Original Article
Toxicology International, *Jan-April 2016 / Vol-23 / Issue-1, 63-72*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2016/v23/i1/146672

Concentration-dependent inhibition of acetylcholinesterase by organophosphate poisoning in dogs: a biochemical and electrographic study

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Abstract

Organophosphate poisoning (OP) is one of the most common poisonings in developing countries. In this study, twenty-four dogs in four groups of six each were used. Control group bathed with water only, group B with 16% Coumaphos (recommended), groups C and D with times 10 and 20 of 16% Coumaphos, respectively. Blood was collected from cephalic vein for biochemical assays. Electrocardiographic parameters were assessed from a Lead-II electrocardiogram. There was a significant increase ($p<0.05$) in total cholesterol in group B and D compared to the control. LDL-cholesterol decreased significantly ($p<0.05$) in all groups compared to the control. The activity of superoxide dismutase (SOD) reduced ($p<0.05$) significantly across all the groups and even after 36 hours of exposure. However, the activity of the glutathione peroxidase (GPx) was not affected following exposure to OP. The serum reduced glutathione (GSH) fell in a concentration dependent manner in all animals exposed to OP. Coumaphos exposure led to a significant ($p<0.05$) increase in serum MDA in a concentration dependent manner after 36 hours post exposure. The serum nitric oxide (NO) and MPO content increased ($p<0.05$) significantly following exposure to different concentrations of Coumaphos. The activity of Acetyl cholinesterase (AchE) fell significantly from the normal concentration of the OP down to the highest concentration. The activity of serum creatine phosphokinase (CK) increased ($p<0.05$) significantly in groups C and D compared to the control and recommended concentration. Electrocardiographic abnormalities recorded included low-voltage R-waves, first degree heart block, significant increased ($p<0.05$) heart rate (HR) and shortened QT interval compared to the control and recommended concentrations. Taking together, coumaphos poisoning caused an inhibition of AchE and significant potentially fatal arrhythmias via the induction of oxidative stress.

Keywords: Coumaphos, Organophosphate poisoning (OP), electrocardiogram (ECG), oxidative stress, acetylcholinesterase (AchE), cardiotoxicity

Received: 04-01-2016 **Revised:** 27-04-2016 **Accepted:** 30-04-2016

1. Introduction

Complications associated with organophosphate (OP) have been documented elsewhere as cardiorespiratory failure, coma, and even death when management is delayed.[1] Human exposure to OP has been reported with clinical signs of vomiting, drowsiness, limb weakness and fasciculation together with low serum cholinesterase levels.[2, 3] The mechanism of OP toxicity has been elucidated to involve the inhibition of cholinesterase; the enzyme that breaks acetylcholine to choline and acetate. The organophosphate (OP) compounds are effective and efficient in inhibiting acetylcholinesterase (AChE). Respiratory failure 24 hours following OP ingestion has been reported in developed and developing countries.[4, 5] Electrocardiographic abnormalities that have been observed and reported in organophosphate poisoning include heart rhythm, sinus tachycardia, QT interval

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prolongation, ventricular arrhythmias and elevation of cardiac troponin I as a diagnostic marker of cardiac injury.[6-11] Acute myocardial infarction after OP exposure has also been reported.[12]

The use of this silent killer is therefore posing a serious threat to global health care system. Poverty, ignorance, lack of regulatory control measures and availability of OP compounds in the developing countries have contributed immensely to the indiscriminate use of these chemicals. Furthermore, many of these compounds bio-accumulate in the environment finding their way to the water, soil and ultimately food chain with attendant negative effects on human and animal health. Since OP is one of the most common forms of poisoning in the developing countries, we therefore investigated the involvement of oxidative stress, the inhibition of acetylcholinesterase as diagnostic markers and electrocardiographic changes as clinical parameters during organophosphate poisoning in experimental animals.

