Research Article

Chlorpyrifos Produces Cardiovascular Risks by Shifting Set Point Lipid Metabolism and Diabetes Mellitus by inhibiting the Insulin Secretion in Rat

Suban Baskey[1], Kaushik Sarkar[1, 2], Mukti Mondal[1], Goutam Paul[1]*

[1]Molecular Neurotoxicology Laboratory, Department of Physiology, University of Kalyani, Kalyani, West Bengal, India.

[2] Department of Physiology, Krishnagar Government College, Krishnagar, Nadia, West Bengal, India.

* Professor, Department of Physiology, University of Kalyani, Kalyani, West Bengal, India.

***Corresponding author's E-mail:** goutampaul.ku@gmail.com

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ABSTRACT

We report here that chlorpyrifos (CPF), an organophosphate insecticide which is used widely in agricultural and domestic sectors, produces cardiovascular risks and diabetes mellitus in rat model. Adult male rats of Charles Foster strain weighing about 120-130 gm were exposed with CPF at a dose level of 19.4mg/kg BW/day, 38.84mg/kg BW/day, 58.24mg/kg BW/day for consecutive 10, 20 and 30 days were considered as test animals. We observed significant increase in the level of triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL) and very low density lipoprotein (VLDL); and significant decrease in the level of high density lipoprotein (HDL) in blood serum of CPF exposed rats dose and duration dependently compared to control groups of rat. This result suggests that CPF might induce the cardiovascular risks by changing the set-point lipid metabolism responsible for maintaining normal level of lipid profile variables. CPF probably induces the genesis of atherosclerosis by increasing the level of TG, TC, LDL and VLDL; and decreasing the level of HDL. We also observed significant increase in the total serum glucose level in CPF exposed rats compared to control rats. From the result, we hypothesize that CPF might induce the genesis of diabetes mellitus probably by inhibiting the β-cell function of the pancreatic islets to synthesize and release insulin, the hormone involved in glucose uptake by the tissues. Considering the above results, it is concluded that CPF induces cardiovascular risks and diabetes mellitus in rats probably by shifting set-point lipid metabolism and inhibiting the insulin secretion.

Keywords: Chlorpyrifos, cardiovascular risks, diabetes mellitus, low density lipoprotein, high density lipoprotein.

INTRODUCTION

Chlorpyrifos is a broad spectrum organophosphate insecticide which is widely used in agricultural and domestic sectors to fight against harmful insects [1].Right now, approximately 800 registered pesticide products in the market contain CPF [2]. Exposure to chlorpyrifos causes harmful effect on animal and humans[3]. Human beings are getting exposed to this chemical through ingestion of vegetables, inhalation and dermal exposure due to indiscriminate use of this chemicals[4]. After entering the body, it is absorbed quickly from the alimentary and respiratory tract, then metabolized and excreted through the urine and faeces[5].A few reports have stated that chlorpyrifos can alter the blood cholesterol and liver glycogen level. Jaroli and Sharma [6] reported that Chlorpyrifos causes significant increase in blood cholesterol level and decrease in liver glycogen level in *Channa punctatus*. From the investigation on rabbit it was found that chlorpyrifos induces the cholesterol and reduces the triglyceride level in the serum[7]. A study on broiler chicks revealed that Chlorpyrifos causes enhancement of TG, TC, LDL and drastically reduces the HDL level[8]. The effect of chlorpyrifos on mammalian body has also been reported by several groups of investigator. A group of scientists reported that chlorpyrifos significantly reduces the VLDL and TC level in treated groups of rats, and the other groups of scientists reported that chlorpyrifos significantly increase the level of VLDL and TC in treated groups of rats[9, 10]. Several research groups in India and abroad reported that chlorpyrifos exerts toxic effects on liver and particularly promotes cellular degeneration of the liver in fish, amphibia, birds and mice[11-16].

It has been well established that HDL is considered as good cholesterol, because it reduces the cardiovascular risks by lowering the LDL cholesterol[17]. Besides, HDL cholesterol plays an important role in ameliorating type-II diabetes mellitus. It helps to synthesize and secret insulin from the β cell of the islets of Langerhans of the pancreas. It has been studied that if the HDL level is lowered than the normal, the blood glucose level is increased due to inadequate amount of insulin in the blood [18], Because, insulin stimulates the synthesis of glycogen in the cells of liver and muscle[19]. The report about the effects of chlorpyrifos in inducing cardiovascular risks and metabolic syndrome like diabetes mellitus have not been reported till date. In this paper we report that chlorpyrifos produces cardiovascular risks and diabetes mellitus in rat.

