

ORIGINAL ARTICLE

Plasma paraoxonase1 activity in rats treated with monocrotophos: a study of the effect of duration of exposure

Raju NAGARAJU^{1,2}, Apurva Kumar R. JOSHI^{1,3}, Sowmya Giriyapura VAMADEVA¹, Rajini Padmanabhan SHARDA¹

¹ Food Protectants and Infestation Control Department, CSIR-Central Food Technological Research Institute, Mysore, India

² Occupational Biochemistry, Regional Occupational Health Centre, Bangalore, Karnataka, India

³ Department of Biochemistry, School of Sciences, Jain (deemed-to-be-university), Jayanagar 3rd Block, Bangalore, India

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ABSTRACT

We have earlier demonstrated the potential of monocrotophos (MCP), a highly toxic organophosphorus insecticide (OPI), to elicit insulin resistance in rats after chronic exposure. Given the understanding of role of paraoxonase1 (PON1) in OPI toxicity and diabetes pathology, this study was envisaged to understand the effect of duration of exposure to MCP on plasma PON1 activity in rats. Rats were administered MCP *per os* at 1/20 and 1/10th LD₅₀ as daily doses for 180 days. Interim blood samples were collected at 15, 30, 45, 90 and 180 d for analysis of plasma parameters. Exposure to MCP for 45 resulted in persistent trend of hyperinsulinemia, while significant increase in fasting glucose levels was observed after 180 days. MCP caused suppression of plasma cholinesterase activity though the study period, albeit extent of inhibition was more severe during the early phase of the study. Exposure to MCP for 180 d resulted in hypertriglyceridemia and marginal decrease in HDL-C levels. MCP failed to modulate PON1 activity in plasma during the early phase of the study (up to 45 d). However, prolonged exposure resulted in significant increase in the plasma PON1 activity. This suggests that manifestation of insulin resistance in rats subjected to chronic exposure to MCP is associated with increase in PON1 activity. Our work provides rationale for studying whether the increase in PON1 activity observed in the present study serves to counter the deleterious effect of long term exposure to organophosphorus insecticides on metabolic homeostasis.

KEY WORDS: paraoxonase1 activity; monocrotophos; organophosphorus insecticide; insulin resistance; chronic exposure; metabolic homeostasis

Introduction

Organophosphorus insecticides (OPI) represent a major class of pesticide employed worldwide either for mitigating pests or preventing vector born disorders in agriculture and public health respectively. Although OPIs are less persistent than organochlorine insecticides, presence of OPI residues in various components of biosphere (Varo *et al.*, 2002; Sanghi *et al.*, 2003; Battu *et al.*, 2004; Kumari *et al.*, 2008; Joko *et al.*, 2018) clearly suggest that OPI are realistic toxicological threat. OPI act by inhibiting the enzyme acetylcholinesterase (AChE) and thereby causing cholinergic stress-mediated neurotoxicity (Fukuto, 1990; Sultatos, 1994; Sogorb & Vilanova, 2002). It is now well recognized that OPI exhibit potential to cause endocrine

disruption (McKinlay *et al.*, 2008; Mnif *et al.*, 2011; Cecchi *et al.*, 2012). Activation of hypothalamus-pituitary-adrenal axis as evidenced by increase in circulating glucocorticoid hormones appears to be one of the most widely observed effects of OPI and other AChE inhibiting chemicals (Spassova *et al.*, 2000; Joshi & Rajini, 2009; Joshi & Rajini, 2012). In addition both clinical (revived by Joshi & Sukumaran, 2019) and experimental studies (Rahimi *et al.*, 2007; Joshi *et al.*, 2009) establish that OPI possess hyperglycemic potential. Cholinergic stress is one of the major factors responsible for hyperglycemic nature of OPI (MCP) (Joshi & Rajini, 2012) and can be completely attenuated by antagonists of cholinergic and adrenergic receptors (Joshi & Rajini, 2012; Joshi *et al.*, 2012). Epidemiological studies on cohorts of farmers and pesticide formulators reveal that chronic exposure to pesticides including OPI is associated with the development of symptoms of diabetes in exposed individuals and the duration of exposure determine the degree of symptoms in exposed individuals (Montgomery *et al.*, 2008; Raafat

Correspondence address:

Raju Nagaraju, PhD.

