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Terbufos-sulfone exacerbates cardiac lesions in diabetic rats: a sub-acute toxicity study

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Organophosphorus compounds (OPCs) have a wide range of applications, from agriculture to warfare. Exposure to these brings forward a varied kind of health issues globally. Terbufos is one of the leading OPCs used worldwide. The present study investigates the cardiac effect of no observable dose of a metabolite of terbufos, terbufos-sulfone (TS), under non-diabetic and streptozotocin-induced diabetic condition. One hundred nanomoles per rat (1/20 of LD_{50}) was administered intraperitoneally to adult male Wister rats daily for fifteen days. The left ventricle was collected for ultrastructural changes by transmission electron microscopy. The blood samples were collected for biochemical tests including RBC acetylcholinesterase, creatinine kinase (CK), lactate dehydrogenase (LDH), cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, ALT, AST, and GGT. The study revealed about 10 % inhibition of RBC-AChE in two weeks of TS treatment in non-diabetic rats whereas RBC-AChE activity was significantly decreased in diabetic TS treated rats. CK, LDH, and triglycerides were significantly higher in diabetic TS treated rats. Electron microscopy of the heart showed derangement and lesions of the mitochondria of cardiomyocytes in the TS treated groups. The present study concludes that a non-lethal dose of TS causes cardiac lesions which exacerbate under diabetic condition. Biochemical tests confirmed the ultrastructural changes. It is concluded that a non-lethal dose of TS may be a risk factor for a cardiovascular disease, which may be fatal under diabetic condition.

KEY WORDS: *acetylcholinesterase; cardiac lesion; cardiotoxicity; diabetes mellitus; lipid profile; myocyte injury markers; organophosphates*

Organophosphorus compounds (OPCs) are used in agriculture as insecticides, pesticides, helminthicides, acaricides, nematocides, and herbicides. Furthermore, they are used as a warfare agent and in controlling disease vectors for public health purposes. Over the years, the use of OPCs has increased dramatically with new applications still being developed. To date, there are over 100 different OPCs available, with a similar generalised chemical structure. Wide accessibility, along with indiscriminate and inadvertent use or exposure, may result in intentional poisoning or unintentional toxicity and it therefore poses a risk to the users and workers involved in their production and application. The acute toxicity of different OPCs ranges from that of an extremely toxic nerve gas to less than that of table salt. Toxicity of all OPCs is due to irreversible inhibition of the neurotransmitter enzyme acetylcholinesterase (AChE).

A significant association between OPC exposure and toxic effects on different organs has been reported in numerous epidemiological studies (1-3). Among several toxic manifestations, cardiotoxicity is believed to be one of the unrecognised risk factors of OPCs. The effects of OPCs on the cardiovascular system are speculative or based on individual clinical case reports (4-7). The risk of exposure to sub-acute low levels of OPCs for the heart has been scarcely addressed and somehow did not garner much attention from researchers. Albeit, only electrophysiological alterations have been reported but morphological maladies have barely been touched upon. The nature of their toxic effects is variable ranging from transient to permanent (8). It is noteworthy that cardiotoxicity with OPCs may be independent of AChE inhibition. For instance, dimethoate toxicity in guinea pigs has been reported to cause cardiac failure without any effect on AChE inhibition (9). Likewise, studies also suggest that toxicity of OPCs causes mitochondrial dysfunction mediating a non-cholinergic mechanism (10).

Simultaneously, cardiovascular diseases are increasing significantly globally, as is the prevalence and incidences of diabetes mellitus (DM), one of the important risk factors for cardiovascular diseases. Taken all together, diabetes and cardiovascular diseases are predicted to increase even further over the next decades. Hence, it is reasonable to

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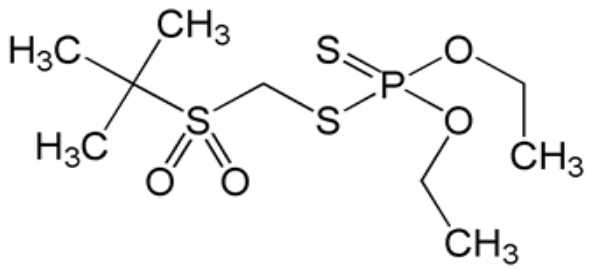

Figure 1 *Structural formula and some chemical properties of terbufos- sulfone*
Molecular weight: 320.43 g/mol; empirical formula: $C_9H_{21}O_4PS_3$
Log P: 2.64; C Log P: 2.58; XLogP3: 2.5
Chemical name/synonyms: Phosphorodithioic acid, S-((1,1-dimethylethyl)sulfonylmethyl) O,O-diethyl ester, Dithiophosphoric acid O,O-diethyl S-[(tert-butylsulfonyl)methyl] ester; S-[(tert-butylsulfonyl)Methyl] O,O-diethyl phosphorodithioate, S-tert-butylthiomethyl O,O-diethyl phosphorodithioate

predict that exposure to OPCs may have a significant impact on the manifestations of cardiotoxicity in patients with diabetes and prediabetes much faster than in normal healthy individuals. It may be noted that the effect of OPCs under diabetic pathological condition is scarcely reported (11, 12).

Therefore, the present study was carried out to investigate the effect of sub-acute exposure to terbufos sulfone (TS), a highly toxic OPC as per the World Health Organization (WHO) classification of insecticides and pesticides. Terbufos is registered in more than 20 countries; however, its usage has been banned in Europe since 2006. The present study was designed to demonstrate whether TS relevant to no-observable adverse effect level (NOAEL) dose could exacerbate cardiac injury in non-diabetic and streptozotocin-induced (STZ) diabetic rats. This study will have significant implications because sub-acute exposure to TS or to any other OPC has inadequately been addressed in the literature.