MATERIALS AND METHODS

Animals

Studies were carried out on adult male albino rats of Charls Foster strain with initial body weight of 120-130gm.The rats were maintained in Animal House as per recommendations of the Kalyani University Animal Ethics Committee (KUAEC). The animals were kept under

standard environmental conditions (12 hours artificially day-night cycle and normal room temperature) during the acclimatization and experimental period. Rats were provided food and water *ad libitum*.

Reagents and chemicals

All the reagents used for this study were of analytical grade. Chlorpyrifos was purchased from Dow Agrosciences Pvt. Ltd, India. Sodium metabisulfate, activated charcoal, basic fuchsin, periodic acid were procured from Merck, Loba Chemie and SRL Diagnostics, India, respectively. Besides, the commercial kits were procured from Span diagnostics Ltd., India and ERBA Diagnostics Mannheim GmbH, Germany.

Animal exposure and grouping

After 2 week of acclimatization to the environment the rats were randomly distributed into four experimental groups along with their respective control groups (vide Table-1). Different doses of chlorpyrifos were selected according to the different percentage of oral LD_{50} value of chlorpyrifos in rats (i.e., 194mg/kgBW/day) and the doses were administered to animals by oral gavage.

Table 1: Experimental design for animal exposure of CPF

Group 1	Animal received distilled water for 10, 20 and 30 days durations- Control groups.
Group 2	Animal received 19.4mgCPF/KgBW/day for 10, 20 and 30 days durations (i.e., 10% of LD_{50} value).
Group 3	Animal received 38.8 mgCPF/KgBW/day for 10, 20 and 30 days durations (i.e., 20% of LD_{50} value).
Group 4	Animal received 58.2 mgCPF/KgBW/day for 10, 20 and 30 days durations (i.e., 30% of LD_{50} value).

Sample collection

After the end of each treatment duration rats were sacrificed by cervical dislocation on the 24th hour after completion of the last dosage. Therefore, the whole blood sample was collected by cardiac puncture. The serum sample was prepared by centrifugation of whole blood sample at 3000rpm for 15min at 4^{0}C at kept in -20^{0}C for further biochemical study. The segregated parts of liver tissues washed in buffer solution and kept in Bouin's fixatives for further histological study.

Biochemical assay

Serum glucose, cholesterol, HDL and triglyceride levels were estimated by using commercial kits (Glucose test kit, Autospan,Newcode:93DP100-74,cholesterol test kit, Autospan, Newcode-71LS300-56, HDL test kit, ERBA Diagnostics Mannheim GmbH, Germany,Cat.no-BLT00028, triglyceride assay kit, ERBA Diagnostics Mannheim GmbH, Germany XSYS0041), according to the kit instruction. VLDL was calculated followed by the method of Friedewald by using Friedewald formula (TG/5) given by Friedewald (20). LDL concentration (mg/dl) was estimated indirectly from the measured level of TG, HDL and cholesterol by using equation LDL= CHOLESTEROL – (VLDL+ HDL) [20].

Histopathological study

After fixing the liver tissues in Bouin's fixatives for 24 hours, the tissues were dehydrated in ascending grades of ethyl alcohol, cleaned in xylene until they become translucent and embedded to paraffin wax (melting point 58-60^{0}C). Uniform section of 5µm thickness were cut and stained with PAS followed by Lillie's Cold Schiff's method [21,22,23] and then observed under the light microscope (Olympus CH20i) fitted with camera (E-620 Olympus digital SLR camera).

Statistical Analysis

All the data obtained from this study were expressed as mean±SEM (n=7). Statistical analysis between the values obtained from control and treated rats were carried out by using ANOVA. p≤0.05 was considered statistically significant.

RESULTS

Effects of chlorpyrifos on some biochemical variables related to cardiovascular risks.

We observed significant increase in the level of LDL, VLDL, TG and TC in a dose and duration response manner in chlorpyrifos expose groups of rats in comparison with control groups of rats (Table-2). We also observed significant decrease in the level of HDL cholesterol in chlorpyrifos treated groups of rats compared with control rats. Further, in 10% exposure group we did not observe significant alterations of LDL, VLDL, TG, TC and HDL level (Table-2).

