https://doi.org/10.48047/AFJBS.6.11.2024.125-143

A THERAPEUTIC NOVEL APPROACH TO ENHANCE ORGANOPHOSPHATE HYDROLASE ENZYME ACTIVITY

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ArticleHistory

Volume:6,Issue7,2024

Received:30May2024

Accepted:26June2024

doi:10.48047/AFJBS.6.11.2024.125-143

ABSTRACT

Objective

Formulation and evaluation of Paraoxonase1 (PON1) microparticles (MPs) efficacy on chlorpyrifos (CPF) induced hepatotoxicity.

Methods

PON1 micro particles are prepared by using solvent evaporation method. The prepared MPs were characterised for entrapment efficacy (EE), drug content, and drug release. The surface morphology was identified using scanning electron microscopy (SEM). *In-vivo* studies were performed biochemical markers such as acetylcholinesterase (AchE), nitric oxide (NO), thiobarbituric acid reactive substances (TBARS), superoxide dismutase **(**SOD), catalase, glutathione peroxidase **(**GPx), and malondialdehyde (MDA) using animal model by CPF induced hepatotoxicity method.

Results

Obtained results revealed that formulated micro particles had 84.37% EE and the drug content was 89.71. The scanning electron microscopy (SEM) images confirm the spherical structure and smooth texture of particles. The maximum % drug was released in 30 days. PON1-MPs supplementation significantly reduces liver weight, liver index, and alanine transaminase (ALT), aspartate transaminase (AST) , alkaline phosphatase (ALP) and AchE, NO, TBARS and ameliorates hepatic toxicity with increased anti oxidant levels.

Conclusion

In the present investigation principal reduction in AchE, elevated levels of anti oxidant enzymes in hepatic tissue by the PON1 and improved efficacy by PON1-MPs contributed hepato protection against CPF induced hepatotoxicity. Further studies have to be emphasizing molecular mechanisms involved in hepato protection.

Keywords: Paraoxonase1, microparticles liver weight, liver index hepatotoxicity, chlorpyrifos,

1. INTRODUCTION

Organophosphates (OPs) are synthetic compounds with a covalent double bond between pentavalent phosphorus and sulphur or oxygen. Early diagnosis and severity assessment improves outcomes[1]. Biochemical indicators such serum acetylcholinesterase (AChE) and pseudocholinesterase are commonly used to assess organophosphorus (OP) compound poisoning severity[2]. Oxime medicines, especially pralidoxime, are used to treat organophosphorus poisoning but lack clinical efficacy. Atropine appears to have no effect on OP-induced oxidative stress. However, oximes are both prooxidative and antioxidative[3].

Wide-spectrum chlorine-containing insecticide chlorpyrifos (CPF) controls infections on a variety of crops, fruits, and vegetables[4]. Its broad-spectrum use is linked to various detrimental health effects due to acute and chronic exposure in agricultural fields, indirect exposure, and trace quantities in agricultural products[5]. This chemical in fruits and vegetables is a global issue since low dosages long-term exposure harms humans and the environment[6]. CPF causes neurotoxicity, cardiovascular problems, endocrine disturbance, developmental malformations, genetic damage, haematological cancers, and immunotoxicity[7]. CPF exposure is a significant health and environmental concern because to its widespread use and various harmful effects on multiple animals and organisms[7].

In biomedical, pharmacological, and medical device applications, microparticles (MPs) improve therapeutic characteristics[8]. Most typically employed for continuous release of therapeutic drugs, MPs produce desirable medicinal effects longer than conventional therapies[9]. Based on where they are injected, polymer utilised to make MPs, and preparation process, the duration can be hours, days, or months. MPs-based drug delivery systems are used for drugs with short plasma half-lives to promote patient compliance by reducing parenteral administration and therapeutic performance[9, 10].

Poly (lactic-co-glycolic) acid (PLGA) is a popular polymer for developing new drug delivery methods[1]. The Uniterd States (US) of Food and Drug Administration (FDA) classifies it as generally recognized as safe (GRAS), a biodegradable, biocompatible polymer with excellent safety. Many drug-loaded nano and microparticle formulations use this polymer[12].

Our present effort also uses PLGA to make PON1-loaded MPs. Minimal study has evaluated the efficacy of micro-PON-1 formulations in acute and chronic OP poisoning. We suggest developing PON1-loaded PLGA-MPs (i.e., PON1-MPs) to sustain therapeutic levels of PON1 in the body for longer due to its ability to guard against OP-induced harmful effects.

2. MATERIALS AND METHODS

2.1 Drugs and reagents

PLGA obtained from Sigma Aldrich, and Paraoxonase1 (PON1) from human plasma was a gift from Zaney Pharmaceuticals, Hyderabad. Alanine transaminase (ALT), aspartate transaminase (AST) , alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, and total protein kits are obtained from Accurex, Mumbai, supplied. Enzyme-linked immune sorbent assay **(**ELISA) kits collected from Thermo-Fisher Scientific, while tri barbituric acid acid (TBA), malondialdehyde (MDA), and haematoxylin & eosin stain and superoxide dismutase (SOD), catalase, and protein carbonyl content test kit and Griss reagent purchased from Sigma Aldrich (USA) & S. D Fine Chemicals Pvt. Ltd, India.