2. Materials and methods

2.1 Care of animals

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments.[13]

2.2 Experimental animals

Twenty-four healthy Mongrel dogs aged 7-8months (8-10 kg) were used. They were grouped in six dogs per group of four. These were housed in the kennel of the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. Dogs in group A received bath with ordinary water and served as control. Group B animals were bathed with 16% Coumaphos whereas dogs in groups C and D received times 10 and 20 of recommended concentration (16% Coumaphos) respectively.

2.3 Serum collection

Blood was collected from the cephalic vein from each dog into sterile plain tubes and left in a tilted position for about 60 minutes to clot. It was centrifuged at 4, 000 rpm for 10 minutes. Serum was decanted into eppendorf tubes and stored at -4°C until the time of analysis.

Note: Blood was collected 2 hours after bathing. Blood was again collected from Groups C and D 36 hours after bathing when signs of toxicity manifested and tagged CT and DT respectively.

2.4 Chemicals

Acetylcholine iodide, Potassium hydroxide, reduced glutathione (GSH), Trichloroacetic acid, sodium hydroxide, 1, 2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), xylenol orange, 30% hydrogen peroxide (H_2O_2), N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% H_3PO_4], 5, 5-Dithiobis-(2-nitrobenzene) DTNB were purchased from Sigma (St Louis, MO, USA). All other chemicals were of analytical grade. Creatine phosphokinase, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) kits were obtained from Randox kit (UK).

2.5 Biochemical assays

Serum samples were used for biochemical assays. Protein concentration was determined by the method of Gornal *et al.*[14] Reduced GSH was determined at 412 nm using the method described by Jollow *et al.*[15] Glutathione peroxidase activity (GPx) was measured according to Buetler *et al.*[16] Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 7.2 at 30°C as described by Misra & Fridovich with slight modification from our laboratory.[17-19] Briefly, 100 mg of epinephrine was dissolved in 100 ml distilled water and acidified with 0.5 ml concentrated hydrochloric acid. 10 µL of serum sample was added to 2.5 ml 0.05M carbonate buffer (pH 10.2) followed by the addition of 300 µL of 0.3mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome during 1 minute. The malondialdehyde (MDA) level was measured according to the method of Varshney and Kale.[20] Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 105 M-1Cm-1. Hydrogen peroxide (H_2O_2) generation was determined as described.[21]

2.6 Measurement of serum nitric acid (NO) contents

Serum NO was measured as described by Olaleye *et al.*[22] Briefly, 500 µL of serum were mixed with 500 µL Griess reagent [0.1% N- (1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% H_3PO_4]. After incubation at room temperature for 20 minutes, the absorbance at 540 nm was measured by spectrophotometer. The concentration of nitrite in the sample was determined from a sodium nitrite ($NaNO_2$) standard curve and was expressed as µmol /ml.

2.7 Marker of inflammation and cardiac damage

Myeloperoxidase (MPO) as marker of inflammation was measured according to the method of Xia and Zweier.[23] To 2 ml of O-dianisidine mixture (16.7 mg of O-dianisidine, 100 ml of 0.05 M potassium phosphate buffer and 50 µL of diluted H_2O_2) into the cuvette, 70 µL of PMF was added. The increase in absorbance was monitored every 30 secs for 1 minute. The absorbance was read at 450 nm. One unit of MPO activity can be defined as the quantity of enzyme able to convert/degrade 1 µmol of hydrogen peroxide to water in one minute at room temperature. Serum creatine phosphokinase (CPK) was measured with Randox kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom by following the manufacturer's instructions.

2.8 Measurement of acetylcholinesterase activity

Acetylcholinesterase activity was assayed colorimetrically in the serum as reported by Whittaker.[24] The reaction mixture contained 0.10 ml of buffered Ellman's reagent, 5,5'-dithiobis-2-nitrobenzoic acid [DTNB (10 mmol/L), $NaHCO_3$ (17.85 mmol/L) in phosphate buffer 100 mmol/L, pH 7.0] and 0.02 ml of acetylthiocholine iodide solution (acetylcholine iodide, 75 mmol/L) were added to 3 ml phosphate buffer in a cuvette and incubated at 25°C for 10 min. A 0.02 ml of diluted serum sample was then added. The absorbance was monitored at 30 seconds interval for 3 minutes at 410 nm.