MATERIALS AND METHODS

Experimental animals

The original stock of Wistar rats were purchased from Harlan Laboratories (Harlan Laboratories, Oxon, England). The animals used in the actual experiments were bred at our own animal facility from the original stock. Male adult rats, weighing 200-250 g were used in the present study. The animals were housed in polypropylene cages (43x22.5x20.5 cm; six rats per cage) in climate and access controlled rooms maintained under standard laboratory conditions (temperature: 23±1 °C; humidity: 50±4 %; day/night cycle: 12h/12h). The rodent pellet diet was procured from the Emirates Feed Factory, Abu Dhabi, UAE and water was available ad libitum. All the experimental procedures were in strict compliance with the protocol approved by the institutional animal ethics committee for the care and use of laboratory animals (Ethical Approval #A2-13).

Induction of experimental diabetes mellitus

To induce type I diabetes, after an overnight fast, the rats were administered a single intraperitoneal (*i.p.*) injection of streptozotocin (60 mg kg^{-1} body weight) prepared freshly in citrate buffer (0.1 mol L^{-1} pH 4.5) solution. The induction of diabetes in rats was confirmed measuring random blood glucose levels using a One-Touch Glucometer (Lifescan Inc., Milpitas, CA, USA). Animals with blood glucose above 235 mg dL^{-1} were considered diabetic and were used further for the experiments.

Experimental design

There were four experimental groups, each containing 6 rats. Terbufos sulfone (TS) was administered *i.p.* at a dose of 100 nmol per rat (1/20 of LD_{50}) following our previous laboratory studies (13). The animals were treated daily for two weeks.
Group 1 (G1): Non-diabetic control (saline-treated non-diabetic rats)
Group 2 (G2): Diabetic control group (No treatment with TS)
Group 3 (G3): Non-diabetic group, treated with 100 nmol of TS
Group 4 (G4): Diabetic group, treated with 100 nmol of TS

Chemicals

The TS stock solution (100 mmol L^{-1}) was prepared in dry acetone. The working solution for the *i.p.* injection was prepared extempore by diluting the stock solution with physiological saline. The chemicals, TS and streptozotocin, were purchased from Sigma-Aldrich Chemie (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Blood glucose measurement

Random blood glucose was checked on day 8 of each week before the injection of TS. Blood samples were collected from the tail vein.

RBC-AChE activity

The blood sample for RBC-AChE measurement was collected from the tail vein of the animal. The RBC-AChE activity was measured in diluted whole blood samples in the presence of a selective butyrylcholinesterase inhibitor, ethoproprazine, as described previously (14).

Blood collection for biochemical tests

The blood samples were collected from rats before decapitation and were subsequently centrifuged at 2555 *g* for 10 minutes. The obtained serum was then stored frozen at -80 °C. The markers for cardiac function viz. myocyte

injury marker enzymes such as lactate dehydrogenase (LDH) and creatinine kinase (CK), for lipid profile such as cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), and liver function enzymes such as alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) were measured by an autoanalyser (COBAS INTEGRA 400 PLUS from Roche Diagnostics, Germany).

Transmission electron microscopy

The left ventricle from the heart of animals of each experimental group was cut into small pieces and fixed for 5 hours in 2.5 % glutaraldehyde in Karnovsky's solution (15). The samples were washed overnight in cacodylate buffer (40 °C) and post fixed with 1 % osmium tetroxide for 1 hour. The samples were further washed six times in cacodylate buffer and dehydrated in ethyl alcohol at graded concentrations. They were later treated with propylene oxide; two changes of 15 minutes and were then immersed in propylene oxide and resin (1:1) for 1 hour followed by pure resin overnight. Afterwards, the samples were embedded and polymerised at 60 °C for 24 hours. Semi and ultrathin sections were cut using a diatome knife procured from Agar Scientific, Essex, England. The semi thin sections were placed on glass slides and stained with toluidine blue. The ultrathin sections were mounted on 3.05 mm 200 mesh copper grids and contrast stained first with saturated aqueous uranyl acetate for 30 min and then with Renold's lead citrate for 5 minutes. The ultrathin sections were viewed under a Philips CM10 transmission electron microscope (Eindhoven, The Netherlands).

Statistical analysis

Statistical analysis was performed for all the parameters obtained from the experimental groups using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). To determine the significance in comparison to control values, the Mann-Whitney order rank test was used. The limit of statistical significance was set at $p<0.05$.

RESULTS

Body weight

Figure 2 shows the change in body weight of experimental animals after the first and second weeks of saline (controls) and TS treatment. The non-diabetic animals increased body weight over time irrespective of whether treated with saline or TS. The diabetic animal groups decreased their body weight. This decrease was more conspicuous in the TS treated than in the saline treated group. However, the change was not found statistically significant compared to the pre-treatment or first-week body weight.

Blood glucose

Random blood glucose levels across G1 to G4, over a period of two weeks, are depicted in Figure 3. The average two-week blood glucose levels in G1 and G2 (control groups) were 100±9 mg dL^{-1} and 473±72 mg dL^{-1}, respectively. The average two-week blood glucose level in G3 was 100±8 mg dL^{-1}. The mean blood glucose level for diabetic rats treated with TS100 (G4) was 511±29 mg dL^{-1} over the two-week period. This increase was statistically significant ($p<0.05$) compared to the pre-treatment value (432±29 mg dL^{-1}).