2.2 Development of PON1 loaded PLGA micro particle formulations

Paraoxonase1 (PON1) loaded PLGA microparticles (PON1-MPs) developed using the human endogenous enzyme of organophosphate hydrolase enzyme **(**OPHE), because of its organophosphate hydrolyzing capacity. OPHE-like PON1 was employed for formulation development. PLGA polymer made micro particles [13]. PON1 loaded MPs were generated by emulsion solvent evaporation method[14]. The PLGA and PON1 polymer solution was emulsified in 2% polyvinyl alcohol (PVA) solution by adding the organic phase to the aqueous phase drop wise while vertexing[14]. Di-chloro methane (DCM) was evaporated from the micro particle formulations overnight while stirring. After centrifuging the MPs formulations at 10,000 rpm, supernatant was collected, and the pellet containing MPs was washed with phosphate buffer saline (PBS) three times. Finally, the pellet was employed for PON1-MPs formulation characterization, *in vitro* drug release, and *in vivo* animal experiments[15].

2.3 Experimental Design

2.3.1 *In vitro* evaluation

2.3.1.1 The PON1-MPs formulations were examined for size, surface characteristics, entrapment efficiency drug content and calibration curve with water using optical microscopy, scanning electron microscopy (SEM), bradford test respectively[13,16-19]. The PON1 entrapment efficiency in the PLGA-MPs was assessed by quantifying the amount of PON1 in all three supernatants and in the MPs by breaking them with acetonitrile. Bradford test evaluated PON1-MP content. Quantification samples were precipitated PLGA polymer with acetonitrile (ACN) and water. The MP formulation's PON1 concentration was quantified using the calibration curve with water. A correlation coefficient (R^2) of 0.984 was found

between 156.32-40,000 µg/ml. EE was calculated by following formula, If 10 mg of MPs contain= X mg of drug [20-23].

Total freeze-dried product (Y) contains=?

Entrapment efficiency= Y*X/10*100 (* means multiplication) [23]

***2.3.1.2 In vitro* release profile of OPHE MPs:** Suspending PLGA-MPs in PBS and packing them in semi-permeable cellulose membranes and shaking at 100 rpm was used to study there *in vitro* OPHE release profile[24]. At 0, 2, 4, 8, 12, 18, and 24 h, and every other day for 30 days, PON1 was collected from the released media and stored at -80 °C until Bradford assay analysis[25]. After the release research ended on the 30th day, all release media samples were tested for PON1. The cumulative drug release profile was displayed versus time points based on concentrations[26].

2.3.2 *In vivo* pharmacological study

2.3.2.1 Experimental animals

Male Wistar Rats, weighing 200–250 g, at the age 4–5 weeks were used in the study and given standard laboratory pellet chow diet; Provimi limited (India), provided water ad libitum and were kept under standard conditions at 23–25 ^{0}C, 35-60% relative humidity (RH), and 12 h light/dark cycle. The rats were acclimatized to the laboratory conditions a week prior to experiment. The experimental protocol (No: CPCSEA/IAEC/JLS/17/03/22/018) was duly approved by institutional animal ethics committee (IAEC) [27].

2.3.2.2 Induction of hepatotoxicity and grouping

Rats were used to study the protective effects of PON1 formulations on CPF induced hepatotoxicity and renal damage model upon chronic exposure with CPF for 28 days. Animals were divided into four groups of each six. Group I was treated PBS: group II was treated with sub-cutaneous injection of CPF for 28 days with the dose of 10 mg/kg. Group III and group IV were pre treated with PON1 free drug and PON1-MPs respectively and toxicity induced by the administration of CPF for 28 days with the dose of 10 mg/kg. On 28th day blood collected by using retro orbital method for hematological parameters estimation and organs such as liver collected for histopathological examination[27, 28].

2.4 Bio Chemical Estimations

2.4.1 Evaluation of liver function tests

All experimental animals' blood plasma was used to estimate ALT, AST, and ALP to assess rats' liver function parameters using standard kits.

2.4.2 Estimation of acetyl cholinesterase (AChE) activity

The AChE activity was measured in plasma by the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of plasma, liver & renal cholinesterase was measured using a spectrophotometer (Shimadzu 1800) at a wavelength of 412 nm[29-30].

2.4.3 Estimation of nitrite levels

Nitrite and nitrate determinations in biological material are increasingly being used as markers of NO production. Nitric oxide production was quantified by measuring nitrite, a stable oxidation end-product of NO[31].

2.4.4 TBRS Assay

Lipid peroxidation was estimated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in tissues by the method. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm [32].

