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# HEPATO-CELLULAR TOXICITY AMONG THE EXPOSURES TO THE DIESEL COMBUSTION NANO-PARTICLES

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ABSTRACT

Toxic organic byproducts of diesel combustion nano-particles are widely spread in the current environment and in more concentration in the congested urban areas and in non ventilated workshops. The present study conducted to evaluate the toxic effect of these nano-particles on hepatic function and biomarkers of oxidative stress among the exposed individuals. Serum sample was analysed for the determination of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvate transaminase (SGPT), total protein, and estimation of catalase (CAT), superoxide dismutase (SOD) and melanadehyde (MDA) among the control and exposed individuals. Our data showed a significant increase in SGOT, SGPT, total protein and albumin and also significant variation in oxidative stress parameters in serum among the cases exposed to diesel combustion nano-particles for more than 5years compared to the control population. These changes were more obvious in long term exposures of 5 and more years. Thus we could able to conclude that toxic organic byproducts of diesel combustion nano-particles affect adversely and alter biochemical markers in vital organ.

## INTRODUCTION

In the current scenario of industrial, and automobile development and misuse of these inventions, various health hazards has been gaining importance due to air pollution in the form of allergic manifestations, degenerative changes and increase incidence of malignancy. Chronic exposure to these nano-particles is related to multiple health, social and economic problems worldwide and adds on to the burden for the crippling health status in developing and under developed countries. Inflammatory process is the final pathway involved in the patho-physiology of these disorders. Various authors have opined that there is a direct or indirect relation between environmental nanoparticles on biochemical parameters related to hepatic function and oxidative stress biomarkers.[1,2,3,4,5]

The diesel combustion nano-particles mediated by oxidative stress factors are the major contributors to the morbidity and mortality of hepatocytes as the liver is the susceptible organ which plays a major role in detoxifying process. In this process the organic molecules are structurally and functionally modified resulting in oxidative stress generation, the reactive oxygen species (ROS) development. Brenner et, al., (1987), Chojkier et, al., (1989) and Houglum et, al., (1991) provided the evidence indicating that aldehyde-protein adducts, including products of lipid peroxidation, modulate collagen gene expressionwhich can cause the tissue injury. 6, 7, 8 It is a known fact that fibrinogenesis is the basis of suppression of inflammatory process. In the current study the diesel combustion nano-particles are the inflammatory agents which trigger the immune system causing various disorders among the apparently normal unprotected exposures. The normal physiology in hepatocytes can be altered due to associated alteration in lipid peroxidation which can occur in various disorders such as viral hepatitis, porphyria, alcoholic liver diseases, and carbon tetrachloride and other polycyclic hydrocarbon induced hepatic injury. [9,10,11,12]

It has been opined that the activation of stellate cells occurs due to oxidative stress induced activation of tumour necrosis factor- (TNF- ) and c-myb nuclear expression. Lipid peroxidation in cells, with the production of malondialdehyde markedly stimulates the activation of hepatic stellate cells and stellate cell entry into S-phase. The impending mediators of this model of oxidative stress in stellate cells include free radicals, reactive aldehydes or cytokines produced by the hepatocytes in response to diesel combustion nano-particles. Thus metabolomics is an emerging tool that can be used to gain insights into cellular and physiological responses.13 Toxic byproducts of diesel combustion nano-particles are widely spread in the environment and in more concentration in the congested urban areas and in non-ventilated workshops. The present study conducted to evaluate the toxic effect of these nano-particles on hepatic function, and biomarkers of oxidative stress in exposed individuals and compared with the control samples. In this context, we studied the levels of malonaldehyde an oxidative stress marker along with antioxidant enzymes like CAT, SOD and total antioxidant capacity (TAC) in relation to the hepato-cellular functional parameters.