Biochemical variables	10 days Treatment duration				20 days Treatment duration				30 days Treatment duration			
	Control	10%	20%	30%	Control	10%	20%	30%	Control	10%	20%	30%
Glucose (mg/dl)	110.43 ±0.46	119.06 ±0.84	120.56± 0.53	125.63 ±0.40	109.13± 0.84	118.23 ±1.009	132.10± 0.88	141.83± 1.12	109.06 ±.80	122.86 ±1.19	136.26± .99	154.50 ±1.38
HDL(mg/dl)	69.26± 0.203	64.80± 0.520	60.83±0 .92	52.36± 0.47	68.33±. 41	61.30± 0.28	54.06±0 .145	46.00±4 .16	68.00±. 723	53.66± 0.52	48.10±0 .252	38.46± 0.40
LDL(mg/dl)	37.25± 0.35	46.40± 0.27	53.04±0 .32	63±1.3 3	37.60±0 .50	52.16± 0.96	59.36±0 .769	68.83±0 .499	38.74±. 091	58.80± 0.91	67.94±0 .38	79.68± 0.48
VLDL(mg/dl)	24.88± 0.24	25.20± 0.16	25.62±0 .14	26.63± 0.14	24.49±0 .20	25.60± 0.29	28.36±0 .25	31.30±0 .12	24.16± 0.24	25.80± 0.15	29.05±0 .15	33.24±. 21
TG(mg/dl)	124.4± 1.22	126±0. 81	128.13± .73	133.16 ±.742	122.46± 10.3	128.03 ±1.48	141.83± 1.28	156.50± 0.62	120.83 ±1.20	129±0. 75	145.26± 0.79	166.23 ±1.09
TC(mg/dl)	131.40 ±0.37	136.40 ±0.55	139.37± 0.53	142±0. 98	130.43± 0.63	139.06 ±0.93	141.13± 1.04	146.06± 0.73	130.90 ±0.40	138.26 ±.570	145.10± 0.43	151.40 ±0.55

Values are represented as Mean ± SEM (n=7), *p<0.05 vs. control group.

Table 2: Showing the changes in the level of some blood biochemical variables. HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density

lipoprotein, TG: Triglycerides and TC: Total cholesterol.10%, 20%, and 30% indicates 10%, 20% and 30% LD50 value of chlorpyrifos respectively in 10,20 and 30 days treatment durations.

Effect of chlorpyrifos on the blood glucose level

We found significant increase in the level of basal blood glucose level in chlorpyrifos treated groups of rat dose and duration dependently in comparison with control group of rats (Table-2).

Histopathological study: (PAS staining of liver tissues)