2.5 Anti-oxidant Activity

2.5.1 Superoxide dismutase (SOD)

For monitoring pyrogallol auto oxidation, 20 to 80 µl of pyrogallol was pipette out and the volume was made up to 100 µl with 0.01 N hydrochloric acid (HCl). To this 600 µl of Tris-HCl buffer, 100 µl of DETPA, 100 µl of Tris EDTA, 100ul of distilled water was added[28]. For the sample assay, 50 µl of pyrogallol and 50 µl of the sample were added and the reaction was initiated and measured at 420 nm at a constant temperature of 25 ºC for 3 min at 0.5 nm bandwidth[33]. SOD activity was determined by the pyrogallol oxidation method. This is an indirect method that is based on the ability of the enzyme to inhibit the auto oxidation of pyrogallol[34].

2.5.2 Glutathione peroxidase (GPx)

GPx was quantified on the basis of its utilization for conversion of glutathione to its oxidized glutathione and it's back converted in the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). Conversion of NADPH to NADP+ is the expression of GPx activity which was measured at 340 nm[35].

2.5.3 Catalase

Add 1 mL of hydrogen peroxide (H_2O_2) to 100 µl of sample and mix well, after 60 sec. Add 1 ml ammonium molybdate. Read the absorbance at 405 nm[36].

2.6 Anti-inflammatory Activity

2.6.1 Enzyme-linked immune sorbent assay (ELISA)

After treatment with PON1 formulations from acute and chronic CPF exposure, liver and kidney were collected for ELISA to estimate tissue pro-inflammatory cytokine levels[37]. Organs were homogenized in tissue lysis solution, supernatants were collected, and protein estimation was done using previously published techniques with modifications[38].

Pro inflammatory cytokines such Interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α were quantified using this approach[39-40]. Thermo Fisher Scientific (USA) ELISA tests were conducted as directed[42]. Cytokine levels will be normalized to protein concentrations and expressed as pg/mg protein from Bradford's technique estimates of supernatant protein concentrations[43].

2.7 Statistical Analysis

The results were expressed as mean ± SEM; the values were statistically analyzed by one way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests[43];

aP< 0.001 indicates the significance on comparison of group II with group I

bP< 0.001 indicates the significance on comparison of group III and IV with group I

cP< 0.001 indicates the significance on comparison of group III and IV with group II

dP< 0.001 indicates the dose-dependent significance on comparing group III and IV

3. RESULTS

3.1 *In vitro* drug release of PON1 from MPs

The optical microscopy, SEM analysisof PON1-MPs size was found 20 µm and clearly demonstrated that spherical shape with uniform distribution. The results were shown in Figure 1 & 2.

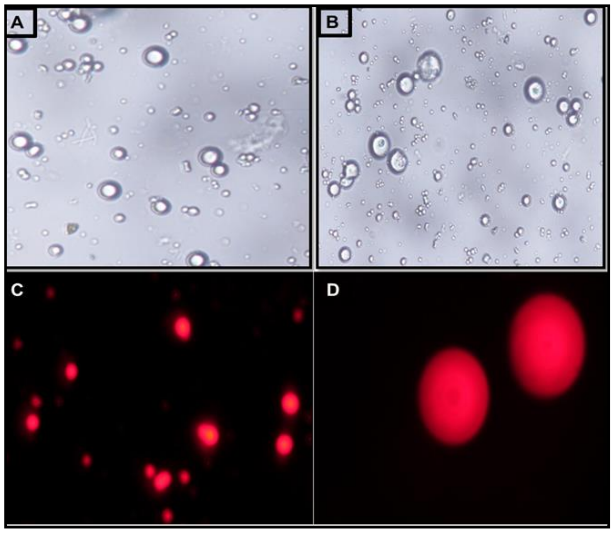


Figure 1: Optical microscopy images of PON1-MPs at different magnifications

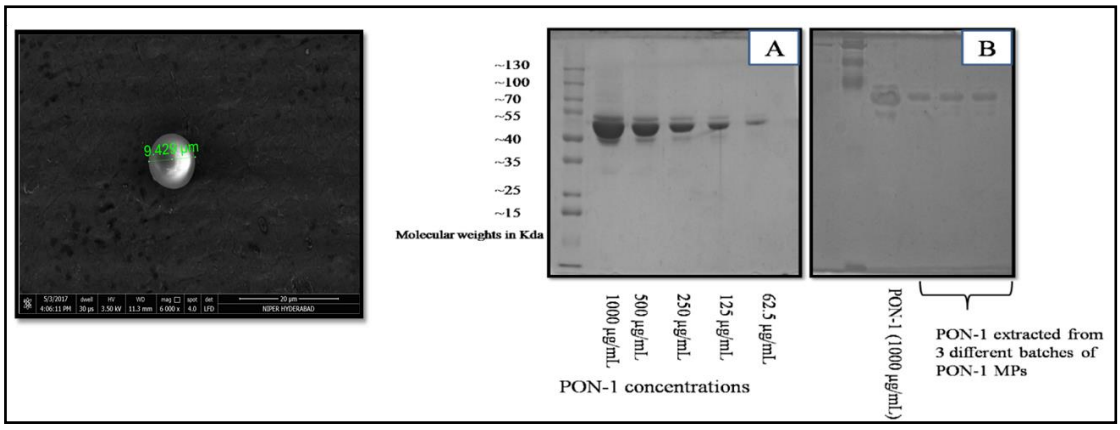


Figure 2: SEM showing smooth surface and spherical morphology of PON1-MPs.