## Materials and Methods

After the ethical clearance from Human Ethical Clearance Committee, University of Mysore, IHEC-UOM No. 123 PhD/2016-17, and consent from the subjects, a cross-sectional study was conducted in the Molecular Reproductive and Human Genetics Lab, Manasagangothri, University of Mysore, Mysore, Karnataka, India. The study population and sample size being 500 male garage workers of age group 25-60 years who were exposed to diesel combustion nanoparticles as a part of their occupation, for 6-8 hrs a day without using any protective aids during work, for 6-12 years. The control sample was 300 males of age 25-60 years. They were apparently healthy, live in hilly areas, had negligible or nil exposure to diesel combustion nanoparticles. The subjects were non-alcoholic, non-smoker, without any associated metabolic disorders. Questionnaires were administered to characterize the work practices, exposure history and use of protective equipments.

Serum sample was analysed for the determination of oxidative stress markers, including SOD, CAT, lipid peroxidation (LP) and hepato-cellular parameters like serum levels of bilirubin, SGOT, SGPT, total protein among the control and exposed individuals. Lipid peroxidation was measured by the melanaldehyde (MDA) level estimation. The results were tabulated and analysed statistically.

## Results

The estimated levels of antioxidant enzymes (SOD and CAT) and the oxidative stress marker (MDA) and hepato-cellular parameters

are depicted in Table 1 and 2. The levels of SOD, CAT, MDA and TAC showed 36.26%, 72.07%, 91.88% and 38.92% of variation respectively among the exposed and non-exposed individuals. (Table 1) The hepato-cellular functional parameters were impaired in the form of rise in the bilirubin, SGOT, SGPT and total protein level with or without clinical significance. Cases with clinical features like nausea, vomiting, pain abdomen showed increased level of serum SGOT and SGPT levels signifying the hepato-cellular toxicity. In such cases there were slight elevations of bilirubin level along with significant variation in serum total protein. (Table 3) Among the study population we could find 6 cases of Type 2 diabetes mellitus (T2DM). Majority of cases showing significant variation in ROS parameters were suffering from some clinical manifestations. (Table 3) Our data showed a significant increase in SGOT, SGPT, total protein and albumin in serum of the cases exposed to diesel combustion nano-particles for more than 5years compared to the control population. There were no other contributing factors like alcoholism, infections, long term usage of hepatotoxic drugs which can cause hepato-cellular toxicity. Among the exposures the activity of oxidative stress markers like SOD, CAT and TAC were reduced and the level of LP was increased. (Table 1)

**Table 1: Mean serum levels of SOD, CAT, and MDA among the exposed (n=500) and control group (n=300)**

| Oxidative stress enzymes and marker | Exposed (n=500) | Control (n=300) | % of variation |
|---|---|---|---|
| SOD% | 36.71 ±6.01 | 57.60±5.4 | 36.26 |
| CAT U/ml | 0.31±0.29 | 1.11 ±0.16 | 72.07 |
| MDA nmol/ml | 4.49±1.405 | 2.34±0.34 | 91.88 |
| TAC µg/ml | 62.3250±10.1549 | 63.3357±19.4038 | 1.59 |

**Independent Samples 'T' Test**

| | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|
| | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | Lower | Upper |
| LP | 16.242 | 798 | < 0 .001 | 1.45482 | 0.08957 | 1.27900 | 1.63065 |
| CAT | 3.490 | 798 | 0.001 | 0.00576 | 0.00165 | 0.00252 | 0.00900 |
| SOD | 5.972 | 798 | < 0.001 | 6.13497 | 1.02724 | 4.11856 | 8.15139 |
| TAC | -0.965 | 798 | 0.335 | -1.01069 | 1.04705 | -3.06598 | 1.04460 |

*p is significant <0.05

In this study significant mean difference was observed in SOD (p=0.000*), CAT (p=0.001) LP (p=0.000*) and TAC (p= 0.335) levels between case and control

**Table 2: Mean serum levels of hepato-cellular functional parameters among the exposed (n=500) and control group (n=300)**

| Hepato-cellular functional parameters | Exposed (n=500) | Control (n=300) | % of variation |
|---|---|---|---|
| Serum Bilirubin | 1.34±0.11 | 0.9±0.07 | 48.88 |
| SGOT (u/l) | 59.5±2.3 | 33±1.7 | 80.30 |
| SGPT (u/l) | 70.8±1.7 | 40±2.5 | 77 |
| Total proteins (g/dl) | 8.8±1.2 | 6.5±1.3 | 35.38 |

