPS treatment, the ALT values were lowered as compared to CPS toxic control group, which could be due to their antioxidant and membrane-stabilizing potential. The total protein concentration serves as an indicator for hepatic damage, as liver is the exclusive site of protein synthesis (Kaneko *et al.* 1997). The study revealed a significant decrease in the concentration of total protein in toxic control group, which could be attributed to the hepatic damage due to the oxidative stress and this is substantiated by the levels of antioxidant enzymes and ALT in CPS toxic control group. The improved protein profile in the groups treated with herbs and herbal combinations reveals therapeutic potential of these herbs.

The concentration of creatinine (mg/dl) recorded at the end of fourth week was increased significantly (P < 0.05) in CPS toxic controls (groups 2 to 15). Following treatment with herbs and herbal combinations, there was a significant (P < 0.05) decrease in creatinine levels in groups 3 to 15 as compared to the CPS toxic control group 2 at the end of sixth week (Table 3). Non-protein nitrogenous (NPN) substances such as serum creatinine and BUN are increased only when renal function is below 30% of its original capacity (Kaneko *et al.* 1997). In the present study, the serum creatinine was significantly increased in the toxic control at the end of fourth week as compared to the basal diet control. Treatment with herbs and herbal combinations following discontinuation of CPS in groups 3 to 15 resulted in significant decrease in serum creatinine as compared to the CPS toxic control group, which confirms the protective/

curative role of the herbs in test. The beneficial renal protective actions of herbs in test may be attributed to their antioxidant and free radical scavenging actions that protect protein thiols from deleterious action of CPS on kidney.

In conclusion, the study revealed that chlorpyrifos induces toxicity by means of oxidative stress by either generating excess free radicals or by disturbing antioxidant defenses and supplementation of herbs with antioxidant potential is useful in the management of accidental chlorpyrifos toxicity in broilers.

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