When we performed the *in vitro* drug release study of PON1 from PON1-MPs, we have observed sustained release of PON1 from PLGA MPs up to 30 days. Whereas, the free drug released the drug within 24 h into the releasing medium after start of the release study.

Figure 3 shows the *in vitro* drug release profile of PON1 in free drug form and PLGA-MPs form. This data clearly suggesting that PLGA-MPs are producing sustained long-term release of entrapped drug from the PLGA-MPs matrix. This kind of sustained release is feasible with PLGA polymer-based MPs formulations.

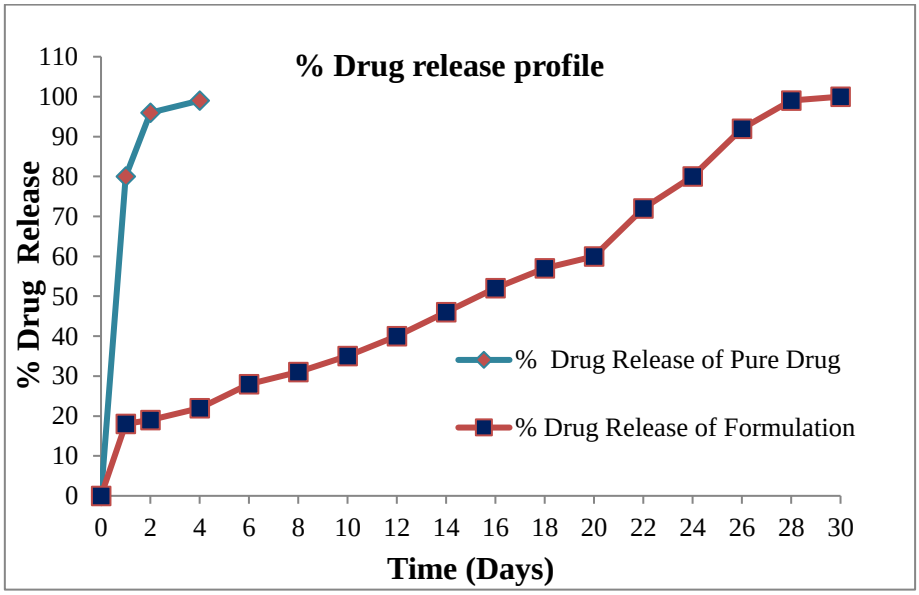


Figure 3: Percentage drug release profile of pure drug and PON1 formulation

Similarly, when conducted the release profile of Rho from PLGA-MPs, the 100% fluorescent dye present in MPs after the start of the release study and after 30 days, there was still some amount of Rho fluorescent observed in the PLGA-MPs matrix, which indicates that either loaded drug or fluorescent dye into PLGA-MPs release their entrapped contents,

slowly in sustained fashion. Figure 3 is representative microscopic images of release of Rho from MPs at different time intervals. These fluorescent intensities retained in the PLGA-MPs also indicate the sustained release pattern from PLGA-MPs. The bar graphs demonstrate the mean fluorescence emitted from Rho-MPs at different intervals of simulated *in vitro* release conditions.

3.1.1 Entrapment efficiency

EE was determined by weighting 0.5 g. of PON-1 MPs and were dissolved using suitable solvent. The sample was allowed for centrifugation for 2 h and the supernatant was filtered using whattman filter paper and was analyzed using UV-spectrophotometer at 405 nm. The % EE in PON-1 formulation was found to be 84.37%.

3.1.2 Drug content

The developed PON-1 MPs drug content was determined by diluting the particles in a suitable solvent and analyzed using UV spectrophotometer. The concentration of drug was found to be 89.71. Increase in the concentration of polymer decreases drug encapsulation of drug in to the matrix.

3.1.3 Calibration curve

A correlation coefficient (R^2) of 0.984 was found between 156.32-40,000 µg/mL using water. Results are shown in figure 4.

