**Supplementary File S1**

**Protective Effects of Aucubin against Nonylphenol-Induced Liver Toxicity by Improving Biochemical, Inflammatory and Histopathological Indices**

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**Biochemical assays**

**Assessment of SOD**

Superoxide dismutase activity was measured by following technique of Kakkar et al. (1984). Reaction solution contained of 1.2 mL of sodium pyrophosphate buffer (0.052 mM; pH 7.0) and 0.1 mL of phenazine methosulphate (186 mM). 0.3 mL of supernatant was centrifuged at 1500g for 10 min and then at 10000g for 15 min and added to the reaction mixture. Then, 0.2mL of NADH (780 mM) was added to start the enzyme reaction, which was later on ended by adding 1 mL of glacial acetic acid. Finally, chromogen was assessed by noticing the change in color intensity at 560 nm.

**Evaluation of CAT**

The activity of catalase was determined by following the technique of Aebi. (1974). 50mL of tissues homogenate was diluted using 2 mL of phosphate buffer with 7 pH. 2 mL of diluted homogenate was added with phosphate buffer (1 mL) of pH 7 comprising 30 mM of H2O2 in the test tube and then, diluted water was added to blanks. After instantaneously mixing, absorbance was noted at 240 nm.

**Analysis of GSH**

GSH was evaluated using the methodology of Jollow et al. (1974) with some modifications. 1000 μL of homogenate mixture was taken and dissolved in 1000 μL 0f 4% sulfosalicylic acid. The solution was incubated at 4°C for one hour followed by centrifugation at 4°C for 20 min at 1200g. 100 μL of supernatant was poured in 2.7 mL of phosphate buffer (pH 7.4) and 200 μL of 100 mM DTNB was added into it. The reaction between GSH and DTNB produced yellow color indicating reduced glutathione. The optical density was measured via spectrophotometer at 412 nm.

**Determination of GPx**

The activity of GPx was determined by the method of Rotruck et al. (1973). GPx converted reduced glutathione (GSH) into oxidized form using hydrogen peroxide during its reaction. The amount of GSH utilized was estimated by measuring it in the assay mixture before and after the enzyme activity. GSH reacted with DTNB to give a yellow color, which was then measured at 412 nm.

**Analysis of GSR**

The methodology of Carlberg and Mannervik (1975) was followed to assess level of GSR. The 0.20 mL of samples were added to 0.10 mL of EDTA (0.50mM), 1.68 mL of phosphate buffer, 0.04 mL of oxidized glutathione (10mM), and 0.10 mL of NADPH (0.10mM). OD was calculated at 340 nm after mixing. GSR was calculated as Nm NADPH oxidized/min/mg protein, using a molar extinction coefficient of 8.22 × 103 M−1.

**Analysis of GST**

GST was determined by the protocol of Habig et al. (1974). The reaction mixture was made using 1.475 mL phosphate buffer (0.1 M, pH 6.5), 0.2 ml reduced glutathione (1 mM), 0.025 mL 1-chloro-2,4-dinitrobenzene (CDNB, 1 mM) and 0.3 mL of liver homogenate. Change in the absorbance was recorded at 340 nm and enzyme activity was calculated as nM CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 × 103M-1cm-1.

**Estimation of MDA**

Lipid peroxidation was estimated by measuring the concentration of MDA in the liver homogenates as per the method of Ohkawa et al. (1979). Liver homogenates were mixed with 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH), and 1.5 mL of a 0.8% aqueous solution of thiobarbituric acid. The volume of the solution was made up to 4 mL with distilled water and heated for 1 hour at 95 °C. After cooling, 1 mL of distilled water and 5 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added before shaking the samples vigorously. After centrifugation at 3500×g for 10 min), absorbance was measured with a spectrophotometer at 532 nm. Lipid peroxidation was calculated from the standard curve using the malondialdehyde tetrabutylammonium salt and expressed as concentration of nmol MDA per g of protein.

**Analysis of ROS**

ROS were assessed from homogenate by following the process explained by Hayashi et al. (2007). 5 mL of homogenate and 140 mL (0.1 M) of sodium acetate buffer with pH 4.8 were mixed and dispensed in 96-well microplate. After incubating at 37°C for 5 min, 100mL of solution of ferrous sulfate and N, N-diethyl-para-phenylenediamine was added to each well, and then incubated at 37°C for 1 minute. At 505 nm, the absorbance was noticed with the help of a microplate reader for 180 seconds with a 15 second interval. Finally, the standard curve was plotted.

**Determination of inflammatory markers**

Level of NF-kΒ (CSB-E13148r), TNF-α (CSB-E07379r), IL-1β (CSB-E08055r) and IL-6 (CSB-E04640r) in addition to activity of COX-2 (CSB-E13399r) were determined by ELISA kits (Cusabio Technology Llc, Houston, TX, USA) and the instructor’s guide was followed. Firstly, 50 mL of sample was dispensed to the microplate wells. After that, 50 mL of antibody cocktail was poured to the wells. Plates were incubated at room temperature for the duration of 1 hour. After washing properly with the help of wash buffer, 100 mL of TMB substrates were dispensed to each well and incubated for about 10 minutes. After the addition of 100 mL of stop solution, the color was developed. The optical density was noted at 450 nm using Tecan Multimode Reader.