Occupational Biochemistry, Regional Occupational Health Centre
Bangalore, 562110 Karnataka, India

TEL.: 91-80-22172500 • FAX 91-080- 22172502

E-MAIL: rajuu86mys@yahoo.com

et al., 2012). Further, it is now established that OPIs elicit insulin resistance in experimental animals (Mostafalou *et al.*, 2012, Nagaraju *et al.*, 2015).

Human paraoxonase1 is a calcium-dependent hydrolytic enzyme and it is speculated that plays an important role in diseases such as diabetes and atherosclerosis. Low PON1 activity is associated with higher risk of cardiovascular diseases (Shunmoogam *et al.*, 2018). PON1 is implicated in hydrolytic detoxification of many OPIs and is known for hydrolyzing paraoxon, phenyl acetate and 4-nitrophenyl acetate as well as lactones (Costa *et al.*, 2005; Ceron *et al.*, 2014). PON is synthesized in the liver and secreted into the plasma where it associates with HDL particles. A 43kDa protein PON1 is known for polymorphisms arising from single nucleotide polymorphisms. While as many as 8 SNPs are known to occur in PON1, important ones are at positions 55 (Leu-Met) and 192 (Gln-Arg). These polymorphisms could either affect substrate specificity or biological activity of PON1 (Humbert *et al.*, 1993; Yamuda *et al.*, 2001; Shunmoogam *et al.*, 2018). PON1 is known for protecting LDL particles from oxidation, a property of PON1 that is majorly responsible for its cardioprotective effects (Mackness *et al.*, 1993; Mackness *et al.*, 2006). Studies suggest PON1 activity may be reduced in diabetic condition and owing to its ability of hydrolyzing OPIs, PON1 serves as a detoxification mechanism against OPI toxicity (see discussion). In our previous study, we observed the course of development of insulin resistance in rats subjected to chronic exposure. Exposure of rats to MCP for 45 days is required to elicit insulin resistance (Nagaraju *et al.*, 2015). Further, we observed that 180 days exposure to MCP is associated with hyperinsulinemic insulin resistance and elevated PON1 activity in rats (Nagaraju & Rajini, 2016). Therefore in this study, we analyzed the effect of duration of exposure to MCP on circulating PON1 activity (as measured by paraoxon hydrolysis) in an effort to understand the status of metabolic homeostasis in relation to circulating PON1 activity.

Materials and methods

Chemicals

Acetylthiocholine iodide (ATCI), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), and paraoxon (O, O-dimethyl-O-p-nitrophenylphosphate) were procured from Sigma Chemicals Co., (St. Louis, MO, USA). Orlistat was procured from Centurion Laboratories, Vadodara, India. Blood glucose, triglyceride, cholesterol and HDL-c kits were procured from Span Diagnostics (India). Insulin kit was procured from Crystalchem.,(USA). Monocrotophos (Technical grade, 75%) was a gift from Hyderabad Chemicals (Hyderabad, India). All other reagents and chemicals used were of analytical grade procured from SRL Pvt. Ltd (Mumbai, India).

Animals and experimental protocol

This study was approved by Institute Animal Ethical Committee, regulated by the CPCSEA, India. Adult

male rats (CFT-Wistar strain, $\sim 150 \pm 5$ g) drawn from the Institute Animal House Facility at CSIR-CFTRI (Mysore) were divided into three groups. Rats of the first group served as control and received distilled water as vehicle, while second and third group were administered MCP at 0.9 and 1.8 mg/kg b.w/d respectively for 180 d. The blood samples were collected after 15, 30, 45, 90 and 180 exposures through orbital sinus method and used for measuring blood glucose, lipid profile, AChE and PON1 activity. The samples were collected 2 h after the last dose (Nagaraju *et al.*, 2015). Separate batches of rats from each group were fasted overnight and blood was collected via tail nick method for the estimation of glucose (Accu-Check) and insulin for analyzing insulin resistance via HOMA method.