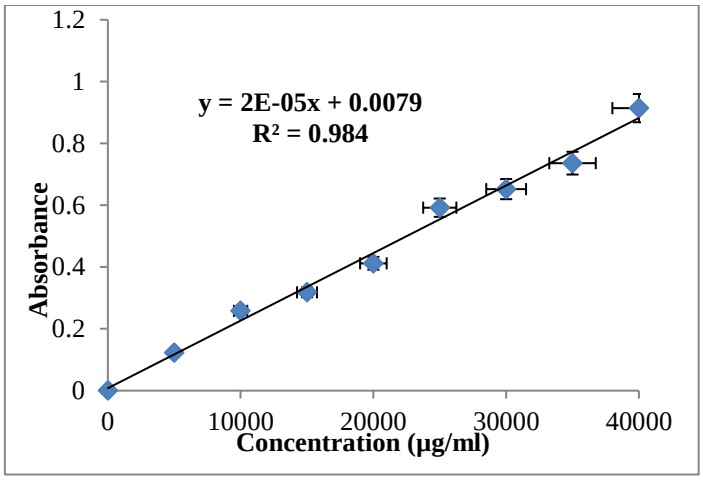


Figure 4: PON1-MPs calibration plot

3.2 *In-vivo* results

3.2.1 Evaluation of hepatotoxicity

3.2.1.1 Effect of PON1-MPs on liver weight / liver Index

Experimental animals liver weights are measured and liver index calculated using the following formula: Liver index (%) = weight of liver/weight of body x 100%. Results are shown in figure 5 and 6 shown.

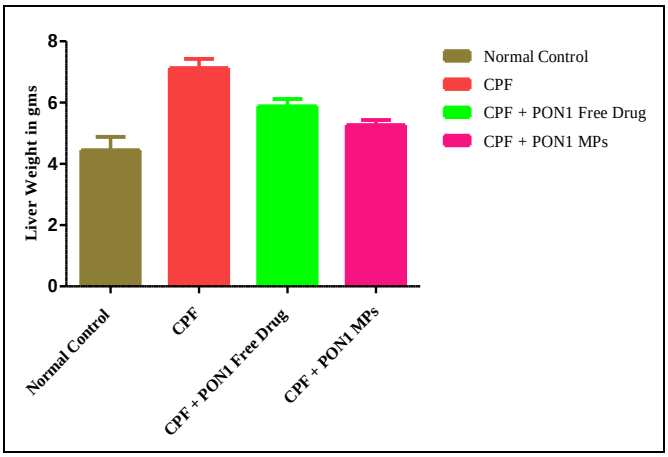


Figure 5: PON1-MPs on liver weight

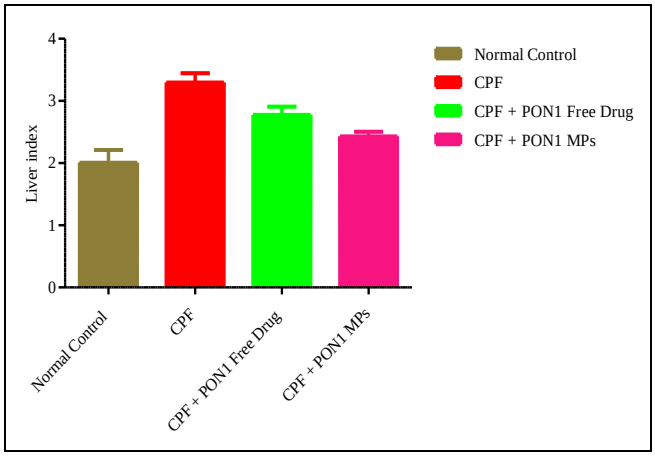


Figure 6: Effect of PON1-MPs liver index

3.2.1.2 Effect of PON1- MPs on liver ALT, AST, ALP

Administration of Chlorpyrifos (CPF) significantly ($P < 0.001$) increased liver ALT, AST and ALP when compared with the vehicle treated control group. Treatment with PON1 and PON1 micro particles ($P < 0.001$) attenuate the increased ALT, AST and ALP levels induced by CPF. Results are shown in table 1.

Table 1: Effect of PON1-MPs liver function test parameters (ALT, AST, ALP)

Experimental Groups	**ALT IU/L (Mean±SEM)**	**AST IU/L (Mean±SEM)**	**ALP IU/L (Mean±SEM)**
Normal Control	33.17±1.701	102.8±10.34	94.67±9.062
CPF	60.83±1.661[a]	183.2±5.319 [a]	179.2±4.571[a]
CPF+PON1 free drug	40.67±2.170[b,c]	154.1±4.575 [c]	140.2±7.115[c]

CPF+PON1-MPs	37.15±1.521[b,c]	146.8±3.361[b,c]	127.2±4.686[b,c]

3.2.1.3 Effect of PON1- MPs on AchE levels

The effect of PON1- MPs on AchE is shown in table 2. Administration of CPF significantly ($P < 0.001$) increased the AchE activity when compared to the vehicle treatedcontrol group. Treatment of PON1- MPs significantly ($P < 0.001$) attenuated the raise in enzyme level in both PON1 and PON1mps treated animals.