2.9 Measurement of lipid profiles

Serum triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL) and high density lipoprotein cholesterol (HDL) were measured with Randox kits (obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom) by following the manufacturer's instructions.

2.10 Electrocardiogram

Standard lead II electrocardiogram was recorded in conscious dogs using a 6/7-lead ECG machine (EDAN VE-1010, Shanghai, China). The machine was calibrated at 10 mm/mV and 50 mm/s paper speed. From the electrocardiogram, parameters such as heart rate, P-wave duration, PR-interval, QRS duration, R-amplitude, QT segment and Bazett's correction of the QT interval were determined. The electrocardiogram also assessed for arrhythmias. The dogs were manually restrained without anaesthesia for the ECG protocol.

2.11 Statistical analysis

All values are expressed as mean±S.D. The test of significance between two groups was estimated by Student's t test. "One-way ANOVA with Dunnett's post test was also performed using GraphPad Prism version 4.00.

3. Results

3.1 The effects of Coumaphos on lipid profiles

The results in Table 1 show that there was a significant ($p<0.05$) increase total cholesterol (TC) in dogs bathed with recommended concentration (16% Coumaphos) and times 20 (of 16% Coumaphos) compared to the control. Similarly, there was an observable significant ($p<0.05$) decrease and increase in TC of dogs bathed with times 10 and 20 (16% Coumaphos) after 36 hours of exposure (Table 1). However, there was no observable difference in serum triglycerides (TG) and HDL-cholesterol in dogs exposed to different concentrations of Coumaphos (Table 1). Furthermore, LDL-cholesterol decreased significantly ($p<0.05$) in all concentrations of Coumaphos compared to the control (Table 1).

3.2 The effects of Coumaphos on enzymic and non-enzymic antioxidant defence system

The activity of superoxide dismutase (SOD) reduced

Table 1. Serum lipid profiles in Coumaphos induced oxidative stress and cardiotoxicity (Mean ± S.D, n=6)

Experimental groups	A	B	C	D	CT	DT
TC (mg/dL)	154.43±10.89	170.83±13.20*	160.80±10.63	166.70±9.37*	154.60±12.64#	171.70±11.29*
TG (mg/dL)	68.00±12.86	70.67±8.12	63.67±9.00	69.17±9.22	65.00±12.76	78.00±18.25
HDL (mg/dL)	49.00±4.80	48.83±3.71	48.33±9.37	51.17±3.97	45.60±4.41	50.29±3.86
LDL (mg/dL)	140.29±17.98	131.00±11.19	101.70±12.31*,#	122.20±13.42*	109.20±19.75*,#	126.40±18.55*

Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C, D, CT and DT respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C, D, CT and DT respectively.
Abbreviations: TC (total cholesterol), TG (Triglycerides), HDL (High density lipoproteins), LDL (Low density lipoproteins)
Dogs in group A received bath with ordinary water and served as control. Group B animals were bathed with normal recommended concentration of OP (16% Coumaphos) whereas dogs in groups C and D exposed to times 10 and 20 of (16% Coumaphos) of recommended concentration.
Note: CT and DT represent Blood collected from Groups C and D respectively 36 hours after exposure when manifesting signs of toxicity.