RBC-Acetylcholinesterase

The activity of RBC-AChE enzyme was determined as a percentage of baseline and is depicted in Figure 4. The

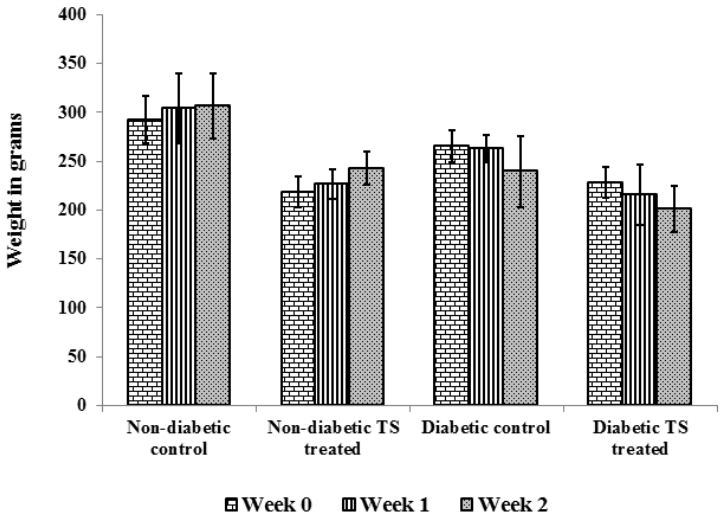

Figure 2 *Changes in body weight of different experimental groups*

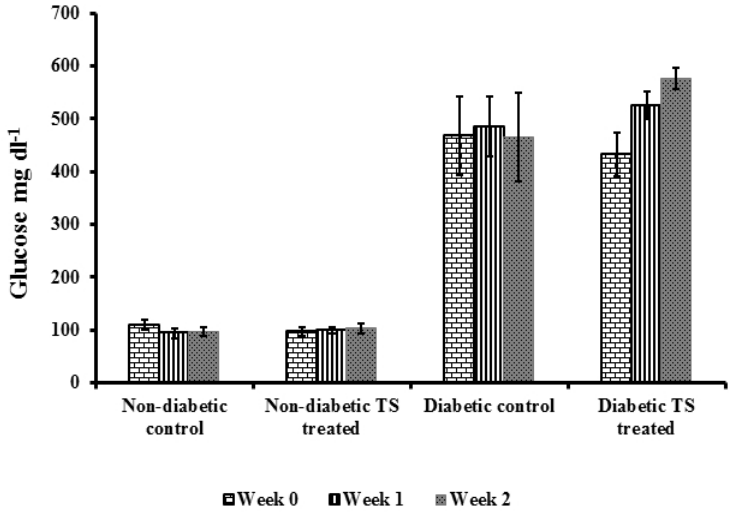

Figure 3 *Changes in blood glucose level over weeks in different experimental groups*

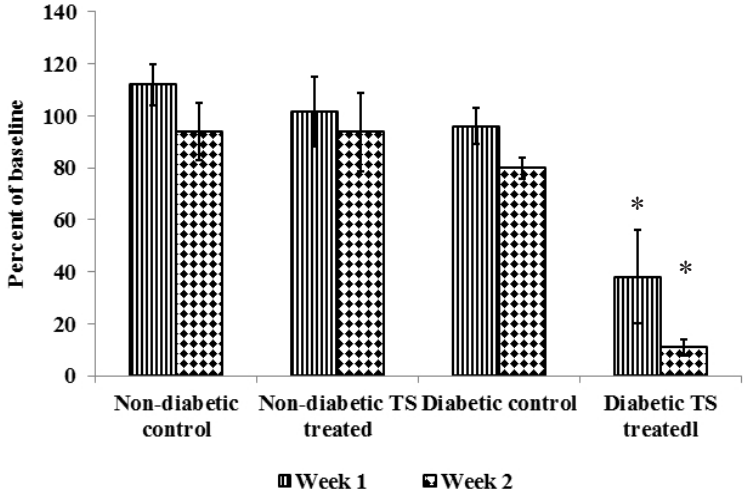

Figure 4 *Changes in RBC-AChE over weeks in control and TS treated groups*
**Statistically significant at* p≤*0.05; Mann-Whitney order rank test*

Table 1 *Serum levels of myocyte injury markers; CK and LDH in different experimental groups. *p≤0.05; Mann-Whitney order rank test.*

	Groups	Non-diabetic control	Non-diabetic TS treated	Diabetic control	Diabetic TS treated
LDH (IU/L)	Mean±SD 95% CI	937±365 (484-1390)	1354±559 (660-2049)	989±301 (615-1362)	1910±179* (1688-2132)
CK (IU/L)	Mean±SD 95% CI	343±117 (198-487)	644±226* (363-924)	404±140 (230-578)	871±88* (762-980)

intraperitoneal injection of 100 nmol of TS administered to non-diabetic rats (G2) did not produce a significant change in the RBC-AChE activity. The same dose in STZ-induced diabetes (G4) produced a sharp and statistically significant decrease (p<0.05) in week 1 (38 %±18) and week 2 (11 %±3). The diabetic control group (G3) showed a moderate decrease which was statistically not significant.