Table 3: Clinical features and mean serum levels of oxidative stress and hepato-cellular parameters among the exposed group (n=500) and controls (n=300) t-test groups=var00001(1 2) /missing=analysis/variables=alp alt dir bil ast total pro

| | Clinical Feature | No. of Cases | SOD% | CAT u/ml | MDA nmol/ml | TAC µg/ml | Serum Bilirubin | SGOT u/l | SGPT u/l | Total proteins g/dl |
|---|---|---|---|---|---|---|---|---|---|---|
| Exposures | T2DM | 6 | 25.33± 6.14 | 0.25± 0.02 | 6.14± 2.12 | 108.4± 23.2 | 0.9± 0.1 | 38± 3.2 | 91.24± 6.4 | 8.5 |
| | Abdominal pain and Diarrhoea | 34 | 41.73± 11.21 | 0.35± 0.07 | 3.94± 0.88 | 160.6± 9.6 | 1.4± 0.23 | 44± 3.4 | 58.12± 3.3 | 8.2 |
| | Nausea | 26 | 42.22± 10.41 | 0.34± 0.097 | 3.87± 1.03 | 183.96± 14.5 | 1.3± 0.12 | 40± 2.3 | 35.45± 1.5 | 7.4 |
| | No clinical features | 434 | 51.70± 3.4 | 0.81± 0.16 | 3.12± 0.16 | 258.96± 14.34 | 0.86± 0.42 | 29± 10.4 | 38.14± 6.4 | 7.8 |
| Controls | | 300 | 57.60± 5.4 | 1.11 ±0.16 | 2.34± 0.34 | 290.45± 40.43 | 0.92± 0.02 | 28± 1.3 | 36± 1.6 | 6.2 |

T-TestNotes

| Notes | | |
|---|---|---|
| Output Created | 28-SEP-2018 18:12:21 | |
| Comments | | |
| Input | Active Dataset | DataSet0 |
| | Filter | <none> |
| | Weight | <none> |
| | Split File | <none> |
| | N of Rows in Working Data File | 800 |
| Missing Value Handling | Definition of Missing | User defined missing values are treated as missing. |
| | Cases Used | Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis. |
| Syntax | T-TEST GROUPS=VAR00001(1 2) /MISSING=ANALYSIS /VARIABLES=ALP ALT DirBil AST TotalPro /CRITERIA=CI(.95). | |
| Resources | Processor Time | 00:00:00.02 |
| | Elapsed Time | 00:00:00.04 |

[DataSet0] Group Statistics

| Group Statistics | VAR00001 | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| ALP | 1.00 | 500 | 101.3819 | 82.92987 | 3.70874 |
| | 2.00 | 300 | 99.2653 | 71.20183 | 4.11084 |
| ALT | 1.00 | 500 | 38.3577 | 15.35435 | .68667 |
| | 2.00 | 300 | 28.3100 | 13.06405 | .75425 |
| Dir Bil | 1.00 | 500 | .8571 | .49559 | .02216 |
| | 2.00 | 300 | .2859 | .23528 | .01358 |
| AST | 1.00 | 500 | 41.3225 | 15.59719 | .69753 |
| | 2.00 | 300 | 31.1733 | 13.39258 | .77322 |
| Total Pro | 1.00 | 500 | 9.9421 | 1.84365 | .08245 |
| | 2.00 | 300 | 7.0979 | .24139 | .01394 |

