Chapter-7
Antioxidant and anti-inflammatory potential of biogenic AuNPI3Cs

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Abstract

To evaluate the antioxidant and anti-inflammatory activities of biogenic gold nanoparticles using indole-3-carbinol in this context, the *in vitro* antioxidant activity was demonstrated by 2,2- diphenyl-1-picrylhydrazyl, hydroxyl radical, nitric oxide, hypochlorous, peroxynitrite, lipid peroxidation scavenging activity. The anti-inflammatory activity was evaluated by studying the membrane of human red blood cells and inhibiting the denaturation of albumin and in vivo anti-inflammatory activity also assessed by carrageenan induced paw edema model.

It was observed that DPPH, nitric oxide, hydroxyl radical, hypochlorous acid, superoxide anion, lipid peroxidation, peroxynitrite scavenging activities of AuNPI3Cs treated groups are almost equal to the standard antioxidant, ascorbic acid. In case of anti-inflammatory activity, AuNPI3Cs exhibited dose dependent HRBC membrane stabilizing activity and inhibition of protein denaturation which was comparable to the standard anti-inflammatory drug, diclofenac sodium. AuNPI3Cs at 500 μg/ml dose showed 76% proteinase inhibitory action comparable to 82% inhibition of indomethacin. AuNPI3Cs also reduced paw edema significantly after the induction of acute inflammation while standard drug indomethacin showed prevention at the dose of 10 mg/kg. The result suggests that the gold nanoparticles synthesized from indole-3-carbinol could be a potent natural antioxidants and anti-inflammatory agent. Our results show that AuNPI3Cs has good antioxidant activity and interesting anti-inflammatory properties.

7.1 Introduction

7.1.1 Oxidative stress and antioxidants

Free radicals are chemical groups containing unpaired electrons and they play a momentous role in chemical reactions. Biological combustion and food spoilage reactions are carried out through free radical reactions which are important in biology and medicine (Prasad et al. 2009). Free radicals such as hydroxyl, peroxyl, singlet oxygen and superoxide radicals (Bellion et al., 2010), often termed as reactive oxygen species (ROS). ROS are endogenously produced from cellular metabolism and inflammatory reactions or by exogenous exposure to ionising radiation and xenobiotics (Bellion et al., 2010). However, imbalance between the production and elimination of free radicals create oxidative stress, which leads to cellular damage. This damage triggers for diseases like inflammation, artherosclerosis, cancer, diabetes, Parkinson's diseases, muscular degeneration (Prasad et al., 2009). Accumulated free radicals bind to DNA, RNA, protein and cell membrane and result in lipid peroxidation and the onset of various diseases (Goze et al., 2009).

Antioxidants have an important neutralizing effect on free radical species which are produced in normal system as end or byproducts of normal biochemical reactions (Lobo et al., 2010). By protecting the damage caused by free radicals antioxidants act as a major defense against radical mediated toxicity. So, ideal antioxidant therapy should be developed by either natural antioxidant enzymes or by agents which are capable to enhance the function of free radical scavenging enzymes.

7.1.2 Inflammation

Inflammation is a fundamental biological response of immune system towards tissue damage and infection (Ashley et al., 2012). Acute inflammatory reactions are characterized by the

increased movement of plasma and cells of innate immune system, such as neutrophils and macrophages, from the blood into the location of tissue damage. Important signs and symptoms of inflammation are edema, hyperalgesia, and erythema and they develop immediately after cutaneous injection of proinflammatory agents like bradykinin, histamine, complement and reactive oxygen, and nitrogen species. Inflammation are also expressed by increased blood flow, elevated cellular metabolism, vasodilatation, and release of prostaglandins, serotonin, cytokines and extravasation of fluids (Ferrero-Miliani et al., 2007). Death and ill health in the world has been increased from the inflammatory diseases such as rheumatoid arthiritis, asthma, colitis and hepatitis (Abreu et al.,2006). Many plants which have saponins have exhibited significant anti-inflammatory activities possibly due to their non-glycosidic moiety (Liu et al.,2012).

The features of inflammation initially increase vascular permeability, the release of prostaglandins and chemotactic substances such as complement factors, interleukin (IL)-1, tumor necrosis factor-alpha (TNF-α), and transforming growth factor beta (TGF-β) (Xing et al.,1994). Expression of inflammatory cytokines such as IL-1, IL-6, and IL-8, increased expression of cyclooxygenase (COX)-2 and nuclear factor kappa B play a vital role in facilitating the interaction between cancers cells and the micro-environment (Maihofner et al.,2003). Non-steroidal anti-inflammatory drugs (NSAID) as well as steroidal anti-inflammatory drugs (SAID) are widely used to treat different inflammatory disease conditions. These drugs are known to be toxic in the body causing severe side effects such as heart attacks and stroke and gastric ulcers (Bally et al., 2017). The adverse effects of the anti-inflammatory drugs have limitations in their clinical use; therefore natural agents may be considered to substitute chemical therapeutics (Rostom et al., 2002). The enzymes cyclooxygenase-1 and -2 are the key enzymes involved in recruiting inflammation. Nevertheless, the proinflammatory cytokines play a crucial role in the initiation and

progression of various cancers. Besides the key role of COX in the initiation and progression of inflammation, overexpression of COX has been considered as the formation of carcinogenic state in the body (Bakhle, 2001). Therefore, inflammation, free radicals and carcinogenesis are closely related with each other.