Table 2. Serum non-enzymic and enzymic antioxidants in coumaphos induced oxidative stress and cardiotoxicity (Mean ± S.D, n=6)

Experimental groups	A	B	C	D	CT	DT
[a]GSH	94.60±6.30	91.20±0.75*	91.20±0.55*	90.00±1.10*	92.20±0.53	95.00±0.87
[b]SOD	2.12±0.07	1.89±0.03*	1.83±0.05*	1.83±0.04*	1.90±0.04*	1.89±0.06*
[c]GPx	14.28±0.94	14.47±0.23	13.67±0.69	13.45±1.57	14.68±1.14	14.40±0.55

Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C, D, CT and DT respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C, D, CT and DT respectively.
[a]GSH (Reduced glutathione; µmole/mg protein), [b]SOD (superoxide dismutase; units/mg protein, [c]GPx (Glutathione peroxidase; (units/mg protein)
Abbreviations: GSH (Reduced glutathione), SOD (Superoxide dismutase), GPx (Glutathione peroxidase).
Dogs in group A received bath with ordinary water and served as control. Group B animals were bathed with normal concentration of OP (16% Coumaphos) whereas dogs in groups C and D received times 10 and 20 of (16% Coumaphos) of recommended concentration.
Note: CT and DT represent Blood collected from Groups C and D 36 hours after bathing when manifesting signs of toxicity.

($p<0.05$) significantly across all the concentrations of the OP and even after 36 hours of exposure (Table 2). However, the activity of the glutathione peroxidase (GPx) was not affected following exposure to 16% Coumaphos. The serum reduced glutathione (GSH) fell significantly in a concentration dependent manner in all animals bathed with the OP (Table 2). However, the observable decrease recorded in the serum GSH 36 hours post exposure was not significantly different from the control and the recommended concentration (Table 2).

3.3 The effects of Coumaphos on electrocardiogram (ECG)

The results obtained from the electrocardiogram (ECG) showed a significant ($p<0.05$) increase in the heart rate (HR) and P-duration in animals bathed with times 10 and 20 (16% Coumaphos) as shown in table 3. There was also a significant ($p<0.05$) decrease in the R-amplitude and QT and QTc (Bazett and Fridericia) values recorded respectively (Tables 3 and 4). Other abnormalities recorded in dogs treated with 20x concentration include supraventricular arrhythmia, atrial fibrillation, first-degree AV-block and low voltage R-wave (Figure 6)

3.4 The effects of Coumaphos on markers of oxidative and nitrosative stress

16% Coumaphos exposure led to a significant ($p<0.05$) increase in serum malondialdehyde (MDA) content in a concentration dependent manner and after 36 hours post Coumaphos exposure (Figure 1). The nitric oxide (NO) content increased ($p<0.05$) significantly following bath with different concentrations of Coumaphos (Figure 2). Furthermore, serum NO decreased and increased significantly 36 hours post exposure in dogs bathed with times 10 and 20 of 16% Coumaphos (Figure 2). The marker of inflammation and cardiac damage was assessed with serum myeloperoxidase (MPO) activity. The serum MPO activity increased ($p<0.05$) significantly and profound toxicity was observable with 10 to 20 folds significant increase in MPO activity 36 hours post exposure (Figure 3).

Table 3. Electrocardiogram (ECG) of coumaphos induced oxidative stress and cardiotoxicity (Mean ± S.D, n=6)

Experimental groups	A	B	C	D
Heart rate (bpm)	136.80±4.86	137.50±7.79	148.67±6.07*,#	145.80±6.71*,#
P wave duration (ms)	44.40±2.694	43.50±6.33	42.67±3.01	49.60 ± 3.48*,#
R amplitude (mv)	0.44880±0.09	0.58650±0.13	0..56±0.04	0.36.±0.12#

Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C and D respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C and D respectively.
Dogs in group A received bath with ordinary water and served as control. Group B animals were bathed with normal concentration of OP (16% Coumaphos) whereas dogs in groups C and D were exposed to times 10 and 20 (16% Coumaphos) of recommended concentration respectively.

