Supplementary Files

S1 Isolation of Monocytes

A 3 ml volume of Histopaque 1083 solution was placed in a 15 ml tube and 3 ml blood was layered on top of this density gradient. After the centrifugation (400×g for 30 min at room temperature) the blood cells were separated into two fractions: an upper white layer consisting of mononuclear cells plus the majority of platelets at the interface region, and a lower layer containing erythrocytes and granulocytes. The plasma layer on top was clear and contained no cells. First the plasma layer was removed and discarded. From the buffy coat, the monocytes were carefully taken off by aspiration and washed with phosphate buffered saline (PBS).

S2 Haemolysis study

Hemolysis was determined by the method of Koziara *et al.,* (2005).

Reagents

a) 0.89% NaCl
b) 0.2% solution of sodium carbonate

Procedure

Human blood was collected from healthy individuals in EDTA containing vials. 0.9 ml blood was mixed with 0.1 ml saline (control), silica nanoparticles (positive control) and 0.1 ml PAH nanocapsule (Test). Samples were incubated at 37^0C for 30 min and vortexed. Equilibrated at room temperature for 2 min and intact red blood cells were separated from plasma by centrifugation at 600 x g for 5 min. supernatant was collected and incubated for 30 min at room temperature to allow for hemoglobin oxidation. Oxyhemoglobin absorbance was measured spectrophotometrically at 540 nm and the percentage of RBC lysis was determined by subtracting the absorbance of the test sample from negative control and further divided by the difference between positive and negative control.

S3 Cytotoxicity studies

Trypan blue dye exclusion method

Trypan blue dye exclusion method (Freshney1993). First, cells were cultured and treated with different concentrations of PAH and silica particle a 96 well plate. Then, the cells were dissociated from the bottom of the plate by trypsin. Finally, 10 µL of the Trypan blue dye was added to each well. The viable cell amount was counted through a hemocytometer, and the viability values were derived by comparing the samples with the negative control. Cells

that showed signs of staining were considered to be dead, whereas those that excluded trypan blue were considered viable.

Lactate dehydrogenase release

The release of LDH was monitored over the PAH concentration range described above. For this, Cyto-Tox 96® Non-Radioactive Cytotoxicity Assay kit (Promega) was used. This is a colorimetric assay that quantitatively measures LDH, a stable cytosolic enzyme that is released upon cell lysis. The LDH released in culture supernatants is measured with a 30 min coupled enzymatic assay (Legrand 1992). The amount of colour developed is proportional to the number of lysed cells. Cells were seeded in 24-wellplates, exposed to PAH and silica particle and incubated for 24, 48 and 72 h. After incubation, the cell media were collected and 50 µL of each sample was plated in a 96-well plate in triplicate. Next, 50 µL of substrate solution was added to the wells, and plates were incubated for 30 min at room temperature. The relative LDH leakage (%) related to control wells containing cell culture medium only was calculated using the relationship, A test/($A_{control}$ +A_{lysed}) x100. Where A test and A control are average absorbances from the spontaneous LDH release of the tested sample and control in the medium, and A lysed is the absorbance from the intentionally lysed cells of the control sample. Upon addition of assay solutions, the media was protected from the light for 30 min. During this inoculation time, NAD is reduced to NADH using the LDH released in the medium. The resulting absorbance was measured at 490 nm using microplate reader (Biorad, USA).

MTT assay

After the treatment of cells with PAH and silica particle with different incubation time, 10 µL stock MTT (5 mg/mL) was added to each well, and cells were incubated at 37^{o}C for 3 h. Cells were lysed with acidulated sodium dodecyl sulfate. Absorbance was measured at 570 nm using microplate reader (Biorad, USA).

Neutral red uptake (NRU) assay

The neutral red cell viability assay provides a quantitative estimation of the number of viable cells in a culture condition (Garcia et al.1993). It is based on the ability of viable cells to incorporate and bind neutral red and predominantly accumulates in lysosomes. Cells were treated with different concentrations of PAH and silica particle incubated for different time intervals. After incubation, the plates were treated with medium containing neutral red for 2 h. The cells are subsequently washed, the dye in each well was extracted and the absorbance at 540 nm, was measured using a micro plate reader (Biorad, USA).