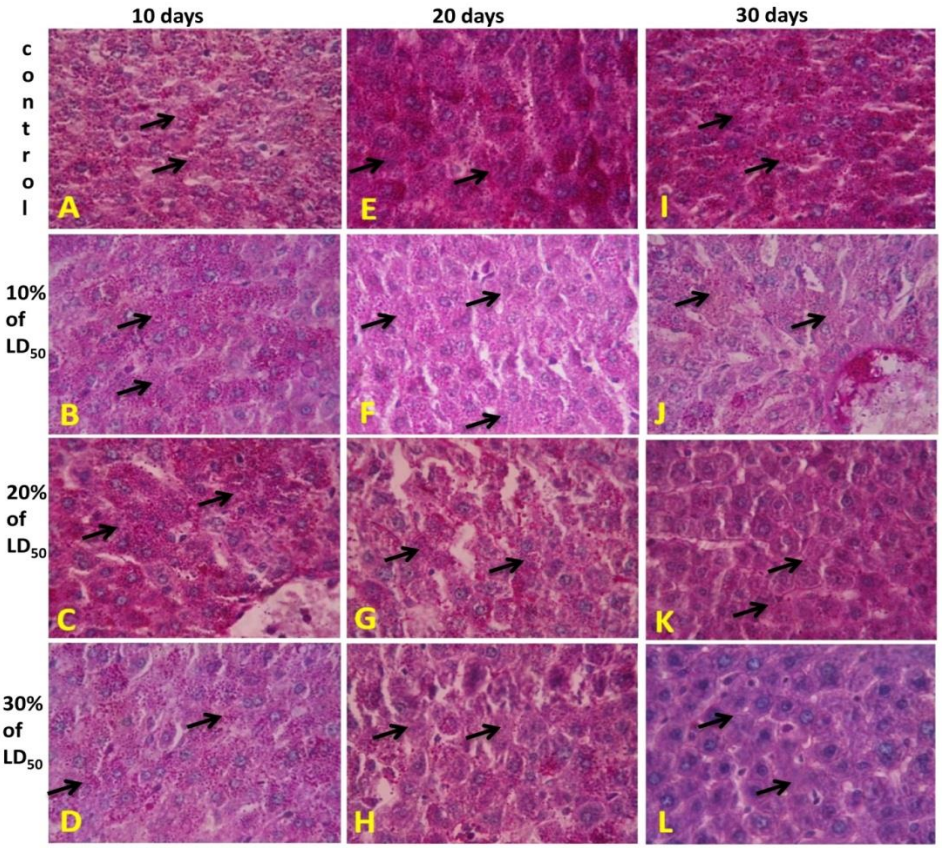

Figure 1: Microphotographs(400 X magnifications)of periodic acid-Schiff stained liver sections showing the density of distributed glycogen granules in CPF exposed and control rats. A,E,I : Control section of 10 days,20 days and 30 days exposure groups; B,F,J : Sections of liver exposed with 10%LD50 of CPF for 10 days, 20 days and 30 days exposure durations; C,G,K: Sections of liver exposed with 20% LD_{50} of CPF for 10 days, 20 days and 30 days exposure durations; D,H,L: Sections of liver exposed with 30% LD_{50} of CPF for 10days, 20 days, 30 days exposure durations. Arrow heads indicate glycogen deposition as granules in hepatocytes.

Effects of chlorpyrifos on the deposition of glycogen granules in liver tissues.

We observed significant decrease in the density of distributed glycogen granules in the liver cells (hepatocytes) in a dose and duration dependent manner, particularly in two higher doses in three exposure durations, in periodic acid-Schiff stained liver section of chlorpyrifos exposed rats compared to control (Fig-1). Though, we have found significant anatomical changes in the structure of liver in CPF treated rats. We observed significant degenerations of tissues, vascular enlargement, pyknosis and dialations of sinusoids.

DISCUSSION

To examine the effects of chlorpyrifos in inducing cardiovascular risks we have determined the lipid profile variable of chlorpyrifos exposed and control groups of rats in three exposure durations. In the present study we have found significant elevation of low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglycerides (TG) and total cholesterol (TC) from their basal levels in chlorpyrifos exposed groups of rats compared to control in a dose and duration dependent manner. Further, the level of HDL was significantly decreased in chlorpyrifos exposed rats compared to control in a dose and duration response manner also. LDL, VLDL, TG and TC are generally called cardiovascular risks generating factors considering their potentials to produce cardiovascular diseases. Because, these variables promote hypertension by forming atherosclerotic plaque in the lumen of the blood vessels. HDL is called good cholesterol because it prevents the formation of atherosclerotic plaque in the blood vessels and thus, counteracts the genesis of hypertension induced by LDL, VLDL, TG and TC. Because, HDL cholesterol reduces the level of LDL, VLDL, TG and TC in

the blood by inducing the transport of those variables to tissues. So, these results suggest that CPF increases the cardiovascular risks probably by decreasing the level of HDL and increasing the level of LDL, VLDL, TG and TC.

We have examined the serum glucose level of CPF exposed rats to find out the probable role of CPF in inducing diabetes mellitus. In our present study we observed a significant increase in the level of serum glucose in CPF exposed groups of rats in both dose and duration response manner. This result suggests that CPF probably decreases the release of insulin from pancreatic β-cells, because insulin normally maintains the blood glucose level at the basal range by inducing transport of glucose from blood to interior of the cells for utilization. The elevated blood glucose level beyond the basal range produces the symptoms of diabetes mellitus.

From the above results it can be concluded that CPF probably induces cardiovascular risks by increasing the level of risk producing lipid variables and lowering the risk minimizing lipid variable; and promotes the development of diabetes mellitus probably by decreasing secretion of insulin. The results may be extrapolated in human beings.

CONCLUSION

It is concluded that CPF induces cardiovascular risks and diabetes mellitus in rat.