7.1.3 Nanoparticles as antioxidant and anti-inflammatory agents

In recent years, several metal nanoparticles have drawn considerable attention in the field of medicine due to their application in drug delivery, diagnosis and treatment of disease such as cancer.

In vitro antioxidant activity studies showed that DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) activities increased in a dose dependent manner in green synthesis of gold nanoparticles using aqueous extract of sumac (Shabestarian et al., 2017).

Gold nanoparticles synthesized using the aqueous extract of red marine algae *Gracilaria corticata* exhibited antioxidant activity by DPPH free radical scavenging assay and Ferric-ion reducing ability (Naveena and Prakash, 2013).

Gold nanoparticles from leaf of *Litchi chinensis* shows strong anti-inflammatory activities (Murad et al., 2018). Gold nanoparticle (AuNP) bioconjugates confirmed their anti-inflammatory affects *in vitro* (Uchiyama et al., 2014).

In vitro antioxidant and anti-inflammatory activity as well as *in vivo* anti-inflammatory activity of biogenic gold nanoparticles synthesized from indole-3-carbinol have been explored in this chapter.

7.2. Materials and methods

7.2.1. Chemicals and reagents

Ascorbic acid, hydrogen peroxide, methanol, phenazinemethosulfate (PMS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), sodium nitroprusside, naphthylethylenediamine-dihydrochloride, orthophosphoric acid, reduced nicotinamide adenine dinucleotide (NADH), Evans blue, taurine, ethylenediaminetetraacetic acid, 2-deoxy-2-ribose,sulphanilamine, trichloro acetic acid (TCA), thiobarbaturic acid (TBA), n-butanol, pyridine, sodium chloride (NaCl), ferric chloride ($FeCl_3$), sulphuric acid (H_2SO_4), potassium chloride (KCl), ferrous sulphate ($FeSO_4$), hydrogen peroxide (H_2O_2), ethylene diamine tetra acetic acid (EDTA), nitrobluetetrazolium (NBT), sodium citrate, citric acid, diethylenetri-amine-penta acetic acid (DTPA), bovine serum albumin (BSA), carrageenan, indomethacin, dextrose, diclofenac sodium and other chemicals were purchased from SRL chemicals, India .

7.2.2. *In-vitro* antioxidant activity

7.2.2.1 DPPH scavenging assay

Firstly, 2.8 ml of AuNPI3Cs were added with 0.2 ml of 1, 1-diphenyl-2-picrylhydrazine (DPPH) (100 µM in methanol) and standard ascorbic acid (10-200 µg/ml in methanol) at concentrations of 50, 100, 250, 500 µg/ml. Then 3 ml of this reaction mixture was incubated for 30 min at 37ºC in dark condition. Then the optical density was noted using spectrometer (ShimadzuU-245) at 517 nm.

The percentage inhibition of DPPH radical was calculated using the following formula (Brand-Williams et al., 1995).

$$\textbf{Percentage inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Absorbance of the control and T = Absorbance of the test sample.

7.2.2.2 Nitric oxide scavenging activity

One ml of 10 mM sodium nitroprusside, 1 ml solution of AuNPI3Cs at each concentration (50, 100, 250, 500 µg/ml) and standard ascorbic acid in phosphate buffer (pH 7.4) were mixed and incubated at 25°C for 2.5 h. Then in 1 ml of this incubated mixture, 1 ml of Griess reagent (1% sulphanilamine, 2 % orthophosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Chromophore was formed by diazotization of nitrite with sulfanilamide followed by coupling with naphthyl ethylene diamine dihydrochloride, and it absorbance was measured at 546 nm (Garrat, 1964) and the percentage inhibition was calculated by the following formula.

$$\textbf{Percentage inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Absorbance of the control and T = Absorbance of the test sample.

7.2.2.3 Superoxide anion scavenging assay

Three ml of AuNPI3Cs at different concentrations, 1 ml of nitrobluetetrazolium (NBT), and 1 ml of NADH were mixed well. Then phenazine methosulfate (100 µl) was mixed to it of and incubated for 5 min at 25ºC to initiate the reaction. The absorbance was measured at 560 nm to determine the percentage inhibition by previously mentioned formula (Dolai et al., 2012).