Table 4. Electrocardiogram (ECG) of coumaphos induced oxidative stress and cardiotoxicity (Mean ± S.D, n=6)

Experimental groups	A	B	C	D
QRS duration (ms)	49.80±7.82	48.75±1.88	47.33±3.68	50.80±5.29
QT SEG (ms)	206.80±22.37	197.75±9.14	180.67±4.95*	167.00±18.42*,#
QTc Baz (ms)	311.20±32.29	298.25±9.39	284.17±9.62	258.00±24.31*,#
QTc Fried (ms)	270.80±28.47	259.25±8.96	243.50±7.50*	222.40±22.20*,#

Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C and D respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C and D respectively.
Dogs in group A bathed with ordinary water and served as control. Group B animals were bathed with recommended concentration of OP (16% Coumaphos) whereas dogs in groups C and D received times 10 and 20 of recommended concentration (16% Coumaphos) respectively.

Table 5. Mean Blood pressure of dogs exposed to varying concentrations of Coumaphos

Mean Blood pressure +SD (mmHg)	Group A Systole	Group A Diastole	Group B Systole	Group B Diastole	Group C Systole	Group C Diastole	Group D Systole	Group D Diastole
	123±8.51	81.55±10.16	115.68±12.67	84±25.02	121.16±19.94	80.5±15.57	124.83±23.77	72.5± 9.31

Syst = Sysolic blood pressure
Dia= Diastolic blood pressure

3.5 The effects of Coumaphos on acetylcholinesterase (AchE) activity

The activity of AchE declined significantly from the normal concentration of the OP down to the highest concentration (Figure 4). Similarly, serum AchE activity declined further after 36 hours post exposure (Figure 4).

3.6 The effects of Coumaphos on serum creatine phosphokinase (CK)

The activity of serum creatine phosphokinase (CK) increased ($p<0.05$) significantly in dogs that were bathed with times 10 and 20 of 16% Coumaphos compared to the control and the animals that received the recommended concentration (Figure 5).

4. Discussion and conclusion

Exposure to OP is known to cause accumulation of acetylcholine in the body with resultant overstimulation of nicotinic expression at the neuromuscular junction. Furthermore, in both vertebrate and invertebrate organisms, the enzyme responsible for the removal of AchE from the synaptic cleft through hydrolysis is inhibited by OP.[25-27] However, at least 1 million incidences of OP poisonings per year is reported globally.[28, 29]

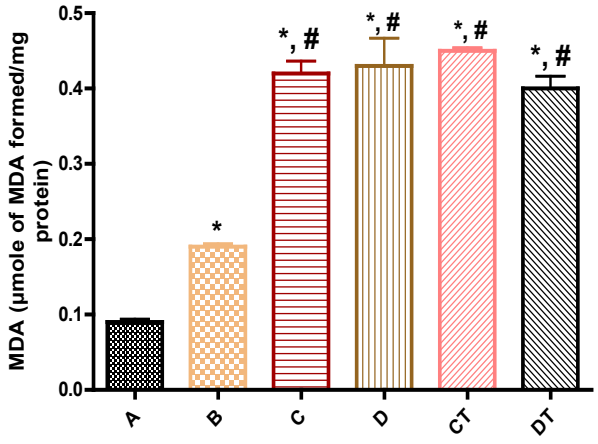


Figure 1. The effect of Coumaphos on serum malondialdehyde content. Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C, D, CT and DT respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C, D, CT and DT respectively.

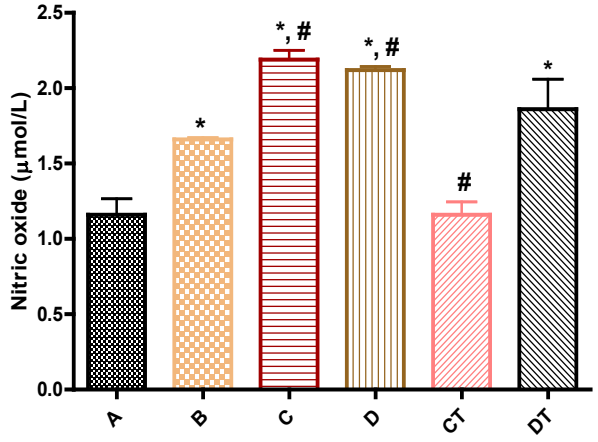


Figure 2. The effect of Coumaphos on serum nitric oxide (NO) content. Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C, D, CT and DT respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C, D, CT and DT respectively.