Plasma parameters

Glucose, total cholesterol, and HDL-C were quantified by employing colorimetric kits based on action respective oxidases to generate hydrogen peroxide, which is quantified by use of peroxidase and a chromogen to obtain a red colored quinoneimine dye. The intensity of the dye was measured at 505 nm and results were expressed as mg/dl. For quantifying HDL-cholesterol, the LDL/VLDL fraction in serum was precipitated by using polyethylene glycol and supernatant was used to measure cholesterol using cholesterol kit. Plasma insulin was quantified using sandwich ELISA kit. Plasma triglyceride levels were measured with correction for free glycerol (Nagaraju *et al.*, 2013) using orlistat as lipoprotein lipase inhibitor and cholinesterase activity was quantified by measuring rate of hydrolysis acetylthiocholine iodide using DTNB (Galgani F, 1991). Paraoxonase activity in plasma was monitored by assessing the hydrolysis of paraoxon in the presence of calcium chloride (Charlton-Menys *et al.*, 2006).

Homeostatic model of insulin resistance (HOMA-IR)

Fasting blood glucose and insulin values were used to calculate insulin resistance and beta cell function by employing HOMA model (Matthews *et al.*, 1985).

Statistical analysis

Mean and standard error (SE) were determined for all parameters and results were expressed as mean \pm SE. The data were analyzed employing ANOVA followed by Tukey's *post-hoc* test for comparison of means to determine the significance of differences between the groups. A *p*-value below 0.05 was considered as significantly different.

Results

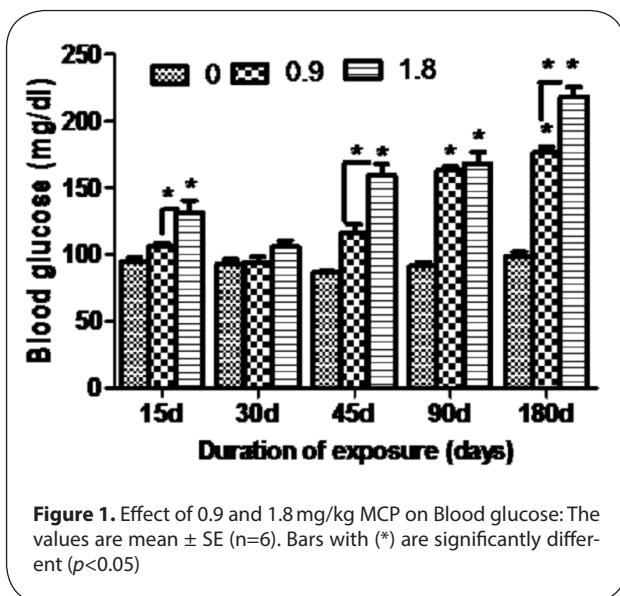
Impact of repeated oral doses of MCP on fasting blood glucose, insulin and insulin sensitivity

The impact of repeated oral doses of MCP on fasting blood glucose, insulin, and status of insulin resistance at different time intervals is tabulated in Table 1. The fasting blood glucose levels were considerably enhanced in treated

Table 1. Impact of repeated oral doses of monocrotophos on fasting blood glucose, insulin and beta cell function in treated rats.