7.2.2.4 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging assay was performed by mixing 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2,0.36 ml of deoxyribose, 1.0 ml of various concentrations (50, 100, 250, 500 µg/ml of AuNPI3Cs) and 0.1 ml of ascorbic acid (50,100,150,200µg/ml) in distilled water along with 0.33 ml of phosphate buffer to perform the assay. After that the mixture was incubated at room temperature for 1 h. The pink chromogen was developed after adding 1.0

ml of TCA and incubated in boiling water bath for 20-30 min. The absorbance was measured at 412 nm and the percentage of inhibition of deoxyribose degradation is calculated by using the previously mentioned formula (Hussein, 2001).

7.2.2.5 Peroxynitrite scavenging assay

Peroxynitrite scavenging activity was measured by Evans blue bleaching assay (Karmakar et al., 2011). In the reaction mixture 50 mM of phosphate buffer (pH 7.4),0.1 mM of DTPA, 90 mM of NaCl, 5 mM of KCl, 12.5 µM of Evans blue and various concentrations (50, 100, 250, 500 µg/ml of AuNPI3Cs),1 mM freshly prepared peroxynitrite were mixed well and final volume is made up to 1 ml. The above mixture was incubated at 25°C for 30 min and observed at 611 nm spectrometrically.

7.2.2.6 Hypochlorous acid scavenging activity

At first hypochlorus acid was freshly prepared using 10% (v/v) solution of NaOCl and 0.6 M H_2SO_4. The concentration was measured at 235 nm using molar extinction coefficient of 100 M^{-1} cm^{-1} (Hazra et al., 1995). After that, 1.5 mM of HOCl, different concentration of AuNPI3Cs and ascorbic acid and was incubated for 1 h at 37°C. After incubation, taurine (30 mM) was added to the mixture and incubated again for 30 min at 37°C followed by the addition of 5-thio, 2-nitro benzoic acid (TNB). Absorbance was determined at 412 nm against blank and percentage scavenging was calculated according to the previously mentioned formula.

7.2.2.7 Lipid peroxidation scavenging assay

At first, young adult male rat liver was dissected and homogenized with an ice-cold Tris-HCl buffer (20mM, pH -7.4) to produce a 1/10 homogenate and centrifuged at 12,000 rpm at 4°C for 15 min to produce supernatant. The supernatant was used for *in vitro* lipid peroxidation assay. Then 1 ml supernatant was mixed with various concentrations (50, 100, 250, 500

µg/ml) AuNPI3Cs in the presence of 30mM KCl, 0.16 mM $FeSO_4$, 0.06 mM ascorbic acid and incubated at 37°C for 1 h. After that the reaction was terminated by adding 1.0 ml of TCA (10% w/v) and 1.5 ml of TBA (1% w/v) and the final volume was make up to 4 ml by adding distilled water and kept it in water bath for 30 min. After cooling of reaction mixture, 1 ml of distilled water and 5 ml of n-butane: pyridine (15:1v/v) was added to it and centrifuged at 4000 rpm for 10 min. Organic layer which was formed due to colored malondialdehyde (MDA)-TBA complex, measured at 532 nm (Ohkawa et al., 1979).The percentage inhibition was determined using previously mentioned formula.

7.2.3 *In-vitro* anti-inflammatory activity

7.2.3.1The human red blood cell (HRBC) membrane stabilization method

Equal volume of Alsever's solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and blood from healthy human individual was mixed and it was centrifuged at 3000 rpm for 10 min. A 10% HRBC suspension was prepared by washing with normal saline. 1 ml of phosphate buffer, 2 ml of hypo saline (pH-4) and 0.5 ml of HRBC suspension were added to each concentration (50,100,150, 200 and 500 µg ml^{-1}) of AuNPI3Cs and incubation was done for 30 min at 37°C and then it was centrifuged at 3000 rpm for 20 min. The absorbance of supernatant was noted spectrophotometrically at 560 nm (Gopalkrishnan et al., 2009). Diclofenac sodium was used as standard drug and the experiment was repeated thrice.
The percentage (%) of HRBC membrane stabilization was measured by the following formula:

$$\text{Percent Protection}(\%) = \frac{100 - \text{OD of drug treated sample} \times 100}{\text{OD of Control}}$$

7.2.3.2. Inhibition of protein denaturation

Firstly, 1% aqueous solution of BSA was added to AuNPI3Cs and all the reaction mixture was adjusted to pH 6.3 using small amount of hydrochloric acid (HCl) and the mixture was incubated at 37°C for 20 min. Then it was again heated at 51°C for 20 min and cooled. The turbidity of the samples was noted spectrophotometrically at 660 nm (Deshpande et al., 2009). Diclofenac sodium was used as standard drug and the experiment was repeated thrice.

The percent inhibition of protein denaturation was measured as follows:

$$\text{Percent Inhibition} = 100 - \frac{(\text{OD of test} - \text{OD of product control}) \times 100}{\text{OD of control}}$$

7.2.4 *In-vivo* anti-inflammatory activity

7.2.4.1 Assay of carrageenan-induced edema in rats

Mice paw edema model was considered to study *in vivo* anti-inflammatory activity of AuNPI3Cs. Group I and Group-II were designated as Distilled water control (10 ml/kg body wt.) group and Carrageenan control group respectively. Group III-VI were considered as different dose treated groups of AuNPI3Cs (0.5, 1.5, 3, 5 mg/kg body wt.) respectively in carrageenan induced mice. Group IV was standard anti-inflammatory drug Indomethacin (10mg/kg body wt.) treated group in carrageenan induced mice.