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | |
|---|---|---|---|---|---|
| | | F | Sig. | T | df |
| ALP | Equal variances assumed | 169.731 | .000 | .368 | 798 |
| | Equal variances not assumed | | | .382 | 704.255 |
| ALT | Equal variances assumed | .358 | .550 | 9.463 | 798 |
| | Equal variances not assumed | | | 9.851 | 708.429 |
| Dir Bil | Equal variances assumed | 182.756 | .000 | 18.731 | 798 |
| | Equal variances not assumed | | | 21.972 | 764.313 |
| AST | Equal variances assumed | 12.435 | .000 | 9.384 | 798 |
| | Equal variances not assumed | | | 9.746 | 704.215 |
| Total Pro | Equal variances assumed | 269.191 | .000 | 26.578 | 798 |
| | Equal variances not assumed | | | 34.014 | 527.202 |

Independent Samples Test

| | | t-test for Equality of Means | | |
|---|---|---|---|---|
| | | Sig. (2-tailed) | Mean Difference | Std. Error Difference |
| ALP | Equal variances assumed | .713 | 2.11655 | 5.75039 |
| | Equal variances not assumed | .702 | 2.11655 | 5.53658 |
| ALT | Equal variances assumed | .000 | 10.04772 | 1.06174 |
| | Equal variances not assumed | .000 | 10.04772 | 1.02001 |
| Dir Bil | Equal variances assumed | .000 | .57115 | .03049 |
| | Equal variances not assumed | .000 | .57115 | .02599 |
| AST | Equal variances assumed | .000 | 10.14915 | 1.08154 |
| | Equal variances not assumed | .000 | 10.14915 | 1.04135 |
| Total Pro | Equal variances assumed | .000 | 2.84425 | .10702 |
| | Equal variances not assumed | .000 | 2.84425 | .08362 |

Independent Samples Test

| | | t-test for Equality of Means 95% Confidence Interval of the Difference | |
|---|---|---|---|
| | | Lower | Upper |
| ALP | Equal variances assumed | -9.17113 | 13.40422 |
| | Equal variances not assumed | -8.75363 | 12.98673 |
| ALT | Equal variances assumed | 7.96358 | 12.13186 |
| | Equal variances not assumed | 8.04513 | 12.05031 |
| Dir Bil | Equal variances assumed | .51129 | .63100 |
| | Equal variances not assumed | .52012 | .62218 |
| AST | Equal variances assumed | 8.02614 | 12.27215 |
| | Equal variances not assumed | 8.10462 | 12.19367 |
| Total Pro | Equal variances assumed | 2.63419 | 3.05432 |
| | Equal variances not assumed | 2.67998 | 3.00852 |

## DISCUSSION

In the present scenario, of industrialization and modernization, the living cells are subjected to enormous stress, to which they respond by altering their cellular metabolism and activating their defence mechanisms. Biochemical system is the first to respond to any stress. The stress response consists of stress proteins, also termed as heat shock proteins and antioxidants, both enzymatic and non-enzymatic, which are the primary protective responses that are highly conserved components of cellular stress.[14] The capacity of diesel combustion nano-particles to increase the oxidative hepato-cellular damage is mainly due to the structural and functional modifications of the organic molecules. These in turn alters the interstitial permeability and translocations of the hepatocytes. Along with the oxidative stress factors, the pro-inflammatory cytokinines like TNF- facilitates hepato-cellular damage.15 The mechanism responsible for oxidative stress induced by diesel combustion nano-particles include intra-hepato-cellular formation of ROS and extra cellular production of ROS by infiltrated inflammatory cell.[15, 16] The ROS production is a natural process for cellular stress, and continual, elevated levels of ROS can produce the ill effect on the cellular system. Thus the antioxidant systems with enzymatic and non-enzymatic mechanisms aim at the neutralization and protection of cells against ROS. These include SOD, which detoxifies the superoxide ion, CAT and the GSH peroxidase system, peroxiredoxins, which inactivate hydrogen peroxide ($H_2O_2$), and glutathione peroxidase, whose function is to detoxify cellular peroxides. There are also non-enzymatic, low-molecular-weight antioxidants, such as GSH, vitamin E, ascorbate (vitamin C), vitamin A, ubiquinone, uric acid, and bilirubin which play a major role in preventing oxidative stress.[17,18]

