In-Vitro Anti-Inflammatory Activity

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The human red blood cell (HRBC) membrane stabilization method

The blood was collected from healthy human volunteer and equal volume of Alsever's solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) was mixed with it and centrifuged at 3,000 rpm for 10 min. The obtained packed cells were washed with normal saline and a 10% HRBC suspension was made. Various concentrations of AOAgNPs were prepared (50,100,150,200 and 500 µg ml-1) using distilled water. A mixture of 1 ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension (of above said various concentrations) was made. It was incubated for 30 at 37°C min and centrifuged at 3,000 rpm for 20 min. The absorbance of supernatant solution was measured spectrophotometrically at 560 nm (Kamalutheen et al., 2009). Diclofenac sodium was taken as standard drug. The experiment was repeated three times.

Inhibition of protein denaturation

To study the inhibitory effect on protein denaturation, AOAgNPs were added to 1% aqueous solution of BSA and pH of the reaction mixture was adjusted. The sample mixtures were incubated at 37°C for 20 min and then again heated to 51°C for 20 min. After cooling the turbidity of the samples was measured spectrophotometrically at 660 nm (Deshpande et al.,2009). Diclofenac sodium was used as a standard drug. The experiment was done in triplicate manner.

Antiproteinase action

Antiproteinase activity of AOAgNPs was estimated according to the modified method of Sakat et al. 2010. At first, 2 ml of reaction mixture was prepared containing0.06 mg/ml trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4) and 1 ml of AOAgNPs at different concentrations (100 - 500 µg ml-1).The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added to it and incubated for 20 min. After that to arrest the reaction,2 ml of 70% perchloric acid was added to it. Then the whole suspension was centrifuged and the absorbance was measured at 210 nm. Indomethacin was used as a standard drug. The percentage inhibition of proteinase inhibitory activity was calculated using following formula.

Anti-lipoxygenase activity

Anti-lipoxygenase activity of AOAgNPs was studied using linoleic acid as substrate and lipoxidase as enzyme (Shinde et al. 1999). Samples were dissolved in 250µl of 2M borate buffer (pH 9.0) and 250µl of lipoxidase enzyme solution (20,000U/ml) and were incubated for 5 min at 25°C. After that, 1.0ml of lenoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as standard drug and the percent inhibition was calculated from the above mentioned formula.