To induce paw swelling, 0.1 ml of 1% sterile carrageenan in normal saline was injected sub-plantarly into the right hind paw of mice. 60 min before carrageenan injection, AuNPI3Cs was administered orally at 0.5, 1.5, 3 and 5 mg/kg body wt., (Winter et al., 1962). Distilled water (10 ml/kg bwt) was given to Control group and indomethacin (10 mg/kg), standard anti-inflammatory drug was used as positive control group. The produced inflammation was determined at 0, 1, 2, 3, and 4 h after carrageenan injection by using vernier calipers and calculating the volume displaced by the paw.

The *in vivo* anti-inflammatory activity was calculated by using the following formula.

$$\%\ \text{of inhibition of edema} = \frac{\text{Thickness of paw in control group} - \text{Thickness of paw edema in the treated group}}{\text{Thickness of paw in control group}} \times 100$$

7.2.5 Statistical analysis

Results were expressed as Mean ± SEM. Results were analyzed using one way ANOVA. Differences were considered significant at $p<0.05$ level.

7.3 Results:

AuNPI3Cs has dose dependent inhibition on DPPH radical scavenging (Figure 7.1) and the IC_{50} value of the AuNPI3Cs was found to be 86.29 μgml^{-1} (Figure 7.1).

Figure 7.2 showed dose dependent inhibition of nitric oxide by AuNPI3Cs and ascorbic acid. The IC_{50} values of AuNPI3Cs and ascorbic acid were 93.56and 88.79 μgml^{-1}respectively.

From the figure 7.3, it is revealed that AuNPI3Cs has prominent superoxide radical scavenging activity. The IC_{50} values of AuNPI3Cs and ascorbic acid were found to be 101.81 and 123.24 μgml^{-1}respectively.

Dose dependent hydroxyl radical scavenging activity of AuNPI3Cs was found in this study (Figure 7.4).The IC_{50} values of AuNPI3Cs and standard antioxidant were 117.81 and 124.25 μgml^{-1} respectively.

Figure 7.5 showed dose dependent inhibition of peroxynitrite by AuNPI3Cs and ascorbic acid. The IC_{50} values of AuNPI3Cs and ascorbic acid were found to be 123.21and 144.05 μgml^{-1} respectively.

The hypochlorus acid scavenging activity was observed (Figure 7.6) in a dose dependent manner compared to ascorbic acid. The IC_{50} values of AuNPI3Cs and ascorbic acid in this assay were 100.26 and 104.25 μgml^{-1}respectively.

AuNPI3Cs also inhibited lipid peroxidation (Figure 7.7) and the IC_{50} values of AuNPI3Cs and ascorbic acid were found to be 180.57 and 120.8 $ugml^{-1}$ respectively.

Anti-inflammatory activity of AuNPI3Cs was found to be concentration dependent and the percentage of protection was increased with the dose dependent manner of AuNPI3Cs. HRBC cell membrane stabilizing activity of AuNPI3Cs was 81.63% at 500µg/ml dose that is comparable to that standard drug diclofenac sodium (87.69%) (Table 7.1).

It was observed that protein denaturation inhibition by AuNPI3Cs was dose dependent and it showed 82.1% inhibition at 500µg ml^{-1} whereas standard drug diclofenac sodium showed 83.53% protein denaturation (Table 7.2).

In this study, *in vivo* anti-inflammatory effect of AuNPI3Cs was compared to carrageenan-control which was comparable to standard drug indomethacin. After 4 h it was observed that, there was significant decrease in paw edema in AuNPI3Cs treated groups at 0.5, 1.5, 3 and 5 mg/kg bwt (Table 7.3) compared to the control group.