Table 2: Effect of PON1-MPs on Biochemical parameters (AchE, NO, TBARS)

Experimental Groups	**AchE (µM/mg protein)**	**NO (µg/mg protein)**	**TBARS (nM/mg protein)**
	(Mean±SEM)		
Normal control	33.17±1.701	2.435±0.214	1.12±0.04
CPF	60.83±1.661[a]	7.351±0.218[a]	5.06±0.22[a]
CPF+PON1 free drug	40.67±2.170[b]	4.864±0.128[b,c]	3.64±0.34[b]
CPF+PON1 -MPs	37.15±1.521[c]	3.412±0.514[c]	2.56±0.18[c]

3.2.1.4 Effect of PON1-MPs on nitrate levels

Administration of CPF significantly ($P < 0.001$) increased liver nitrite levels when comparedwith the control group. Treatment with PON1 and PON1-MPs significantly ($P < 0.001$) attenuated the increase in tissue nitriteinduced by CPF. Results are shown in table 2.

3.2.1.5 Effect of PON1-MPs on TBARS

Administration of CPF significantly ($P < 0.001$) increased TBARS when compared with the control group.Treatment with PON1 and PON1-MPs significantly ($P < 0.01$) attenuated the increase in TBARS induced by CPF. Results are tabulated in table 2.

3.2.1.6 Effect of PON1-MPs on SOD

Administration of CPF significantly ($P < 0.001$) decreased the activity of SOD when compared to the vehicle treated control group. Treatment with PON1 and PON1-MPs doses showed a significant increase ($P < 0.01$ and $P < 0.001$) in activity of SOD, respectively comparedto negative control group. The results are shown in table 3.

Table 3: Effect of PON1-MPs on biochemical parameters (SOD, catalase, GPx & MDA)

Experimental groups	**SOD (µg/mg protein)**	**Catalase (U/mg Protein)**	**GPx (U/mg protein)**	**MDA nM/mg protein**
	(Mean±SEM)			
Normal Control	22.18±0.541	1.68±0.08	26.12±1.21	36.24±0.88
CPF	8.148±0.422[a]	0.78±0.04[a]	15.35±1.89[a]	94.04±0.92[a]
CPF+PON1 free drug	16.32±0.327[b,c]	1.29±0.07[b]	21.08±0.98[b,c]	76.81±0.69[c]
CPF+PON1-MPs	21.32±0.824	1.46±0.06[c]	24.32±0.46[c]	41.68±0.98[b]

3.2.1.7 Effect of PON1-MPs on GPx

Administration of CPF significantly ($P < 0.001$) decreased the activity of GPxwhen compared to the vehicle treated control group. The twogroups of animals treated with PON1 and PON1-MPs showed a significant increase ($P < 0.001$) in activity of glutathione peroxidase, respectively compared to CPF treatedgroup. The results are shown in table 3.

3.2.1.8 Effect of PON1-MPs on catalase

Administration of CPF significantly ($P < 0.001$) decreased the activity of catalase when comparedto the vehicle treated control group. The two groups of animalstreated with PON1 and PON1-MPs doses showed a significant increase ($P < 0.001$) in activity of catalase compared to CPF treated group. The results are shown in table 3.

3.2.1.9 Effect of PON1-MPs on MDA

Administration of CPF significantly ($P < 0.001$) increased MDA levels when compared with the control group.Treatment with PON1 and PON1-MPs significantly ($P < 0.01$) attenuated the increase in MDA induced by CPF. Results are tabulated in table 3.

3.2.1.10 Effect of PON1-MPs on TNF-α level

Administration of CPF significantly ($P < 0.001$) increased TNF-α level when compared with the control group. Treatment with PON1 and PON1-MPs significantly ($P < 0.01$) attenuated the increase in TNF-α induced by CPF. Results are shown in figure 7.

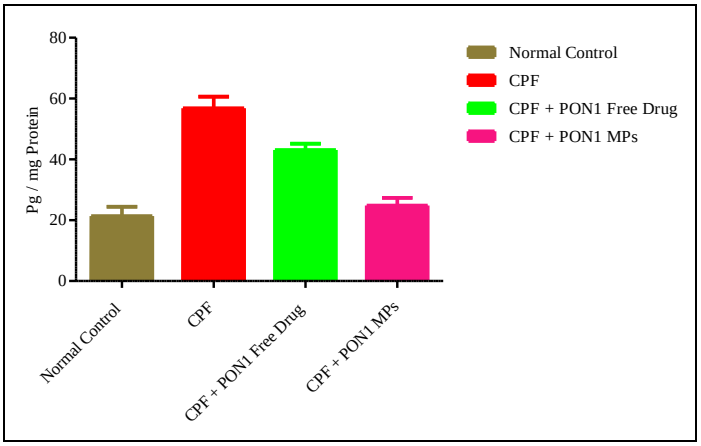


Figure 7: Effect of PON1-MPs on hepatic TNF-α level

3.2.1.11 Effect of PON1-MPs on IL-1β level

Administration of CPF significantly ($P < 0.001$) increased IL-1β levels when compared with the control group. Treatment with PON1 and PON1-MPs significantly ($P < 0.01$) attenuated the increase in IL-1β induced by CPF. Results are shown in figure 8.