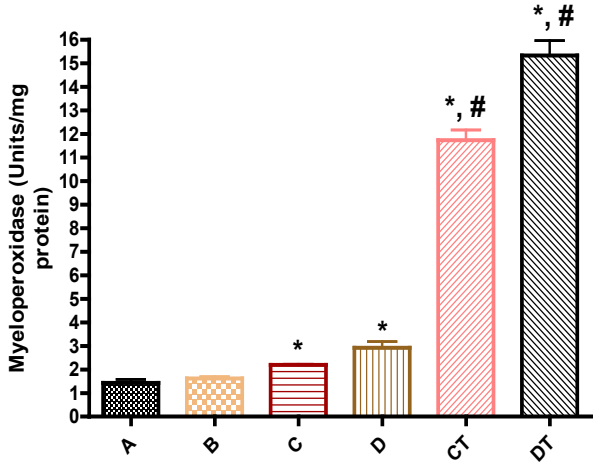


Figure 3. The effect of Coumaphos on serum myeloperoxidase (MPO) activity. Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C, D, CT and DT respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C, D, CT and DT respectively.

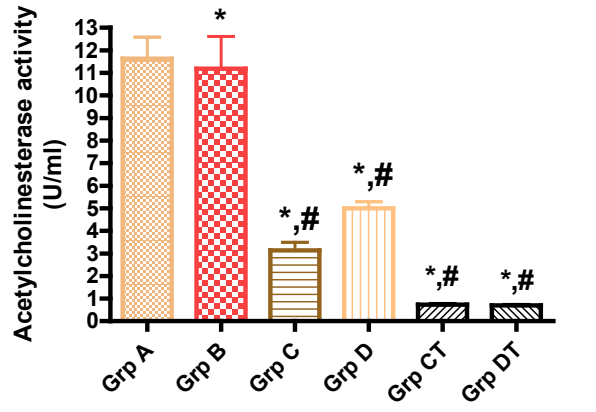


Figure 4. The activity of acetylcholinesterase (AchE) on different concentrations of organophosphate bath. Asterisks (*) indicate significant difference ($p<0.05$) when Group A was compared with Groups B, C, D, CT and DT respectively whereas (#) indicate significant difference ($p<0.05$) when Group B was compared with C, D, CT and DT respectively.

In this study, we observed inhibition of AchE in a concentration dependent manner with the highest concentration having the most profound effect of AchE. At the 36 hours of post OP exposure, about 95% of the AchE activity had been inhibited by the highest concentration of the exposed OP. Substantial evidence has shown that oxidative stress contributes significantly to OP toxicity in both chronic and sub chronic conditions.[30-31] Therefore, our results are consistent with the previous findings of the inhibitory effects of OP on AchE activity.

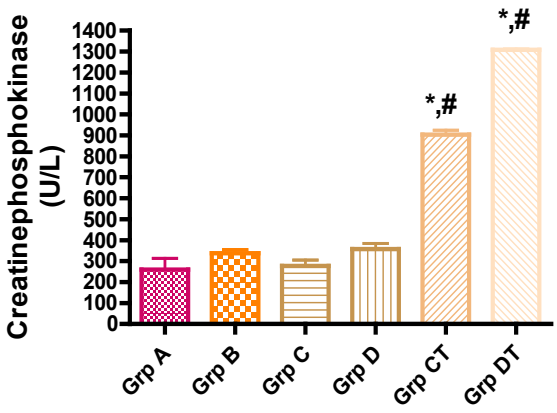
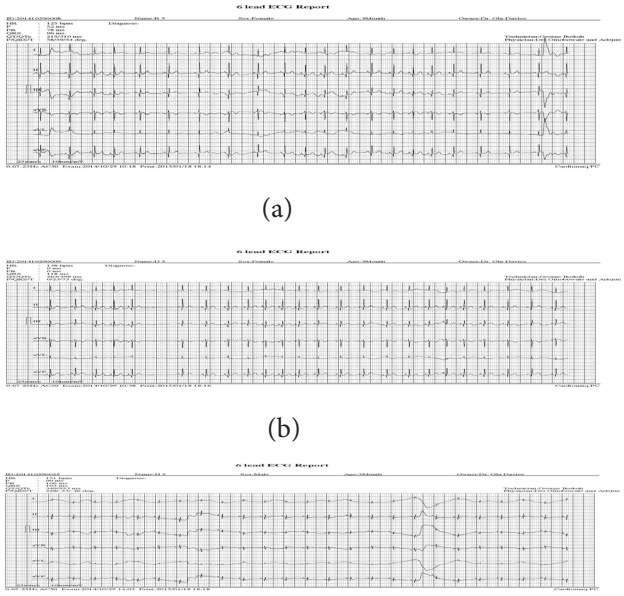