S4 Reactive oxygen species (ROS)

Direct evidence of intracellular oxidation using the oxidant-sensitive probe 2'.7'-dichlorofluorescin diacetate (DCFDA) was used to measure the level of ROS, which is expressed as fluorescence intensity/mg protein. cells were seeded at a concentration 1x 10^6 cells per ml in six well culture plates and incubated in a humidified condition at 37^0C and 5% CO_2 for 4 h. PAH and silica particle were dispersed in RPMI medium to a final concentration and added to cell suspensions, then allowed to incubate for 6 and 24 h.. 150 µl of the cell suspension after PAH and silica particle treatment was taken and made up to 990 µl with phosphate buffer. 10 µl DCFDA in ethanol was added and incubated at 37^oC for 30 min. Supernatant obtained after centrifugation at 10,000 rpm for 15 min was analysed for fluorescence at an excitation wavelength of 502 nm and emission wavelength of 523 nm using a spectrofluorimeter (Perkin-Elmer LS-3B, Norwalk, NJ, USA).

S5 Lipid peroxidation level in PBMCs

The content of lipid peroxides in cultured cell lysate was determined by the classical method of measuring TBARSs. Briefly, cells (in a P100 dish) were preincubated for 1 hour in the presence of BSA followed by a 3-hour incubation in phenol red free medium (to avoid interference with absorption at 532 nm), in the continued presence of BSA or FA/BSA. Some wells were supplemented with the antioxidants indicated in Results. Cells were then washed twice in cold PBS, scraped off the dishes, and then suspended in 12 ml of PBS supplemented with BHT (to prevent any further lipid peroxide formation). After centrifugation at 2,000 g for 5 minutes, the supernatant was discarded, and the cells were resuspended in PBS with BHT. The cell suspension was combined with freshly made 30% trichloroacetic acid, 0.75% thiobarbituric acid, and 0.5N HCl, and incubated for 15 minutes at 100°C. The reaction mixture was then centrifuged for 8 minutes at 13,500 g. The absorbance of the supernatant was measured at 532 nm (the contribution of absorbance by a peak at less than 532 nm was deducted). The concentration of MDA equivalents was calculated using an extinction coefficient. The rest of the cells were used for protein determination using the DC protein assay kit (Bio-Rad, Hercules, California, USA). Similar assays were performed on stock solutions of FA/BSA complexes to detect pre-existing lipid peroxidation, or on medium placed in wells without cells to detect lipid peroxidation independent of cellular metabolism. In both cases, there were no TBARSs above background

S6 Inflammatory markers status

Assay of Cyclooxygenase

The assay of COX were measured by TBA method (Shimazu et al, 1981). The assay mixture contained Tris-HCl buffer, glutathione, hemoglobin and enzyme. The reaction was started by the addition of arachidonic acid. Then terminated after 1 minutes incubation at 250C by addition of 0.2ml of 10% TCA in 1N HCl, mixed and 0.2ml of TBA was added. The contents heated in a boiling water bath for 20 minutes, cooled and centrifuged at 1000 rpm for 3minutes. The supernatant was measured at 532nm for COX activity.

Assay of Lipoxygenase

The assay of LOX was measured by Axelrod. B *et al.* method (1981). 70mg of linoleic acid and equal weight of Tween 20 was dissolved in 4ml oxygen free water and mixed back and forth with a pipette avoiding air bubbles. Sufficient amount of 0.5N NaOH was added was added to yield a clear solution (0.55ml) and then made upto 25ml using oxygen free water. This was divided into 0.5ml portions and flushed with nitrogen gas before closing and kept frozen until needed.

S7 Toxicity markers status *in vivo*

Estimation of the activity of Serum Glutamate Oxaloacetate Transaminase

Activity of SGOT was measured by the method (Reitman and Frankel 1957).