The ultrastructure of the heart (transmission electron microscopy)

The ultrastructure of the heart by transmission electron microscopy is represented in Figure 5. A representative image of the ultrastructure of the heart of non-diabetic control rats is represented in Figure 5A. Myofibrils were observed intact with thick and definite architecture and linearly arranged mitochondria. Figure 5C shows the ultrastructure of the heart of non-diabetic TS treated rats. A distorted architecture of myofibrils, large inter-myofibrillar spaces and swollen mitochondria are conspicuous and are the effect of TS treatment. Figure 5B shows the diabetic control group. Thin myofibril, deranged mitochondria, and inter-myofibrillar spaces are a characteristic pathological effect of diabesity. The mitochondria (m) of the cardio myocytes of the heart of diabetic rats treated with TS (Figure 5D) contain deranged mitochondria (m) and large muscle inter-fibre spaces. Mitochondrial lesions were more conspicuous in diabetic rats (Figure 5D) when compared to those of the non-diabetic and diabetic control groups (Figure 5A & B), and TS treated non-diabetic (Figure 5C) rats. A damaged architecture of myofibrils was clearly observable between the TS treated and non-treated groups though diabetes itself damages the heart structure.

CK and LDH

Table 1 shows the creatine kinase (CK) and lactate dehydrogenase (LDH) levels in the control and TS treated groups after two weeks of treatment. CK and LDH levels were found significantly elevated (p<0.05) in the diabetic TS treated groups, indicating a cardiac manifestation of TS treatment.

Lipid profile

Table 2 shows the measurement of cholesterol, HDL, LDL, triglycerides, ALT, AST, and GGT in the serum of the treated and non-treated groups. The non-diabetic TS treated groups did not show statistical significance (Table 3) in the alteration of the tested parameters when compared to their corresponding non-diabetic saline control except for AST, which decreased significantly. However, diabetic rats treated with TS showed marked and statistically significant (p<0.05) changes in all the tested parameters except for ALT, which was found to be non-significantly different than in the diabetic saline control group. Among all the parameters of the lipid profile, triglycerides markedly surged in the diabetic TS treated group (mean±SD: 1.61±0.79) compared to the diabetic and non-diabetic controls (mean±SD: 0.60±0.12, 0.90±0.40 respectively).

DISCUSSION

The present study demonstrates that even a relatively non-lethal dose of terbufos sulfone and sub-acute exposure to it causes toxic effects to the heart of non-diabetic and STZ-induced diabetic rats. Terbufos belongs to an extremely toxic group of OPCs as per the WHO's classification of hazardous chemicals (16) and its metabolites are also

Table 2 *Blood lipid profile in different experimental groups. *p≤0.05; Mann-Whitney order rank test. *Statistically significant when compared to non-diabetic saline control (p<0.05). **Statistically significant when compared to diabetic saline control (p<0.05)*

Experimental groups		Non-diabetic saline control	Non-diabetic TS treated	Diabetic saline control	Diabetic TS treated
Cholesterol (mmol L^{-1})	Mean±SD	1.26±0.23	1.22±0.17	1.60±0.27	1.20±0.21**
	95 % CI	0.97-1.55	1.04-1.40	1.32-1.88	0.98-1.42
HDL (mmol L^{-1})	Mean±SD	0.84±0.08	0.77±0.31	0.96±0.13	0.58±0.26**
	95 % CI	0.74-0.94	0.44-1.09	0.82-1.09	0.32-0.86
LDL (mmol L^{-1})	Mean±SD	0.11±0.02	0.16±0.06	0.30±0.10	0.07±0.04**
	95 % CI	0.08-0.14	0.09-0.23	0.19-0.41	0.03-0.11
Triglyceride (mmol L^{-1})	Mean±SD	0.90±0.40	0.79±0.08	0.60±0.12	1.61±0.79**
	95 % CI	0.39-1.40	0.71-0.87	0.48-0.72	0.78-2.43
ALT (IU L^{-1})	Mean±SD	40.20±4.44	37.67±13.90	135.83±62.97	96.00±34.29
	95 % CI	34.69-45.71	23.08-52.25	69.75-201.92	60.02-131.98
AST (IU L^{-1})	Mean±SD	97.60±11.63	159.83-31.49*	711.17±258.65	224.50±71.71**
	95 % CI	83.16-112.04	126.78-192.88	484.46-937.84	149.24-299.76
GGT (IU L^{-1})	Mean±SD	5.00±0.71	5.50±0.55	1.50±0.55	4.67±2.50**
	95 % CI	4.12-5.88	4.93-6.07	0.93-2.07	2.04-7.29

Table 3 *p-values of multiple comparison of Table 2.*

		Non-diabetics saline control	Non-diabetic TS treated	Diabetics saline control	Diabetic TS treated
Cholesterol	Non-diabetic saline control	--	0.925	0.701	0.062
	Non-diabetic TS treated	0.925	--	0.019	0.019
	Diabetics saline control	0.701	0.019	--	0.023
	Diabetic TS treated	0.062	0.019	0.023	--
HDL	Non-diabetics saline control	--	0.715	0.008	0.144
	Non-diabetic TS treated	0.715	--	0.199	0.199
	Diabetics saline control	0.008	0.199	--	0.004
	Diabetic TS treated	0.144	0.199	0.004	--
LDL	Non-diabetics saline control	--	0.142	0.117	0.006
	Non-diabetic TS treated	0.142	--	0.020	0.020
	Diabetics saline control	0.117	0.020	--	0.004
	Diabetic TS treated	0.006	0.020	0.004	--
Triglyceride	Non-diabetics saline control	--	0.715	0.045	0.068
	Non-diabetic TS treated	0.715	--	0.016	0.016
	Diabetics saline control	0.045	0.016	--	0.006
	Diabetic TS treated	0.068	0.016	0.006	--
ALT	Non-diabetics saline control	--	0.647	0.010	0.035
	Non-diabetic TS treated	0.647	--	0.030	0.030
	Diabetics saline control	0.010	0.030	--	0.150
	Diabetic TS treated	0.035	0.030	0.150	--
AST	Non-diabetics saline control	--	0.011	0.006	0.006
	Non-diabetic TS treated	0.011	--	0.006	0.006
	Diabetics saline control	0.006	0.006	--	0.016
	Diabetic TS treated	0.006	0.006	0.016	--
GGT	Non-diabetics saline control	--	0.219	0.402	0.005
	Non-diabetic TS treated	0.219	--	0.003	0.003
	Diabetics saline control	0.402	0.003	--	0.007
	Diabetic TS treated	0.005	0.003	0.007	--

