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IN-VITRO AND IN VIVO ANTI-INFLAMMATORY POTENTIAL OF SILVER NANOPARTICLES SYNTHESIZED FROM CALOTROPIS GIGANTEA LATEX EXTRACT

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ABSTRACT

In recent years plant mediated biological synthesis of nanoparticles is gaining importance due to its simple synthetic procedure and eco-friendliness. The present study describes *in-vitro* and *in vivo* anti-inflammatory activitiesof biogenic stable silver nanoparticles synthesized by using ethanolic extract of *Calotropis gigantea* latex (Ag NPCGs). The silver nanoparticles were previouslycharacterized by UV-visible spectroscopy, dynamic light scattering (DLS), zeta-potential measurements, Fourier transform infrared spectroscopy and transmission electron microscopy. The dose-dependent anti-inflammatory activity of silver nanoparticles was assessed by human red blood cell membrane stabilization, inhibition of protein denaturation, proteinase and lipoxygenase inhibitory action. The percentage of HRBC membrane stabilization of Ag NPCGs at 500µg ml^{-1} was found to be 75.28%. At the concentration of 500 µg ml^{-1}, Ag NPCGs showed 74% proteinase inhibitory action. Lipoxygenase activity was inhibited by Ag NPCGs at the concentration range of 50-500µg ml^{-1}. Ag NPCGs also exhibited better percentage of inhibition in the carrageenan induced right hind paw edemathan that of standard drug indomethacin. On the basis of present study, it is concluded that Ag NPCGs possess potent anti-inflammatory activity.

KEYWORDS: Silver nanoparticles, *Calotropis gigantea*, Anti-inflammatory activity, Protein denaturation.

INTRODUCTION

Inflammation is a process of body defense mechanism, which includes the increase in vascular permeability, protein denaturation and membrane alteration. The process is characterized by swelling, redness, pain, heat, and loss of function of injured area (Iwalewa et al. 2007). Inflammation may occurdue to chemical agent, physical agents as well as microbes. Cytokines, kinins, prostroglandins and histamine are released due to the injury of cell by inflammation. The release of these mediators induces vasodilation, increase capillary permeability leading to increased blood flow to the injured site. Inflammations are classified in acute and chronic inflammations. Acute inflammation is considered as initial response of the body to injurious stimuli and movement of plasma and leukocytes is increased from the blood into the injured tissues (Iwalewa et al. 2007; Khan et al. 2010). A cascade of biochemical events propagatesand matures the inflammatory response, by the participation of local vascular system and the immune system. Chronic inflammationcauses to a progressive shift in the type of cells present at the site of inflammation manifested by simultaneous destruction and healing of the tissue from the inflammatory process. Presently used anti-inflammatory drugs manifest severe side effects; this resulted in a search for other alternative anti-inflammatory drugs (Iwalewa et al. 2007; Khan et al. 2010; Haddad 2007). Traditional medicines are being used right from the beginning of history of civilization to cure human aliments in every possible condition. In modern era, we have the option to use these traditional medicines due to their lesser side effects over the synthetic molecules (Bhattacharya and Mukherjee 2008).

Modern era is of nanomedicine owing to their various therapeutic applications with more efficacies and lesser side effects. The wide range of acceptance of metal nanoparticles is due to their potential for achieving specific process and selectivity in pharmacological action (Verma et al.2013). Various conventional methods employed for the synthesis of silver nanoparticles include chemical (Sun et al. 2002), electrochemical (Yin et al. 2003), radiation (Dimitrijevic et al. 2001), photochemical methods (Callegari et al. 2003) and biological techniques (Naik et al. 2002).In the present study, we have developed a rapid, eco-friendly and convenient method for the green synthesis of silver nanoparticles using latex extracts of

Calotropis gigantea and an attempt has been made to evaluate *in-vitro* and *in-vivo* anti-inflammatory activity of synthesized biogenic silver nanoparticles (AgNPCGs).

MATERIALS AND METHODS

Chemicals

Diclofenac sodium, carrageenan, indomethacin, linoleic acid, lipoxidase enzyme, boric acid, were purchased from Sigma Aldrich Co., U.S.A. Bovine serum albumin (BSA), sodium chloride (Na Cl), dextrose, sodium citrate, perchloric acidand other chemicals were purchased from Merck India, Ltd., Mumbai, India.