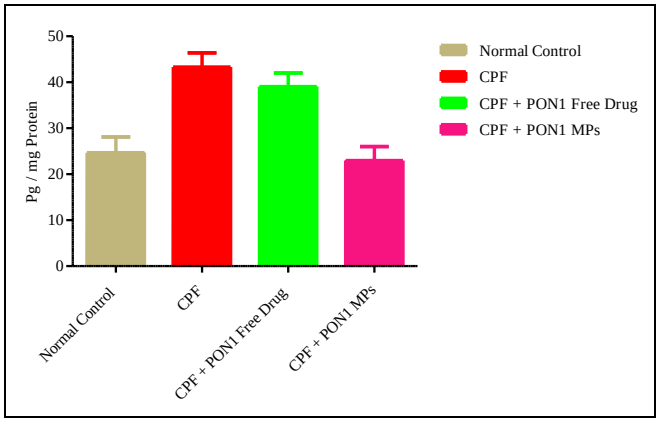


Figure 8: Effect of PON-MPs on IL-1β level

3.2.1.12 Effect of PON1-MPs on IL-6 level

Administration of CPF significantly ($P < 0.001$) increased IL-6 levels when compared with the control group. Treatment with PON1 and PON1-MPs significantly ($P < 0.01$) attenuated the increase in IL-6 induced by CPF. Results are shown in figure 9.

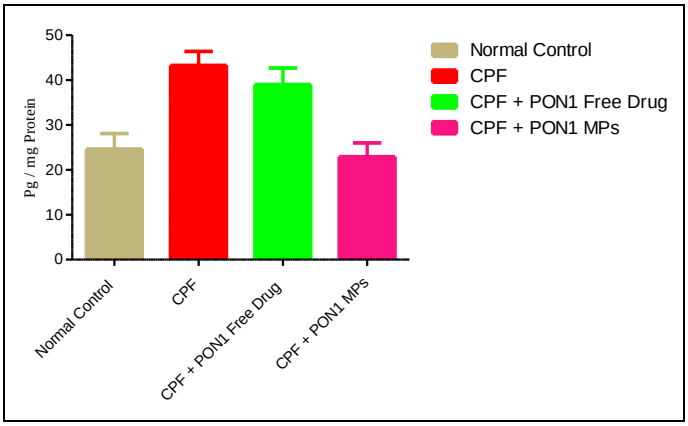
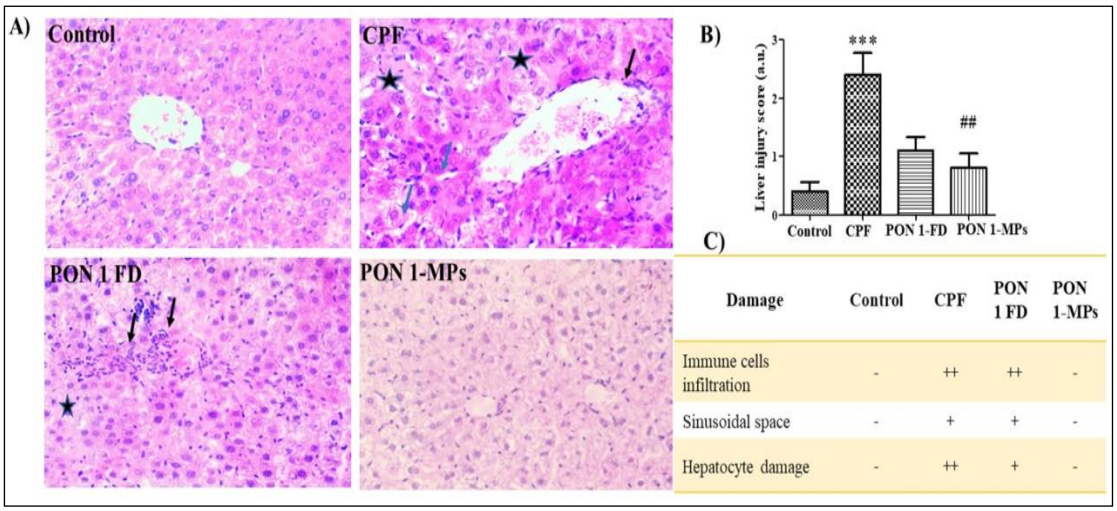


Figure 9: Effect of PON1-MPs on IL-6 level

3.2.1.13 Histopathological evaluation on liver toxicity & renal toxicity

Histopathological changes in liver tissue were shown in figure 10.



Damage	Control	CPF	PON 1 FD	PON 1-MPs
Immune cells infiltration	-	++	++	-
Sinusoidal space	-	+	+	-
Hepatocyte damage	-	++	+	-

Figure 10: Effect of PON1 mps on histopathological changes in liver Histopathological changes in liver tissues & (B, C) graphical and tabular representation of liver injury scoring of control; Immune cells infiltration (black arrow), sinusoidal space (blue arrow), and hepatocyte damage (star). A scoring system was followed to examine the severity of liver injury which includes 0 = no or minimum damage; 1 = mild; 2 = moderate; 3 = severe, 4 = more severe. All values are expressed as the mean ± SEM analyzed by one-way ANOVA with Tukey's multiple comparison test; ***P < 0.001 vs. Control; #P < 0.05; ##P < 0.01; ###P < 0.001 vs. DC.