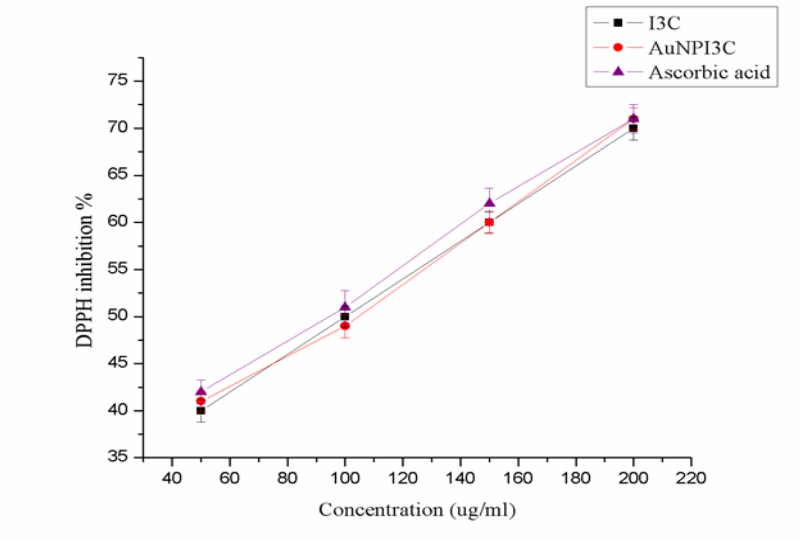

Figure 7.1.Diphenyl-2-Picrylhydrazine (DPPH) scavenging activity at different concentrations of AuNPI3s and ascorbic acid. Results are expressed as Mean ±SEM.

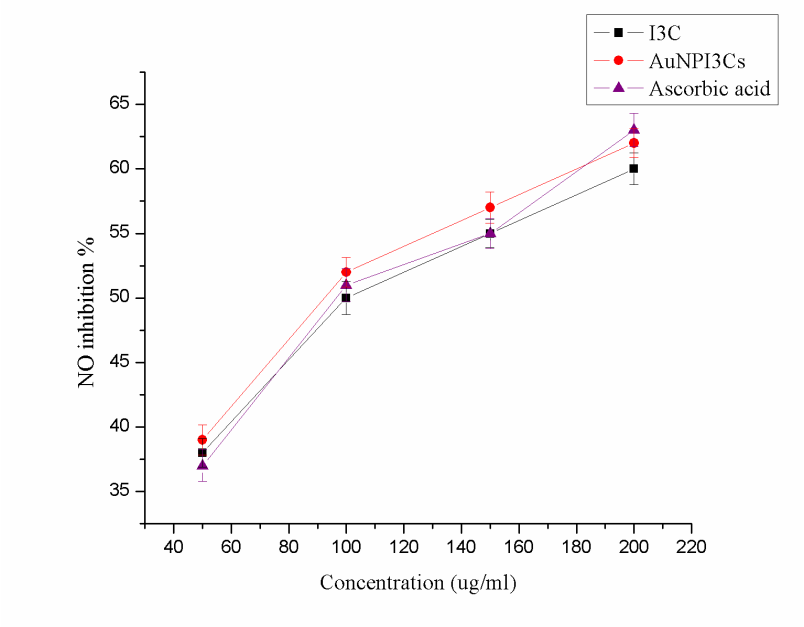

Figure 7.2 showed nitric oxide scavenging activity at different concentrations of AuNPI3Cs and ascorbic acid. Values are expressed as Mean ±SEM.

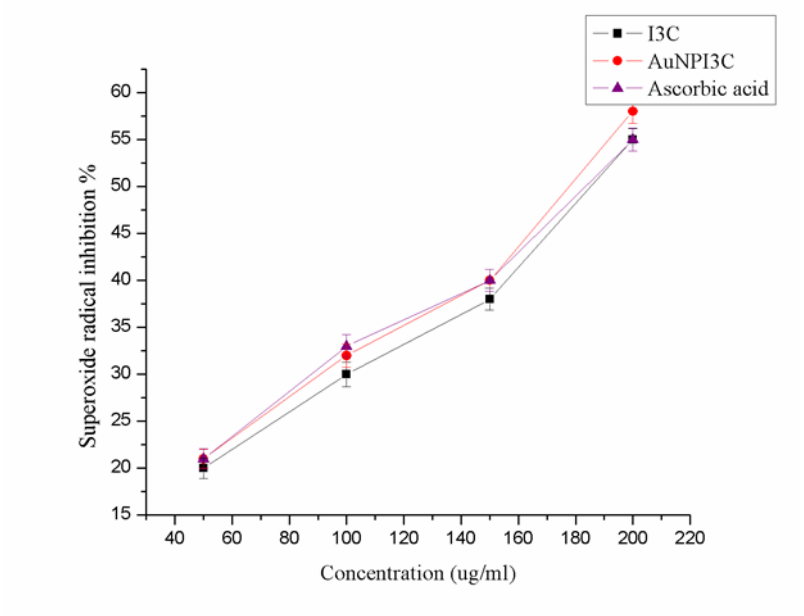

Figure 7.3 Superoxide anion scavenging activity of AuNPI3Cs and ascorbic acid at different concentrations. Results are expressed as Mean ±SEM.