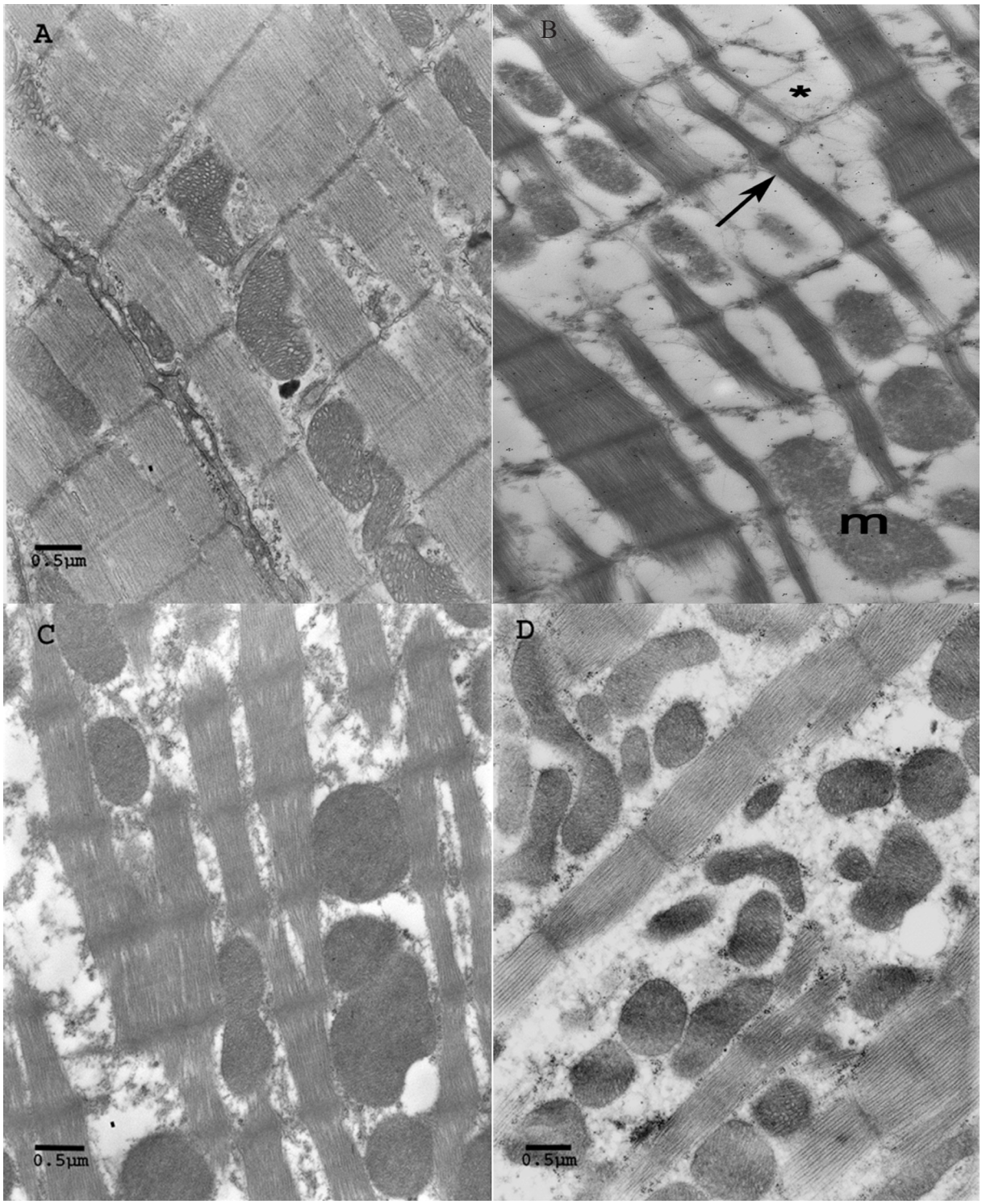

Figure 5 *Electron microscopic changes in the heart tissue. Scale bar=0.5 µm.*
A=non-diabetic control; B=diabetic control; C=non-diabetic TS treated, and D=diabetic rat treated with TS; m=mitochondrion

persistent in the environment (http://www.abcbirds.org/abcprograms/policy/toxins/profiles/terbufos.html). TS is the active metabolite of terbufos, the fourth most frequently used pesticide in the USA, reported for its use in fish kill incidents, which is also suggestive of its presence in the food chain (17). To the best of our knowledge and based on the available literature, no data on the cardiovascular condition following sub-acute exposure to this compound has been reported. Hundreds of different OPCs share a common mechanism of action but are toxicologically different one from the other, ranging from deadly poison nerve agents to those with virtually no toxic potential (18). According to the website Scorecard (19), the list of more than 500 cardiovascular toxins causing different types of cardiovascular complications contains only few OPCs. This list reflects the paucity of studies with OPCs. Asymptomatic low-level exposure to OPCs has been reported to cause various health effects including cardiac lesions (8, 20, 21). Different individual toxicokinetics of OPCs necessitates investigating the effect on the heart under normal and diabetic conditions (22). The cardiovascular manifestation of OPC poisoning is mainly attributed to acute poisoning (23–27), which comprises bradycardia or tachycardia or impaired heart rate. There are reports showing an increased level of creatine kinases and lactate dehydrogenases, besides myocardial necrosis (28). The results of the present study reveal that TS causes a leakage of myocyte injury marker enzymes into the serum, as evidenced by the elevated levels of serum CK and LDH. The structural change in the heart was further correlated with the myocyte injury marker enzymes' leakage from cardiomyocytes to the serum. Abdou et al. (29) reported increased LDH and histologically large areas of degenerating muscle fibres with an evident loss of transverse striations and wide inter-fascicular spaces when treated with diazinon, an OPC. Velmurugan (30) used 1/50th of LD_{50} dosage of monochrotophos against Wister rats and found elevated LDH and CK-MB in the blood plasma along with signs of nonspecific inflammatory changes and oedema between muscle fibres of the heart.