Variable	MCP exposure (mg/kg b.w)	15	30	45	90	180
Fasting blood glucose (mg/dl)	0	77.00±1.1 ^a	84.01±2.0 ^a	74.67±1.2 ^a	74.67±2.91 ^a	71.00±2.08 ^a
	0.9	65.50±2.0 ^b	78.33±1.9 ^a	77.33±2.3 ^a	70.67±2.73 ^a	85.33±2.73 ^b
	1.8	61.88±5.0 ^b	73.67±1.8 ^a	77.67±4.2 ^a	71.33±3.14 ^a	94.33±2.85 ^b
Fasting plasma insulin (mu/L)	0	8.53±0.8 ^a	8.29±0.6 ^a	7.60±0.6 ^a	8.33±0.3 ^a	7.72±0.24 ^a
	0.9	4.10±0.5 ^b	8.35±0.8 ^a	10.90±0.2 ^b	13.08±0.3 ^b	16.44±0.54 ^b
	1.8	3.62±0.4 ^b	8.31±1.2 ^a	12.73±0.9 ^b	15.85±0.9 ^b	26.33±1.92 ^c
Insulin resistance (HOMA)	0	1.06±0.1 ^a	1.03±0.1 ^a	0.94±0.1 ^a	1.02±0.1 ^a	0.94±0.03 ^a
	0.9	0.47±0.1 ^b	1.04±0.1 ^a	1.35±0.1 ^b	1.57±0.1 ^b	2.03±0.05 ^b
	1.8	0.43±0.1 ^b	1.01±0.1 ^a	1.47±0.1 ^b	1.90±0.1 ^c	3.35±0.26 ^c
Beta cell function (HOMA)	0	138.8±5.6 ^a	114.6±1.9 ^a	138.1±11.0 ^a	147.3±12.0 ^a	124.13±8.9 ^a
	0.9	119.3±8.1 ^a	133.2±10.7 ^a	155.7±12.1 ^a	223.1±18.9 ^b	208.3±14.7 ^b
	1.8	123.1±10.6 ^a	149.6±14.1 ^a	180.5±16.0 ^b	247.5±19.5 ^b	200.6±1.9 ^b

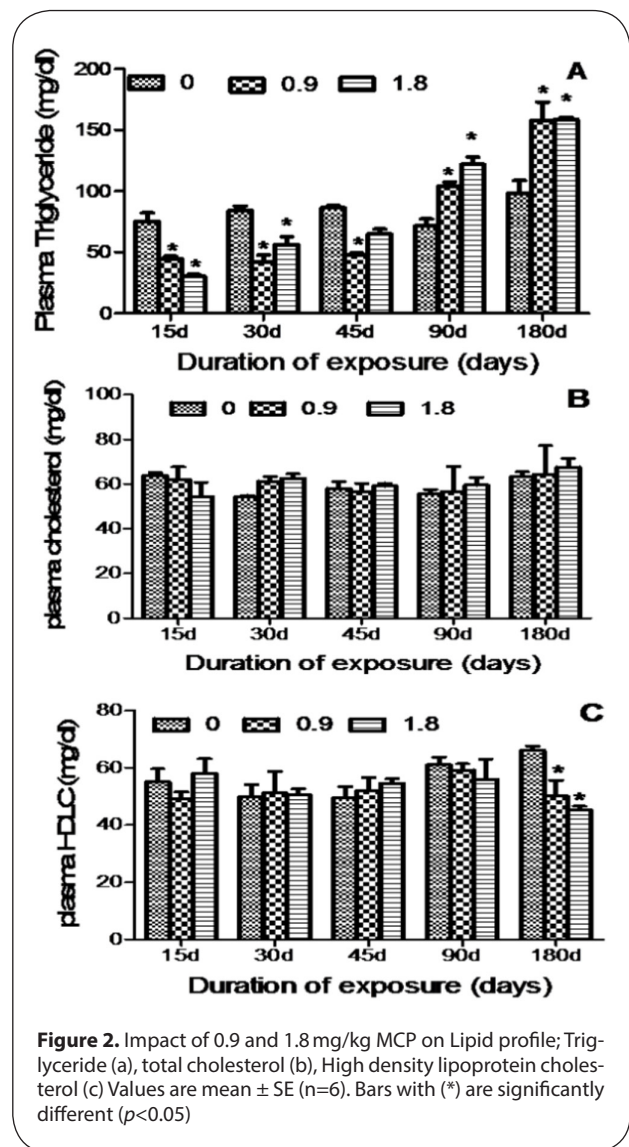
Values are expressed as mean ± S.E (n=3); The columns with different alphabets are statistically different ($p < 0.05$).