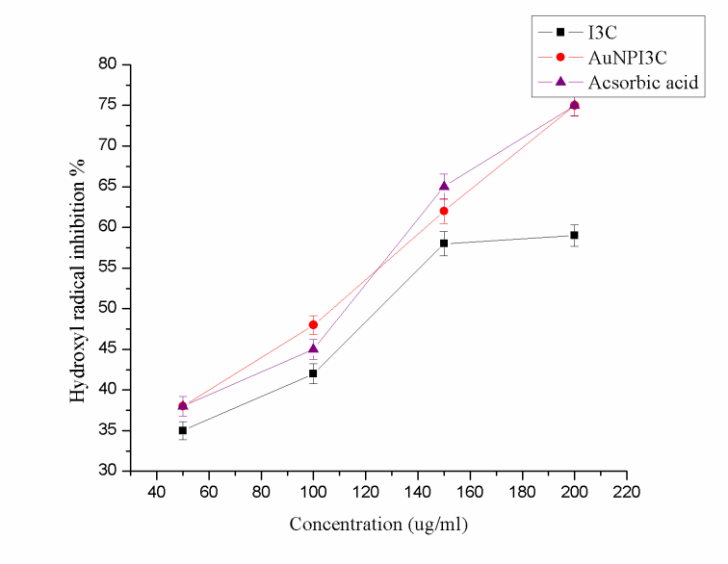

Figure 7.4 illustrates hydroxyl radical scavenging activity at different concentrations of AuNPI3Cs and ascorbic acid. Values are expressed as Mean ±SEM.

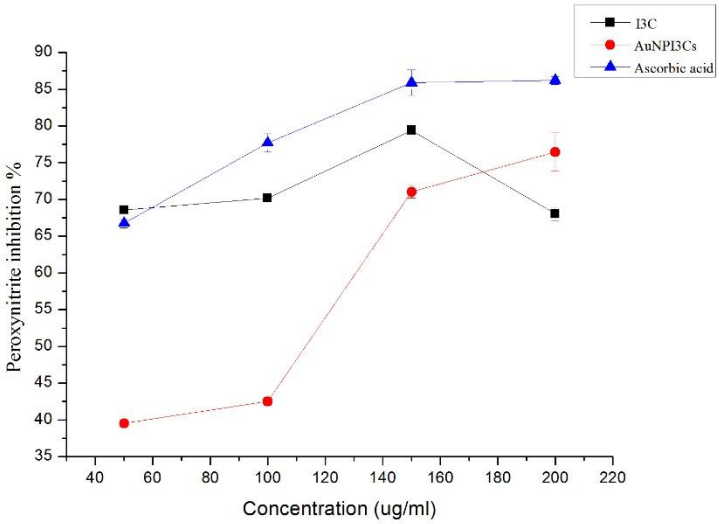

Figure 7.5.Peroxynitrite scavenging activity at different concentrations of AuNPI3Cs and ascorbic acid. Values are expressed as Mean ±SEM.

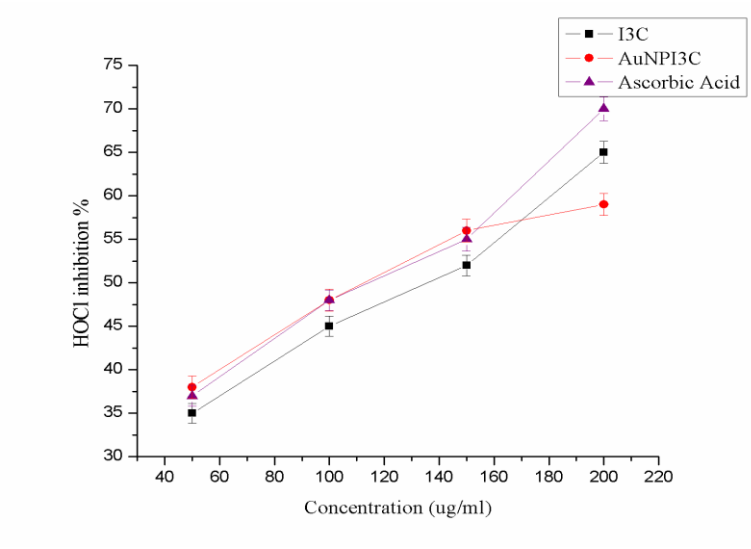

Figure 7.6 showed hypochlorous acid scavenging activity at different concentrations of AuNPI3Cs and ascorbic acid. Results are expressed as Mean ±SEM.

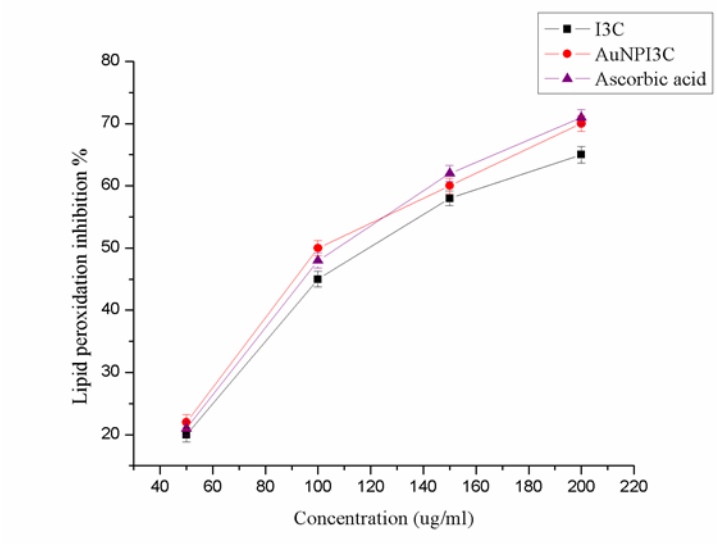

Figure 7.7.Lipid peroxidation scavenging activity at different concentrations of AuNPI3Cs and ascorbic acid. Values are expressed as Mean ±SEM.