The present study also investigated ultrastructural changes following the administration of a non-lethal dose of TS to non-diabetic and STZ-induced diabetic rats. Ultrastructural changes after acute OPC poisoning have been reported by numerous clinical case studies (31–34). We also observed similar cardiac lesions in diabetic rats with sub-acute and apparently non-lethal exposure to TS. The injurious effect on the heart in diabetic rats was further confirmed by low HDL and high triglycerides. These observations are in line with other previous studies which report that exposure to repeated doses of profenfos, an OPC, significantly increased LDH and CK enzymes (35). The probable reason for this leakage could be cell death as Razavi et al. (36) reported with sub-chronic exposure to diazinon, an organophosphate. In the present study, we also observed that TS administration led to a structural impairment of the mitochondrion in non-diabetic TS treated rats, but the lipid profile was not significantly affected. This observation was supported by a previous study wherein a higher but non-lethal dose of 20 mg kg^{-1} body weight of diazinon administered for four weeks did not reveal a change in the cholesterol and triglyceride levels (37).

OPC elicits its toxicity by inhibiting acetylcholinesterase. The severity of poisoning depends upon the extent of AChE inhibition and consequent cholinergic crises. This may be correlated with the observation that inhibition of AChE was severe in diabetic rats at the end of the second week and it resembled acute toxicity. The effect of anticholinesterases on the heart is mainly attributed to acute toxicity, especially the delayed or late phase effect (5). Different mechanisms and pathways have been reported to be involved in the cardiotoxicity of acute OPC poisoning. One of the studies suggests that morphologic damage may be instigated by uninterrupted vagus nerve stimulation of the accumulated acetylcholine (5). Herein, the magnitude of myocardial damage correlated with the duration of exposure. It has also been found that cardiac damage may be independent of the extent of AChE inhibition contrary to the high level inhibition of AChE in acute poisoning. Since acetylcholine receptors have a high binding capacity for calcium ions (38), the disorder of cellular morphology may be ascribed to a massive influx of Ca^{2+} into the cytoplasm. The results also signify that TS even after two weeks has a potential to raise triglyceride levels, cause myocyte injury and subsequent myocardial injury. Interestingly, the changes observed were irrespective and independent of the degree of AChE inhibition, as it was observed to be high in diabetes and low in non-diabetic rats. This suggests that occupational exposure to TS, even at low doses, may pose a risk in those who are prone to develop diabetes and can worsen cardio-toxic manifestations in diabetics. In addition, ultra-structural studies may be a vital tool in identifying the non-lethal dose associated cardiac effect, since biochemical changes may be not evident at such dose or can manifest after very long exposure. Considering the high rise of diabetes and cardiovascular diseases, the exposure to TS may impact cardiovascular health, worsen the disease condition and accelerate progression.

CONCLUSION

TS at a relatively non-lethal dose may be associated with cardiovascular complications including myofibril degeneration, wide inter-fascicular spaces, and mitochondria mutilation. The impact may become exacerbated under diabetic condition, which may lead to mortality and morbidity. The effect may be independent of ACh inhibition as observed in non-diabetic rats or might be related to ACh inhibition, as was seen in diabetic rats.

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Authors' contribution

All the authors (SMN, MS, JY, AA, JA, ST, EA, SO) provided an important intellectual content, reviewed the content, and approved the final version of the manuscript. SMN, AA, JA, EA, SO contributed significantly, read and approved the manuscript. SMN,JA, EA, SO conceived and designed the experiments. SMN and MS performed the animal experiments. SMN and JY performed laboratory investigation. SMN and ST performed electron microscopy. SMN, EA and SO analysed the data. SMN, JA, EA and SO contributed with reagents/materials/analysis tools. SMN, EA and SO wrote the paper.

Conflict of interest

The authors declare no conflict of interest. There are no patents, products in development or marketed products to declare. The research grant funding organisations had no role in the study design, data collection and analysis, decision to publish or the preparation of the manuscript.