REFERENCES

1. Sancherz-santed F, Candas F, Flores P, Lopez-grancha M,Cardona D, Long-term functional neurotixicity of paraoxon and chlorpyrifos: behavioural and pharmacological evidence, NeurotoxicologyTeratology, 26, 2004, 305-317.
2. Dutta J, Gupta (Dasgupta) J, Sarkar A, Sengupta D, Effect of organophosphorous insecticide phosphomidon on antioxidant defence components of human erythrocyte and plasma, Indian Journal of Experimental Biology, 30, 1992, 65-67.
3. Zhao Q, Gadagbui B,Dourson M,Lower birth weight as a critical effect of chlorpyrifos acomparison of human and animal data , Regulatory Toxicology and Pharmacology, 42(1), 2005, 55-63.
4. Caroline C,Pesticide and birds from DDT to todays's poison, Journal of pesticide Reform, 11(4), 1991, 3-5.
5. Davide LE, Robert BD, Herman A, James B, Patricia B, Lucio GC,Josep C, Hermann RS,Peter SS, Review of toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment, Critical review in Toxicology, 2, 2008, 1-125.
6. Jaroli DP, Sharma BL, Effect of organophosphate insecticide on the organic constituents in liver of *channa punctatus*,Asian Journal of Experimental Sciences,19, 2005, 121-129.
7. Solati A, Tavasoly A, Koohi MK,Marjamehr SH, Rezanjoo B, Effect of dermal exposure to chlorpyrifos on liver and brain structures and biochemicals parameters in rabbits, Comparative clinical pathology, 21, 2005, 1211-1217.
8. Bharati P, Reddy AG, Reddy R and Alpharaf M, A study of certain herbs against chlorpyrifos induced changes in lipid and protein profile in poultry, Toxicology international 11, 2011, 44-46.
9. Ambali SF, Shittu M, Ayo JO, Esievo KAN, Ojo SA, Vitamin C alleviates chronic chlorpyrifos induced alterations in serum lipids and oxidatives parameters in male wister rats, American Journal of Pharmacology and Toxicology. 6(4), 2011, 109-118.
10. Uchendu C, Ambali SF, Ayo JO, Umosen AJ,Subacute chlorpyrifos induced alteration in serum lipids and some oxidative stress biomarkers in male wister rat: benificial effect of acetyl-L-Carnitine, Toxicological and Environmental Chemistry, 95(3), 2013, 484-494.
11. Devi Y, Mishra A, Histopathological alteration in gill and liver anatomy of fresh water,air breathing fish *channa punctatus* after pesticide Hilban(chlorpyrifos) treatment, Advances in Bioresearch, 4(2), 2013, 57-62.
12. Aniladevi Kunjamma KP, Philip B, Bhanu SV, Jose J, Histopathological effects on *Oreochromis mossambicus* (Tilapia) exposed to chlorpyrifos, Journal of Environmental Research and Development, 4, 2008,553-559.
13. Khan S, Sharma N, Hepatocellular changes induced by chlorpyrifos in liver of *gambusia affinis*, International Journal of Research in Pharmacy and Life Science, 1(1), 2013 21-25.
14. Bandra MGDK, Wijesing MR, Ratnasooriya WD, Priyani AAH, Chlorpyrifos –induced Histopathological alteration in *duttaphrynus melanostictus*(Schneider 1799) tadpole : Evidence from empirical trials, Journal of Tropical forestry and Environment, 2(2), 2012, 27-36.
15. Kammon AM, Brar RS, Banga HS, Sodhi S, Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens, Veterinarski Arhiv, 80(5), 2010, 663-672.
16. Sharma A, Sodhi S, Apminder SB, Ghai JK, Chlorpyrifos –induced hematological,biochemical and histoarchitectural alterations in albino mice, International Journal of Advanced Research 1(10), 2013, 825-835.
17. Zhang Q, Zhang Y, Feng H, Guo R, Jin L, Wan R, Wang L, Chen C, Li S, High density lipoprotein (HDL) promotes glucose uptake in adipocytes and glycogen synthesis in muscle cells, PLoS one, 6(8), 2011, e23556.
18. Fryirs MA, Barter PJ, Appavoo M, Tuch BE, Tabet F, Heather AK, Rye KA, Effect of high-density lipoproteins on pancreatic β-cell insulin secretion, Arteriosclerosis Thrombosis and Vascular Biology, 30, 2010, 1642-1648.
19. Perry RJ, Samuel VT, Petersen KF, Shulman GI, The role of hepatic lipids in hepatic insulin resistant and type 2 diabetes, Nature, 510, 2014, 84-91.
20. Friedewald WT, Levy RI, Fredrickson DS, Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge , Clinical Chemistry, 18, 1972, 499-502.
21. Sheehan D, Hrapchak B, Theory and practice of histotechnology, 2nd Ed, Battelle Press, Ohio, 1980, 164-166.
22. Bancroft J, Steven s A, Theory and Practice of Histological Techniques, 2nd Ed, Churchill Livingstone, NY, 1982, 188-190.
23. Crookham J, Dapson R, Hazardous Chemicals in the Histopathology Laboratory, 2nd Ed, 1991, Anatech.

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