rats after 180th dose (20–33% above control, $p < 0.05$), while plasma insulin levels were significantly enhanced during the 45th dose of MCP administration (50–70% above control, $p < 0.001$) in treated rats. The plasma insulin level was highly intensified as the duration of exposure continued and attained 2.7–3.5 fold above controls after 180 days exposure. Since enhanced fasting insulin is the surrogate marker of IR, we calculated IR using the HOMA model. MCP treated rats exhibit insulin resistance after 45 d of MCP exposure and insulin resistance worsened as the experimental regime continued and at the end of regime insulin resistance was found to be 3–4 fold above controls in treated rats. Similarly, aggravation of insulin resistance due to chronic exposure is associated with an increase in beta cell function (60–80%).

Effect of chronic exposure to MCP on plasma parameters.

Effect of duration of exposure to MCP on blood glucose, lipid profiles AChE, and PON1 activity in rats are depicted in Figures 1–4 respectively. The data represented in these



figures are from samples collected 2 h after the respective last dose. Impact of MCP on blood glucose levels was persistent after 45 days of exposure and after 180 days

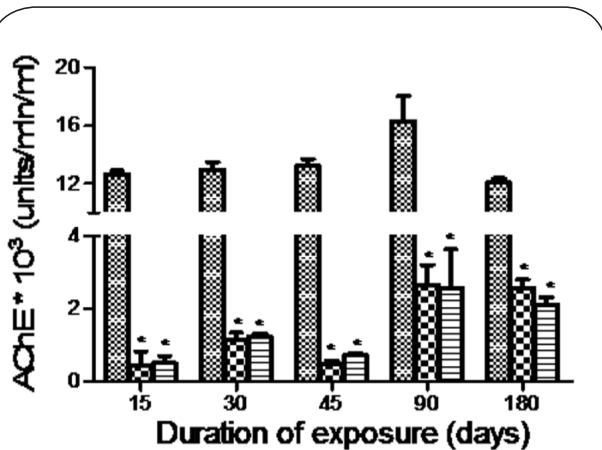


Figure 3. Effect of 0.9 and 1.8 mg/kg MCP on AChE activity: The Values are mean ± SE (n=6). Bars with (*) are significantly different (p<0.05)

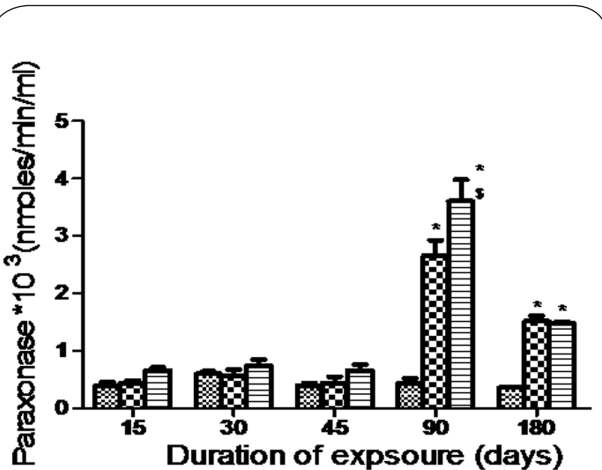


Figure 4. Paroxonase activity in control and 0.9 and 1.8 mg/kg MCP treated rats: The Values are mean ± SE (n=6). Bars with different symbols are significantly different (p<0.05)