Table 7.1: Effect of AuNPI3Cs on human red blood cell (HRBC) membrane stabilization

Concentration (µg/ml)		HRBC membrane stabilizing activity (%)	
	Diclofenac sodium	I3C	AuNPI3Cs
50	75.45±.55	50±1.02	61±1.1
100	82.2 ±0.95	58.2±1.15	67.4±1.12
150	83.2 ±1.04	63.75±1.25	72.4±0.85
200	85.8 ±0.95	72.3±1.35	78.45±0.82
500	89.2 ±1.07	80.2±0.83	84.2±0.53

Values are expressed as the Mean±SEM.

Table 7.2 Effect of AuNPI3Cs on protein denaturation

Concentration (µg/ml)		Percentage of Protein denaturation (%)	
	Diclofenac sodium	I3C	AuNPI3Cs
50	21.3±0.9	15.8±0.58	21.7±0.7
100	37.9±1.1	28.3±0.65	34.4±0.95
150	42.8±0.95	35.2±1.05	45.2±1.13
200	57.3±1.19	48.4±0.78	53.4±0.87
500	85.5±1.08	55.5±0.54	83.2±0.55

Results are expressed as the Mean±SEM.

Table 7.4 Effect of AuNPI3Cs and indomethacin as compared to carrageenan control in carrageenan induced paw edema model

Groups	Paw oedema (mm)					
	0 hr	30 min	1hr	2hr	3hr	4hr
Carrageenan	1.61±0.022	3.4±0.083	4.1±0.12	4.02±0.15	3.9±0.09	3.98±0.12
Carrageenan + Indomethacin (10mg/kg b wt)	1.63±0.01	3.206±0.017	3.173±0.017	3.023±0.014	2.8±0.014**	2.526±0.014**
Carrageenan + I3C (50 mg/kg b wt)	1.65±0.07	3.3±0.05	3.21±0.01	3.156±0.023	3.03±0.08	2.92±0.02*
Carrageenan + I3C (75 mg/kg b wt)	1.65±0.03	3.3±0.03	3.22±0.02	3.1±0.04	3.05±0.01	2.95±0.05*
Carrageenan + I3C (100 mg/kg bwt)	1.68±0.03	3.35±0.03	3.28±0.03	3.1±0.02	3.01±0.01	2.92±0.05*
Carrageenan + I3C (150 mg/kg bwt)	1.6±0.017	3.38±0.014	3.32±0.04	3±0.01	3.07±0.04	2.85±0.01**
Carrageenan + AuNPI3Cs (0.5 mg/kg bwt)	1.65±0.017	3.283±0.020	3.16±0.04	3.056±0.023	2.9±0.028*	2.8±0.028**
Carrageenan + (AuNPI3Cs 1mg/kg bwt)	1.75±0.03	3.31±0.03	3.18±0.023	3.1±0.04	2.9±0.01*	2.8±0.05**
Carrageenan + AuNPI3Cs(2 mg/kg bwt)	1.65±0.017	3.1±0.014	3.3±0.014	3±0.011	2.7±0.014*	2.5±0.011**
Carrageenan+ AuNPI3Cs (4 mg/kg bwt)	1.623±0.014	3.05±0.017	3.1±0.011	3±0.011	2.6±0.02*	2.45±0.014**

Data are expressed as mean ± SEM. * indicates significant difference at p< 0.05 significant level- compared to carrageenan treated group. ** indicates significant difference at p< 0.01- significant level compared to carrageenan treated group.

7.4. Discussion:

In the present study the antioxidant activity of the biogenic AuNPI3Cs was ascertained.

DPPH is a nitrogen-centered free radical, the color of which changes from deep purple to yellow for the reduction by either the process of hydrogen or electron donation. Substances which are able to execute this reaction can be considered as antioxidants and therefore, radical scavengers (Dehpour et al., 2009). Radical scavenging activities of I3C and AuNPI3Cs as well as ascorbic acid increased with increasing concentration of its dose dependent nature. Figure 7.1 shows the DPPH radical scavenging ability of AuNPI3Cs and ascorbic acid in a dose dependent manner. The effects of antioxidant on DPPH activity is occurred due to their hydrogen donating activity (Gangwar et al., 2014).