REFERENCES

1. Carmichael SL, Yang W, Roberts E, Kegley SE, Padula AM, English PB, Lammer EJ, Shaw GM. Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. Environ Res 2014;135:133-8. doi: 10.1016/j.envres.2014.08.030
2. Hrabetz H, Thiermann H, Felgenhauer N, Zilker T, Haller B, Nährig J, Saugel B, Eyer F. Organophosphate poisoning in the developed world - a single centre experience from here to the millennium. Chem Biol Interact 2013;206:561-8. doi: 10.1016/j.cbi.2013.05.003
3. Lim Y-P, Lin C-L, Hung D-Z, Ma W-C, Lin Y-N, Kao C-H. Increased risk of deep vein thrombosis and pulmonary thromboembolism in patients with organophosphate intoxication: a nationwide prospective cohort study. Medicine (Baltimore) 2015;94:e341. doi: 10.1097/MD.0000000000000341
4. Bar-Meir E, Grubstein A, Givony S, Tadmor B. [Cardiac manifestations of organophosphate intoxication, in Hebrew]. Harefuah 2001;140:764-9. PMID: 11547484
5. Roth A, Zellinger I, Arad M, Atsmon J. Organophosphates and the heart. Chest 1993;103:576-82. doi: 10.1378/chest.103.2.576
6. Zoltani CK. CHAPTER 34 - Cardiovascular System as a Target of Chemical Warfare Agents. In: Gupta RC, editor. Handbook of Toxicology of Chemical Warfare Agents. San Diego: Academic Press; 2009. p. 493-507. [displayed 23 May 2015]. Available at http://www.sciencedirect.com/science/article/pii/B9780123744845000341
7. Baskin SI, Whitmer MP. The cardiac toxicology of organophosphorus agents. In: Baskin SI, editor. Principles of cardiac toxicology. Boca Raton (FL): CRC Press, Taylors and Francis Group; 1991. p. 275-294.
8. Ray DE, Richards PG. The potential for toxic effects of chronic, low-dose exposure to organophosphates. Toxicol Lett 2001;120:343-51. doi: 10.1016/S0378-4274(01)00266-1
9. Marosi G, Iván J, Nagymajtényi L, Csatlós I, Tószegi A. Dimethoate-induced toxic cardiac failure in the guinea pig. Arch Toxicol 1985;57:142-3. doi: 10.1007/BF00343126
10. Ballantyne B, Marrs TC. Overview of the biological and clinical aspects of organophosphates and carbamates. In: Ballantyne B, Marrs TC, editors. Clinical and experimental toxicology of organophosphates and carbamates. Oxford: Butterworth– Heinemann; 1992, p. 3-14.
11. Jaacks LM, Staimez LR. Association of persistent organic pollutants and non-persistent pesticides with diabetes and diabetes-related health outcomes in Asia: A systematic review. Environ Int 2015;76:57-70. doi: 10.1016/j.envint.2014.12.001
12. Nurulain SM, Petroianu G, Shafiullah M, Kalász H, Oz M, Saeed T, Adem A, Adeghate E. Sub-chronic exposure to paraoxon neither induces nor exacerbates diabetes mellitus in Wistar rat. J Appl Toxicol 2013;33:1036-43. doi: 10.1002/jat.2794
13. Lorke DE, Nurulain SM, Hasan MY, Kuča K, Petroianu GA. Prophylactic administration of non-organophosphate cholinesterase inhibitors before acute exposure to organophosphates: assessment using terbufos sulfone. J Appl Toxicol 2014;34:1096-103. doi: 10.1002/jat.2939
14. Worek F, Mast U, Kiderlen D, Diepold C, Eyer P. Improved determination of acetylcholinesterase activity in human whole blood. Clin Chim Acta 1999;288:73-90. doi: 10.1016/S0009-8981(99)00144-8
15. Karnovsky M. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 1965;27:137A-8A.
16. The WHO recommended classification of pesticides by hazard and guidelines to classification 2009 [displayed 29 May 2015]. Available at http://apps.who.int//iris/handle/10665/44271
17. Bonner MR, Williams BA, Rusiecki JA, Blair A, Freeman LEB, Hoppin JA, Dosemeci M, Lubin J, Sandler DP, Alavanja MC. Occupational exposure to terbufos and the incidence of cancer in the Agricultural Health Study. Cancer Causes Control 2014;21:871-7. doi: 10.1007/s10552-010-9514-9
18. Nurulain SM. Different approaches to acute organophosphorus poison treatment. J Pak Med Assoc 2012;62:712-7. PMID: 23866522
19. Cardiovascular Toxicants. Suspected Cardiovascular Or Blood Toxicants [displayed 23 May 2015]. Available at http://scorecard.goodguide.com/health-effects/chemicals-2.tcl?short_hazard_name=cardio&all_p=t
20. Eskenazi B, Kogut K, Huen K, Harley KG, Bouchard M, Bradman A, Boyd-Barr D, Johnson C, Holland N. Organophosphate pesticide exposure, PON1, and neurodevelopment in school-age children from the CHAMACOS study. Environ Res 2014;134:149-57. doi: 10.1016/j.envres.2014.07.001