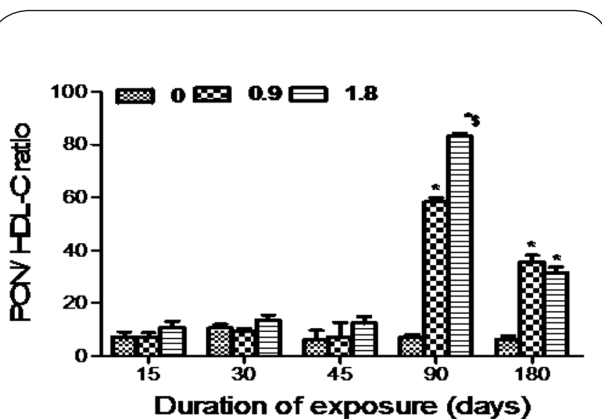


Figure 5. PON activity/HDL cholesterol ratio in control and 0.9 and 1.8 mg/kg MCP treated rats: The Values are mean ± SE (n=6). Bars with different symbols are significantly different (p<0.05)

of exposure, glucose levels were 90–110% above control (Figure 1). Figure 2 depicts data on triglycerides, total and HDL-cholesterol in plasma of rats from different groups. The plasma triglyceride levels were significantly declined in earlier phase (35–45% and 50–60% of control at 15 and 30 d respectively), while enhanced plasma triglycerides were noticed after 180 d which was found to be 50–60% above control. The plasma cholesterol levels remain unaltered, while a marginal decrease in HDL-c levels was noticed (60–75% of control) after 180 d and which might demonstrate the possibility of symptoms of dyslipidemia induced by chronic exposure to MCP in treated rats.

The AChE activity was suppressed throughout the study period in MCP-treated rats, although extent of inhibition was more severe up to 45 days (Figure 3). Interestingly, plasma PON1 activity remained comparable in rats of all groups up to 45 days of the study period. However, there was a phenomenal increase in circulating PON1 activity in plasma of rats exposed to MCP for 90 days. In rats exposed to MCP for 180 days, the plasma PON1 activity remained elevated, although extent of elevation was less compared to that of 90 days exposure group (Figure 4). Consequently, PON1 activity/HDL-c ratio was significantly increased in rats exposed to MCP for 90 and 180 days, although extent of elevation was more pronounced in rats exposed for 90 days.

Discussion

hyperglycemia and dyslipidemia are the common metabolic impairments associated with OPI toxicity in humans and experimental animals (Rahimi *et al.*, 2007; Karami-Mohajeri *et al.*, 2011; Joshi & Sukumaran, 2019). Many OPIs including MCP are known to induce hyperglycemia in experimental animals after exposure to a single dose (Seifert, 2001; Lasram *et al.*, 2008; Joshi & Rajini, 2009, Joshi & Rajini 2012; Joshi *et al.*, 2012; Acker *et al.*, 2012). An important feature of hyperglycemia induced by OPI appears to be its transient nature (Seifert, 2001; Lasram *et al.*, 2008; Joshi & Rajini, 2009, Joshi & Rajini 2012). In this study, we observed that the chronic exposure was associated with increased glycemic responses in the earlier phase which gradually enhanced and aggravated as the duration of exposure continued. These results are in agreement with our previous observations (Nagaraju *et al.*, 2015) of hyperglycemia caused by chronic exposure which was found to be associated with up regulation of substrate driven glucose synthesis and enhanced glycogenolysis in MCP treated rats. Further, in this study, MCP treated rats exhibited dyslipidemia, characterized by hypertriglyceridemia with marginal lower levels of HDL cholesterol. These results are in agreement with observations made by others (Slotkin *et al.*, 2005; Rezg *et al.*, 2010) on the effect of OPIs on lipid profile.