Reagents

a) 0.15 M phosphate buffer at pH 7.5
b) Substrate: dissolved 0.3 g L-Aspartate and 50 mg of α-oxoglutarate in 20-30 ml of phosphate buffer and to this added10% NaOH to bring the pH to 7.5. The volume was made up to 100 ml with phosphate buffer.
c) Aniline-Citrate reagent: dissolved 50 g citric acid in 50 ml of distilled water and to this add an equal volume of redistilled aniline.
d) DNPH reagent: dissolved 200 mg of 2-4-dinitrophenyl hydrazine in 85 ml of concentrated HCl and make up to 1 L with distilled water
e) 0.4 N NaOH
f) 1% Pyruvate (in water)

Procedure

1 ml of substrate was incubated at 37°C for a few minutes. To this, 0.2 ml of serum was added and shaken gently. No serum was added to the control tubes. Exactly after 1 h, 0.07 ml of aniline-citrate reagent was added and 0.2 ml serum to the control tube after the addition of aniline-citrate reagent. After 20 min, added 1 ml of DNPH reagent to all the tubes and

incubated for another 20 min. Removed the tubes from the bath and added 10 ml of 0.4 N NaOH and absorbance was read at 520 nm after 10 min against reagent blank taking distilled water instead of serum. A pyruvate standard was prepared and activity was calculated using a standard activity chart.

Estimation of the activity of Serum Glutamate Pyruvate Transaminase (SGPT)

Activity of SGPT was measured by the method (Reitman and Frankel 1957).

Reagents

a) 0.15 M Phosphate buffer at pH 7.5
b) Substrate: Dissolve 5.0 g of DL-alanine and 20 mg of α-oxoglutaric acid in 20-30 ml of phosphate buffer and added 0.5 ml of10% NaOH to bring the pH to 7.5.
c) Aniline-Citrate reagent: Dissolve 50 g citric acid in 50 ml of distilled water and to this add an equal volume of redistilled aniline.
d) DNPH reagent: dissolved 200 mg of 2-4-dinitrophenyl hydrazine in 85 ml of concentrated HCl and made up to 1 L with distilled water.
e) 0.4 N NaOH
f) 1% Pyruvate (in distilled water)

Procedure

2 ml of substrate was incubated at 37°C for a few minutes. To this, 0.2 ml of serum was added and shaken gently. No serum was added to the control tubes. Exactly after 30 min, 0.07 ml of aniline-citrate reagent was added and 0.2 ml serum to the control tube after the addition of aniline-citrate reagent. After 20 min, added 1 ml of DNPH reagent to all the tubes and incubated for another 20 min. Removed the tubes from the bath and added 10 ml of 0.4 N NaOH and absorbance was read at 520 nm after 10 min against reagent blank taking distilled water instead of serum. A pyruvate standard was prepared and activity was calculated using a standard activity chart.

Estimation of the activity of Creatine kinase (CK) in serum

CK was assayed by the spectrophotometric, kinetic procedure described (Rosalki 1967).

Reagents

a) Homogenising solution: 0. 25 M sucrose, 0.001M EDTA and 0.1 mM mercaptoenthanol.
b) Buffer: 0.2% bovine serum albumin (BSA) and 0.01 M Tris, pH 7.4.
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c) 1 mM ADP
d) 10 mM Creatine phosphate
e) 10 mM Glucose
f) 30 mM MgCl2
g) 0.8 mM NADP
h) 10 mM AMP
i) 5 mM cysteine-HCI
j) 0.8 IU hexokinase
k) 0.3 IU glucose-6-phosphate dehydrogenase

Procedure

CK activity in the supernatant fraction was assayed after appropriate dilutions of sample/serum were made in a buffer containing 0.2% bovine serum albumin (BSA) and Tris 0.01 M, pH 7.4. Samples were assayed at 30°C in a reaction mixture containing Tris, 50 mM; ADP, 1 mM; creatine phosphate, 10 mM; glucose, 10 mM; MgCl2, 30 mM; NADP, 0.8 mM; AMP, 10 mM; cysteine-HCI, 5 mM; hexokinase, 0.8 IU; glucose-6-phosphate dehydrogenase, 0.3 IU; and supernatant fraction protein final volume of 1.25 mL. (IU: International Unit). Enzyme reaction rates were linear for at least 15 min after an equilibration period of 5 min.