Excess production of nitric oxide (NO) leading to tissue damage may cause several diseases. So scientists are concerned to develop potent inhibitors of NO for therapeutic use (Nowakowska, 2007). In this study, AuNPI3Cs shown to have potent nitric oxide scavenging activity in a dose dependent manner (Figure 7.2). The nitric oxide scavenging activity of nanoparticles is evident by its ability of inhibition of the generation of nitrite molecules through direct interaction with oxygen and oxides of nitrogen (Hazra et al., 2008).

Superoxide anions produced from dissolved oxygen by PMS-NADH coupling reaction reduced from NBT. In the reaction mixture superoxide anion is consumed when there is decrease of absorbance with antioxidants (Elmastas et al., 2006). Superoxide radical scavenging activity of AuNPI3Cs is occurred at dose dependent manner and compared with the standard ascorbic acid (Figure 7.3).

The hydroxyl radical formed in the fenton reaction in the presence of reduced transition metals such as Fe2+ and H_2O_2 which are known to be the most reactive is capable of damaging of almost all molecules found in living cells (Rollet-Labelle et al. 1998). Increased radical scavenging activities with increasing concentration of AuNPI3Cs under study were noticed. Percent of inhibition for OH radical scavenging activity are tabulated in Figure7.4.

Peroxynitrite (ONOO-) causes oxidative damage (Dolai et al., 2012) and bleaches Evans blue. AuNPI3Cs inhibits bleaching of Evans blue by scavenging peroxynitrite at a dose dependent manner (Figure 7.5).

At sites of inflammation, myeloperoxidase enzyme of neutrophil causes oxidation of Cl^- ions and results the production of hypochlorous acid (Aruoma et al., 1989). HOCl inactivate catalase by breaking heme prosthetic group. AuNPI3Cs indicates its HOCl scavenging activity (Figure 7.6).

Various pathological events such as atherosclerosis, inflammation and liver injury are related to lipid peroxidation of cell membrane (Singh et al., 2012). Different studies reported that various terpenoid compounds provided inhibitory effect against lipid peroxidation (Hazdu et al., 2007). The present study confirms the efficacy of AuNPI3Cs as a significant source of natural antioxidant which have different ROS scavenging activity.

In vitro anti-inflammatory activity of AuNPI3Cs was studied by the inhibition of protein denaturation and stabilization of HRBC membrane activity. Some reports (Chandra et al., 2012) proved that destabilization of cell membrane especially lysosomal membrane, denaturation of proteins particularly blood proteins are the major causes of arthritis and inflammation. Erythrocyte membrane is analogous to the lysosomal membrane and AuNPI3Cs stabilize erythrocyte membrane. So, it may be concluded that AuNPI3Cs can also

stabilize lysosomal membranes (Kumar et al., 2011). Stabilization of lysosomal membrane is significant to prevent the inflammatory process by the prevention of the release of lysosomal constituents of activated neutrophil that contains bactericidal enzymes and proteases and cause further inflammation and tissue damage after extracellular release (Yurugasan et al., 1981). Hypotonicity dependent haemolysis may be happened due to cell shrinkage as a result of osmotic loss of intracellular electrolytes and fluid components. AuNPI3Cs may arrest the process by decreasing the efflux of these intracellular components (Yang et al., 2010).

This study indicated that AuNPI3Cs was capable to inhibit protein denaturation (Table 2). Neutrophils are known to be a good source of serine proteinase which exists at lysosomes. In the course of inflammatory reactions leukocyte proteinase plays a substantial role in the progress of tissue damage and proteinase inhibitors provides a significant level of protection against it (Das and Chatterjee, 1995).

On the basis of the findings in the in-vitro anti-inflammatory study of AuNPI3Cs, *in vivo* anti-inflammatory activity was performed. Carrageenan-induced paw edema in rodent is an ideal model to study the acute anti-inflammatory effect of any compound (Amdekar et al., 2015). AuNPI3Cs significantly inhibit paw volume in the carrageenan induced mice paw edema (Table 3). It was observed that anti-inflammatory effect of synthesized AuNPI3Cs started at 1 h and reached at peak at 4 h and the maximum inhibitory effect was found between 2 and 4 h. Carrageenan induced paw edema is a biphasic event involved different inflammatory mediators. In the first phase of inflammation that is after 2 h of carrageenan injection, histamine and serotonin play an important role and in the second phase kinin and prostaglandins play vital role. Histamine, serotonin, kinin and prostaglandins (Shenoy et al., 2010) act as a chemical mediators. In the study it was observed that after administration of AuNPI3Cs, edema was inhibited from the first hour and the inhibition was continued in all

phases of inflammation. Both in *in-vitro* and *in-vivo* studies, it is evident that AuNPI3Cs possess potent anti-inflammatory effect.

7.5. Conclusion:

AuNPI3Cs exhibited *in vitro* antioxidant activity and *in vitro* and *in vivo* anti-inflammatory potential and it suggests that biogenic AuNPI3Cs could be a good source for the controlling of oxidative stress and inflammation. This finding suggests a novel pharmacological approach for the treatment of various inflammatory disorders which generally potentiate the initiation of many life-threating diseases.