Figure 5. The activity of Creatine phosphokinase (CPK) on different concentrations of organophosphate bath. Asterisks (*) indicate significant difference ($p<0.05$) when compared Group A with Groups B, C, D, CT and DT respectively whereas (#) indicates significant difference ($p<0.05$) when compared Group B with C, D, CT and DT respectively.

In this study, increase in serum MPO observed may also contribute significantly to oxidative stress via oxidation of LDL-cholesterol. The oxidation of low-density lipoprotein (LDL) by MPO has been shown to play a crucial role in the initiation and progression of atherosclerosis.[32-36] Elevated levels of the heme enzyme myeloperoxidase (MPO) are associated with adverse cardiovascular outcomes.[37, 38] It has been reported that Dichlorvos markedly lowered both serum low density lipoprotein (LDL-C), cholesterol contents, triglycerides as well as esterified fatty acids, whereas high density lipoprotein (HDL-C) concentration increased and very low density lipoprotein (VLDL) remained and free fatty acid concentrations were unaffected.[39] MPO predominantly catalyses formation of the oxidants hypochlorous acid (HOCl) from Cl^-, and hydrogen peroxide as a substrate.

The superoxide dismutase (SOD) catalyses the conversion of superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2), which serves as a substrate for MPO. In our study, SOD activity was significantly inhibited by the OP, causing accumulation of superoxide anion radicals. The superoxide anion radicals may combine with nitric oxide (NO) to form peroxynitrite ($ONOO^-$) which has more damaging effects than the NO or O_2^- alone. This interaction between NO and (O_2^-) reduces the bioavailability of NO thereby attenuating the beneficial effect of NO on vascular system.



(a)

(b)

(c)

Figure 6. a. Electrocardiogram showing arrhythmia in dogs treated with 20x organophosphate
b. 10 AV block (single arrow) and supraventricular tachycardia double arrows in dogs treated with 20x organophosphate
c. Low-voltage R-wave in dogs treated with 20x organophosphate

The increase in the generation of reactive oxygen species (ROS) /free radicals and reduction in antioxidant defence system is known as oxidative stress. In this experiment, OP administration reduced serum GSH and nitric oxide (NO) contents, serum superoxide dismutase (SOD) activity was also significantly dropped in a concentration-dependent manner. However, it was noted that the activity of glutathione peroxidase (GPx) was unaffected by the exposure to OP. The GPx functions to decompose H_2O_2 to water (H_2O) and O_2 as the second line of defence. Pronounced oxidative stress was observed in

higher OP concentration exposure than the recommended dose. Elevated serum MPO and MDA are major players in contributing to inflammation, oxidative stress and free radical generation with ultimate lipid peroxidation and inactivation of antioxidant defence system. Altogether, these may also contribute to cardiovascular dysfunction via death of cardiomyocytes due to excessive generation of ROS by membrane peroxidation and the action of peroxynitrite which is a cytotoxic molecule.