Plasma cholinesterase activity, a marker of OPI toxicity, was significantly decreased in MCP treated rats. Interestingly, the extent of inhibition is more severe in the early phase of the present study than chronic exposure

which might be due to enhanced PON1 activity in these rats. Mice lacking serum paraoxonase demonstrated more sensitivity to OPI toxicity via inhibition of acetylcholine esterase activity at the dose which is ineffective in wild animals (Shih *et al.*, 1998). Therefore, the enhanced PON1 activity might have an impact on AChE activity after chronic exposure. We observed that fasting blood glucose and insulin levels were significantly increased in treated rats after chronic exposure. Scientific studies have shown that chronic exposure to organophosphorus insecticides in farmers and pesticide formulators is likely to be associated with insulin resistance and type 2 diabetes which correlates with duration and frequency of exposures to such chemicals in their lifetime (Montgomery *et al.*, 2008; Raafat *et al.*, 2012). Further, OPI's may increase the risk of gestational diabetes (Saldana *et al.*, 2007). Gifford *et al.*, (2019) reported that OPI toxicity is associated with acute dysregulations in glucose homeostasis linked to changes in insulin action and secretion.

PON1 is a serum enzyme closely associated with high-density lipoproteins and is known for its antioxidant properties and metabolism of toxic lipid molecules associated with LDL and HDL molecules (Mackness *et al.*, 2006). Further, it also hydrolyzes various organophosphorus insecticides and lactone containing pharmaceutical compounds. PON1 polymorphism and activity are known to be a determinant or biomarker for the sensitivity to organophosphorus insecticides in human subjects. General observation from these studies is that lower activity or phenotypes with lower activity are associated with high risk for OPI toxicity (Lee *et al.*, 2003; Sirivarasai *et al.*, 2007). Further, mice lacking PON1 are reported to exhibit higher sensitivity of OPI toxicity and fail to prevent LDL oxidation when fed high levels of fat and cholesterol in diet (Shih *et al.*, 1998). In addition to modulating OPI toxicity, the role of PON1 as a critical determinant of metabolic health is now being understood. Studies have reported that diabetes is associated decreased PON1 activity (Mackness *et al.*, 1991; Abbott *et al.*, 1995; Inoue *et al.*, 2000; Flekač *et al.*, 2008; Gupta *et al.*, 2011; Shakeri *et al.*, 2017). Animal studies shed light on association of PON1 activity with metabolic dyshomeostasis. Streptozotocin (STZ), a pharmacological diabetogen used for generating experimental models of insulin-dependent diabetes, is known to cause hyperglycemia and decreased PON1 activity (Patel *et al.*, 1990). Administration of recombinant PON1 has been reported to reduce incidence of diabetes, lower glucose and increase circulating insulin levels in STZ-treated rats (Koren-Gluzer *et al.*, 2011). PON1 deficiency (knockout) has been reported to cause increase in fasting glucose and insulin levels in both normal diet and high fat diet fed mice (Koren-Gluzer *et al.*, 2013). Further, PON1 deficiency has been reported to aggravate STZ-induced diabetes in mice, while PON1 over expression offers protection against diabetes incidence and mortality in mice (Rozenberg *et al.*, 2008). Thus, PON1 activity may be perceived to play a major role in regulation of metabolic homeostasis. In our previous study, we reported that chronic exposure

to MCP in rats is associated with the onset of insulin resistance after 45 days of exposure to daily doses and insulin resistance worsened as the duration of exposure continued (Nagaraju *et al.*, 2015). We demonstrated that insulin resistance after 180 days exposure to MCP is associated with augmented pancreatic beta cell response presumably to counter the metabolic effects of MCP (Nagaraju *et al.*, 2015; Nagaraju & Rajini, 2016). Further studies are needed to understand whether increase in PON1 activity reported by us serves as a counter against chronic MCP-induced metabolic dysregulations.

Conclusion

Many scientific studies have established a strong correlation between PON1 activity and the prevalence of metabolic syndrome in humans and animal studies. PON1 is also a determinant of OPI toxicity. In this study, plasma PON1 activity was significantly increased after chronic exposure to MCP which coincides with decrease in the extent of AChE inhibition along with an increase in fasting plasma insulin levels. Further studies are needed to understand the impact of increase in PON1 activity on metabolic status in rats subjected to chronic MCP exposure.

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RN, AKRJ and PSR conceived and designed the study. Experiments and data acquisition were performed by RN and SV. RN, AKRJ and PSR wrote the manuscript. All authors have read and approved the manuscript.

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