21. Melgarejo M, Mendiola J, Koch HM, Moñino-García M, Noguera-Velasco JA, Torres-Cantero AM. Associations between urinary organophosphate pesticide metabolite levels and reproductive parameters in men from an infertility clinic. Environ Res 2015;137:292-8. doi: 10.1016/j.envres.2015.01.004
22. Pope C, Karanth S, Liu J. Pharmacology and toxicology of cholinesterase inhibitors: uses and misuses of a common mechanism of action. Environ Toxicol Pharmacol 2005;19:433-46. doi: 10.1016/j.etap.2004.12.048
23. Joshi P, Manoria P, Joseph D, Gandhi Z. Acute myocardial infarction: can it be a complication of acute organophosphorus compound poisoning? J Postgrad Med 2013;59:142-4. doi: 10.4103/0022-3859.113843
24. Pankaj M, Krishna K. Acute organophosphorus poisoning complicated by acute coronary syndrome. J Assoc Physicians India 2014;62:614-6. PMID: 25672037
25. Kiss Z, Fazekas T. Arrhythmias in organophosphate poisonings. Acta Cardiol 1979;34:323-30. PMID: 317206
26. Kiss Z, Fazekas T. Organophosphate poisoning and complete heart block. J R Soc Med 1983;76:85-6. PMCID: PMC1438580
27. Kiss Z, Fazekas T. Organophosphates and torsade de pointes ventricular tachycardia. J R Soc Med 1983;76:984-5. PMID: 6631883
28. Bayir A, Kara H, Köylü O, Kocabas R, Ak A. The effects of ubiquinone (CoQ10) on heart tissue in cardiac toxicity related to organophosphate poisoning. Hum Exp Toxicol 2013;32:45-52. doi: 10.1177/0960327112455070
29. Abdou HM, ElMazoudy RH, el-Mazoudy RH. Oxidative damage, hyperlipidemia and histological alterations of cardiac and skeletal muscles induced by different doses of diazinon in female rats. J Hazard Mater 2010;182:273-8. doi: 10.1016/j.jhazmat.2010.06.026
30. Velmurugan G, Venkatesh Babu DD, Ramasamy S. Prolonged monocrotophos intake induces cardiac oxidative stress and myocardial damage in rats. Toxicology 2013;307:103-8. doi: 10.1016/j.tox.2012.11.022
31. Britt JO, Martin JL, Okerberg CV, Dick EJ. Histopathologic changes in the brain, heart, and skeletal muscle of rhesus macaques, ten days after exposure to soman (an organophosphorus nerve agent). Comp Med 2000;50:133-9. PMID: 10857003
32. Pimentel JM, Carrington da Costa RB. 14 - Effects of organophosphates on the heart. In: Marrs BBC, editor. Clinical and experimental toxicology of organophosphates and carbamates 1992. p. 145-8 [displayed 29 May 2015]. Available at http://www.sciencedirect.com/science/article/pii/B9780750602716500206
33. Póvoa R, Cardoso SH, Luna Filho B, Ferreira Filho C, Ferreira M, Ferreira C. [Organophosphate poisoning and myocardial necrosis, in Portuguese]. Arq Bras Cardiol 1997;68:377-80. PMID: 9497529
34. Tryphonas L, Veinot JP, Clement JG. Early histopathologic and ultrastructural changes in the heart of Sprague-Dawley rats following administration of soman. Toxicol Pathol 1996;24:190-8. doi: 10.1177/019262339602400207
35. Zaki NI. Evaluation of profenofos intoxication in white rats. Nat Sci 2012;10:67-77. doi: 10.7537/marsnsj101212.11
36. Razavi BM, Hosseinzadeh H, Movassaghi AR, Imenshahidi M, Abnous K. Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure. Chem Biol Interact 2013;203:547-55. doi: 10.1016/j.cbi.2013.03.010
37. Hariri AT, Moallem SA, Mahmoudi M, Memar B, Hosseinzadeh H. Sub-acute effects of diazinon on biochemical indices and specific biomarkers in rats: protective effects of crocin and safranal. Food Chem Toxicol 2010;48:2803-8. doi: 10.1016/j.fct.2010.07.010
38. Rübsamen H, Hess GP, Eldefrawi AT, Eldefrawi ME. Interaction between calcium and ligand-binding sites of the purified acetylcholine receptor studied by use of a fluorescent lanthanide. Biochem Biophys Res Commun 1976;68:56-63. doi:10.1016/0006-291X(76)90009-7

Terbufos sulfon pogoršava srčane lezije u štakora koji boluju od dijabetesa: studija subakutne toksičnosti

Organofosforni spojevi (eng. *Organophosphorous Compounds - OPCs*) imaju široku primjenu, od one u poljoprivredi do one u vojne svrhe. Izlaganje takvim spojevima izaziva niz različitih zdravstvenih problema od globalnog značaja. Terbufos je jedan od vodećih OPC-a koji se koriste diljem svijeta. U ovom je istraživanju na modelu štakora bez dijabetesa i sa streptozotocinom izazvanim dijabetesom ispitivan metabolit terbufos-sulfon (TS) u najvišoj dozi koja ne izaziva učinak te njezin utjecaj na srce. Odrasli mužjaci štakora soja Wistar dobivali su petnaest dana dnevnu dozu od 100 nmol štakor^{-1} (1/20 LD_{50}) intraperitonealno. Transmisijskim elektronskim mikroskopom istražene su ultrastrukturne promjene lijeve klijetke. Na krvnim uzorcima provedeni su biokemijski testovi, uključujući aktivnost acetilkolinesteraze u crvenim krvnim stanicama, razinu kreatinin kinaze (CK), laktat dehidrogenaze (LDH), kolesterola, lipoproteina visoke gustoće (HDL), lipoproteina niske gustoće (LDL), triglicerida, ALT, AST i GGT. Istraživanjem je otkriveno oko 10 % inhibicije AChE-a u crvenim krvnim stanicama nakon dva tjedna izlaganja štakora bez dijabetesa TS-u, dok je u štakora s dijabetesom aktivnost AChE-a bila značajno smanjena. Razine CK, LDH i triglicerida bile su značajno više u TS tretiranim štakorima s dijabetesom. Elektronsko-mikroskopska analiza srca upućuje na narušenu strukturu i lezije u mitohondrijima u kardiomiocitima skupina štakora koji su tretirani TS-om. Zaključuje se da nesmrtonosna doza TS-a uzrokuje srčane lezije koje se pogoršavaju u prisutnosti dijabetesa. Biokemijski testovi potvrdili su ultrastrukturne promjene. Navedena doza TS-a može biti rizični čimbenik za kardiovaskularne bolesti, koje se mogu pokazati smrtonosnima uz istovremeno postojanje dijabetesa.

KLJUČNE RIJEČI: *acetilkolinesteraza; diabetes mellitus; kardiotoksičnost; lipidni profil; marker oštećenja miocita; organofosfati; srčana lezija*