The significant increase in malondialdehyde (MDA), nitric oxide (NO) and decrease in the levels of glutathione (GSH) in the lung, liver, and kidney tissues in OP poisoning has been reported.[25] Furthermore, elevated activities of malondialdehyde (MDA) and reduction in superoxide dismutase in the plasma of experimental animal exposed to Dichlorvos poisoning have been reported[40]. Mishra and Srivastava reported that decrease in the levels of GSH and the corresponding increase in the levels of GSSG, decreasing the GSH/GSSG ratio in OP poisoning is an index of on-going oxidative stress.[41] The inhibition of AchE has been associated with increased erythrocyte MDA level, SOD, CAT and GPx activities suggestive of an adaptive measure against pesticide accumulation.[42] Shafiee *et al.* reported that the activities of SOD, CAT, and GPx did not change significantly in acute Malathion poisoning and that $MgSO_4$ had no considerable improvement on the oxidative stress parameters.[43] In another experiment, it was reported that serum xanthine oxidase (XO) and malondialdehyde (MDA) activities were higher and the serum superoxide dismutase (SOD), paraoxonase-1 (PON1), butyrylcholinesterase (BChE) activities were lower in acute organophosphorus pesticide poisoning (AOPP) as a mechanism of toxicity.[40]

Monitoring of serum CTnT and CK-MB levels in OP exposure is an important diagnostic tool for cardiac damage.[44] Atale *et al.* reported the contribution of oxidative stress in malathion-induced death of cardiac myocytes.[45] OP can result in tissue hypoxia which results in a reduction of heart contractility and cell damage.[46] Aghabiklooei *et al.* reported cardiac arrest and arrhythmia with significantly higher levels of systolic blood pressure as one of the cardiac complications in OP including.[47] The increase in serum activity of creatine phosphokinase observed in this present study might also contribute to cardiotoxicity following the OP exposure.

Our results showed that OP caused a significant increase in total cholesterol (TC) and triglycerides (TG) but reduced low density lipoprotein (LDL)-cholesterol while the serum content of high density lipoprotein (HDL)-cholesterol was not significantly affected. We proposed that the oxidation of (LDL)-cholesterol might contribute to the reduction in serum (LDL)-cholesterol. Oxidation of (LDL) has been shown to contribute to endothelial cell (EC) dysfunction thereby leading to atherosclerotic development and progression.[48] This phenomenon has also been reported to impair NO generation and bioavailability.[48] Recently, Aydin *et al.* reported the association between oxidized-LDL and inflammatory markers in ST elevation during myocardial infarction.[49]

The elevations in plasma lipid levels and the inflammatory markers including MPO have been reported in OP poisoning.[50] Myeloperoxidase (MPO) catalyses the formation of a wide variety of oxidants, including hypochlorous acid (HOCl), and contributes to cardiovascular disease progression.[51] Some recent data also suggest that higher MPO concentration may predict the development of coronary events in apparently healthy people with evidence of subclinical atherosclerotic plaque.[52]

The ECG changes included increase in heart rate, P wave duration, PR interval and QRS duration. A decrease in R-wave amplitude was also observed in dogs exposed to OP. Contrary to some other studies in which a prolongation of the shortened QT interval followed by the polymorphic ventricular tachycardia, torsades de pointes, was reported (Karki *et al*., 2004, Anand *et al*., 2009). [53, 54] We observed a dose-dependent decrease in the QT and QTc values in dogs exposed to OP. Several mechanisms have been identified in OP-induced myocardial damage and these include sympathetic and parasympathetic overactivity, acidosis, hypoxaemia and electrolyte derangement (Karki *et al*., 2004).[53] Hyperkalemia and acidosis have both been incriminated as secondary causes of short QT interval and these have been reported as complications in OP toxicity.[55, 56] We speculate that the shortened QT interval observed in this study might have arisen from complications of OP such as hyperkalemia and acidosis.[57, 58] Combining all, caution should be taken in the use of organophosphate insectide on pets as toxicity may progress from tachycardia to cardiac arrest and death.

5. Conflict of Interest

None Declared

6. References

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