4. DISCUSSION

The liver is the most prominent digestive gland that metabolizes drugs via oxidation, reduction, hydration, hydrolysis, condensation, conjugation, or isomerization. Two stages of hepatic drug metabolism convert pharmaceuticals into conjugated water-soluble substances via P450 enzymes, which are excreted out in urine or bile. Although the liver metabolizes drugs, disruption of these processes can lead to hepatotoxicity. Hepatotoxicity occurs through numerous mechanisms: disassembly of hepatocytes, apoptosis of hepatocytes, and injury to bile duct, inhibition of mitochondria, and cytolytic T-cell activation. Hepatotoxicity, or liver damage, is caused by hepatotoxins, & other sources are chemicals, dietary supplements, pharmaceutical drugs, and medicinal plants. Once hepatotoxicity is initiated, the following symptoms are observed: hepatic necrosis, fibrosis, vomiting, bleeding, swelling of the legs and feet, elevated serum transaminases, bilirubin, or cholestasis, liver cirrhosis, liver failure, and hepatic veno occlusive disease. Cirrhosis is marked by the degeneration of nodules enclosed by the fibrous glands of the liver, causing high portal blood pressure, and ultimately liver disease, due to deformity of hepatic vasculature. There are two forms of hepatotoxin-induced liver injury: Idiosyncratic injuries resulted from the formation of reactive metabolites and activation of the immune system. It is dose-independent and predictable. Intrinsic liver injuries are dose-dependent and reproducible. In the present investigation hepatoprotective function of PON1-MPs with improved efficacy was assessed by using *in-vivo* parameters. The paraoxonase (PON) gene family includes three members (PON1, PON2 and PON3) that are located next to each other on the long arm of human chromosome 7. PON1 and PON3 are mainly found in the bloodstream and bind to high-density lipoprotein (HDL) while PON2 is an intracellular enzyme. All three members of this family have antioxidant properties and play an important role in retarding the oxidative modification of low-density lipoprotein (LDL) and cell membranes. The term paraoxonase is originally derived from the enzyme's ability to hydrolyze paraoxon (diethyl p-nitrophenyl phosphate), which is metabolically produced from the insecticide parathion. PON1 is a calcium-dependent esterase / lactonase that is produced mainly in the liver, and is secreted into the blood, where it binds physically to the HDL particles. The antioxidant and antiatherogenic properties of HDL are largely attributed to PON1 among all the HDL-associated proteins. PON1 micro particles are showing % drug release in 30 h which indicated prolonged sustainable efficacy. The %percentage entrapment efficacy of PON1-MPs formulation was found to be 84.37% and the drug content was 89.71. Chlorpyrifos (CPF) is an organophosphate insecticide commonly used to treat fruit and vegetable crops. CPF can cause severe adverse effects on body organs

including the liver, kidney and central nervous system. CPF induced liver inflammation observed by using the various parameters which includes liver weight, liver weight index, ALT, AST and ALP. The experimental data revealed significantly ($P < 0.001$) decrease in liver weight and liver index and ALT, AST, ALP indication of hepatoprotective activity improved by PON1-MPs. Induction of hepatotoxicity by administration of CPF in experimental animals results decreased levels of antioxidant parameters, such as SOD, catalase, GPx, MDA and improved levels of NO & TBARS compared to vehicle treated animals. Supplementation of PON1 and PON1-MPs significantly counteracted the all changes in oxidative markers. CPF administration enhances the activity of AchE. PON1 and PON1-MPs administration inhibit the AchE activity due to elevated levels of potential anti-oxidant markers. The experimental data revealed that the PON1 MPs formulation possesses free radical scavenging effects. Nitric oxide formed during their reduction with oxygen or with superoxide, such as NO_2, N_2O_4, and $N_3O_4$is very reactive. These radicalsare responsible for altering the structure and functional behavior of hepatocytes. The PON1 MPs showed better activity incompeting with oxygen to react with nitric oxide and thus inhibitedthe generation of anions. Liver inflammatory mediators such as Tnf-α, IL-6 and IL-β counteracted significantly by the supplementation of PON1 and PON1-MPs. From these studies, it was finding that the PON1 MPs enhance the efficacy in terms of hepatoprotection.

CONCLUSION

In the present investigation principal reduction in AchE, elevated levels of anti-oxidant enzymes in hepatic tissue by the PON1 and improved efficacy by PON1 MPs contributed hepatoprotection against CPF induced hepatotoxicity. Further studies have to be emphasizing molecular mechanisms involved in hepatoprotection.

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