The activity of Cytochrome P450 in particular enables the formation of free radicals. All these metabolic events contribute together and exacerbate the hepatocyte response for the endotoxins. This cellular toxicity has a proportionately high effect on the cells which are exposed to the stress of nano-particles. The capacity of diesel combustion nano-particles to trigger the immune system can be observed by protein adducts with hepatic proteins. (Table 2) Oxidative stress has been implicated in the induced immunity through the detection of antibodies. The increased circulating antibody titres can be induced by the modifications of proteins and derived products of lipid peroxidation like melanaldehyde and hydroperoxides that are prevalent in advanced liver disorders.19, 20 However condensation products will be formed from the residues of protein molecules and melanaldehyde that further triggers the immune system aggravating the disorders.21, 22

Oxidative hepatic damage is also explained by adenosine monophosphate activated protein kinase (AMPK) pathway which helps in metabolic homeostasis, redox status and mitochondrial respiration in cells and prevent oxidative stress induced cell death.22 AMPK activation is mediated by different stimuli like hyperglycemia, Type 2 diabetes mellitus (T2DM), which has to be treated pharmacologically.[23, 24, 25] In the present study we could appreciate this condition so that we could be able to detect 6 cases of newly identified T2DM cases which could be correlated to AMPK inhibition and ROS activation occurs by different pathways, as per Moreno and Reyes.22 Choi et al revealed that the AMPK cascade was highly sensitive to ROS.26 Common vascular disease states including diabetes, hypertension and atherosclerosis are associated with endothelial dysfunction, characterised by reduced bioactivity of oxidative stress markers and the vascular endothelium in diabetic vessels is a net source of superoxide.27 The severity of reduction in SOD, CAT and TAC activities was proportionately reduced as the severity of illness increased. In chronic disorder like chronic obstructive pulmonary disorder, T2DM and hypertension these parameters were significantly reduced when compared with the cases with simple clinical ailments. This was in consistent with Moller28 and Steiner et., al.29 where the effects of diesel combustion nanoparticles on the activities of antioxidant enzymes, SOD and CAT, in blood was altered by 21.2% and 35.1% respectively, when compared with the controls and the MDA concentration was increased by 81%. Moller28 and Radu et al.,30 concluded that there exists a reliable relationship between exposure to diesel combustion derived nanoparticles and oxidative markers in urine, blood and exhaled-breath condensate. Diesel combustion nanoparticles and their constituents, generate oxidative stress in a number of cell types crucial to the development of disorders. MDA levels were significantly increased (Table 1) reflecting the more amount of LP.

In the present study there is convincing evidence that oxidative stress markers are significantly altered among the exposures which can be declared as due to the diesel combustion nanoparticle. The antioxidant scavenging enzymes SOD and CAT activities in blood were reduced due to the oxidative stress among the exposures along with increase in the LP and associated reduction of TAC. This reveals significant plasma membrane destruction due to the nanoparticle induced inflammation and reduced scavenging activity in plasma which accords with the conclusion of Gill-villa et al., here they assessed for LP.31 In clinically presenting cases along with the treatment proper, co-administration of free radical scavengers or other antioxidant compounds like SOD, N-acetyl cystine, tiron, cysteine prodrugs prevents particulate matter-induced cardiovascular impairment and early cell degeneration and also aging.32, 33, 34 This will have the capacity to reverse some aspects of the particulate matter-induced cardiovascular impairment suggesting that oxidative stress is an important mechanism in the cardiovascular disorders.35, 36, 37

Thus in the present scenario of industrial and automobile health hazards it is mandatory to recommend the usage of antioxidants in such to reduce the ROS generation.

## Conclusion:

Chronic exposure to diesel combustion nano-particles has an important role in oxidative stress induction and in turn generation of hepato-cellular damage. Because of multi- factorial etiology liver damage has become a difficult strategy to treat. Thus to improve the functional status of liver, the toxicity has to be blocked, early and periodic screening for oxidative stress factors in relation to the hepatic parameters and primary treatment with antioxidants to reduce ROS and detoxifying agents has be considered as a primary preventive measure of hepato-toxicity among the exposures to diesel combustion nano-particles.

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