Collection of plant latex and preparation of ethanolic extract

Latex of *Calotropis gigantea* was collected from the out fields of Vidyasagar University, Midnapore, West Bengal, India during March and May and authenticated from Botanical Survey of India, Ministry of Environment and Forest, Govt. of India, Howrah (Identification No. CNH/2014/Tech.II/55). Then 250 g dried latex was dissolved in 450 ml of ethanol, incubated at room temperature for 48 h and filtered. Then the filtered ethanolic extract was concentrated in a rotary evaporator (EYELA CCA 1110) at 60°C. The concentrated filtrate was dried by incubating at 37°C to produce crude ethanolic extract (EECGL; yield- 4.5% w/w) (Sanyal et al. 2016) and was stored in air tight container at 4^0C.

Synthesis of silver nanoparticles

EECGL (2mg/ml) was mixedwith 2mM $AgNO_3$ solution in a ratio of 1:1 and incubated at 60°C temperature by the help of magnetic stirrer at 600 rpm for 1 hto accomplish the reduction of Ag+ ions. Formation of biogenic AgNPCGs was indicated by the brown-yellow colored solution suggesting that aqueous silver ions can be reduced by the latex extract of *C. gigantea* (Kumar et al. 2012).

Characterization of silver nanoparticles

The silver nanoparticles were characterized (Maity et al. 2017) by UV–visible spectrophotometer (UV-1800 Shimadzu, Japan), DLS particle size and zeta-potential analyzer (Malvern Instruments, U.K.), Fourier transform infrared spectroscopy (Perkin Elmer Spectrum Express Version 1.03.00 FTIR spectrometer) and X-ray diffraction (XRD) analysis, transmission (CM12 PHILIPS) and scanning electron microscopy.

Animals

Male Swiss albino mice (22-25g) were purchased from enlisted supplier of Vidyasagar University.Animals were accommodated in polypropylene cages with sixanimals per cage un der standard laboratory conditions (25°C ± 2°C) with standard laboratory diet and water *ad libitum*. The Institutional Animal Ethical Committee (IAEC), registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests & Climate Change, Govt. of India, approved the study (approval No. IEC/7-12/C12) and the relevant laws and guidelines of the CPCSEA were followed.

***In-vitro* anti-inflammatory activity**

The human red blood cell (HRBC) membrane stabilization activity

The blood was collected from healthy human volunteer and mixed with equal volume of Alsever's solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm for 10 min. The packed cells were washed with normal saline and a 10% HRBC suspension was made. Various concentrations of AgNPCGs were prepared (50,100,150,200 and 500 µg ml^{-1}) using distilled water and 1 ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min and the supernatant solution was estimated spectrophotometrically at 560 nm (Kamalutheen et al. 2009). Diclofenac sodium was used as standard drug. The experiment was performed in triplicate manner.

The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula:

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

Inhibition of protein denaturation

The reaction mixture was consisting of AgNPCGs and 1% aqueous solution of BSA, pH of the reaction mixture was adjusted using small amount of hydrochloric acid (HCl). The sample mixtures were incubated at 37°C for 20 min and then heated to 51°C for 20 min. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm (Deshpande et al. 2009). Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicate manner.

Percent inhibition of protein denaturation was calculated as follows:

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

Antiproteinase action

The test was performed according to the modified method of Sakat et al. 2010. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM TrisHCl buffer (pH 7.4) and 1 ml of test sample of 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. The mixture was incubated for an additional 20 min. After that 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance was measured at 210 nm. Indomethacin was used as a standard drug. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated using following formula.

$$\% \text{ of inhibition} = \frac{\text{Abs Control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Anti-lipoxygenase activity

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

***In-vivo* anti-inflammatory activity**

Assay of carrageenan-induced edema in rats

For screening *in vivo* anti-inflammatory activity of Ag NPCGs

Group I: Treatment with control (Distilled water); 10 ml/kg body wt.

Group-II: Treatment with carrageenan

Group III: Treatment with Ag NPCGs; 1, 5, 10mg/kg body wt.

Group IV: Treatment with standard drug, indomethacin (10mg/kg body wt.)

Paw swelling was induced by sub-plantar injection of 0.1 ml of 1% sterile carrageenan in normal saline into the right hind paw. Ag NPCGs at dose of 1, 5, 10 mg/kg body wt. were

administered orally 60 min before carrageenan injection (Winter et al. 1962). Indomethacin (10 mg/kg) was used as standard anti-inflammatory drug. Control group received thevehicle only (10 ml/kg body wt. distilled water). The inflammation was measured by calculating the volume displaced by the paw, using vernier calipers at time 0, 1, 2, 3, and 4 h after carrageenan injection.

The *in vivo*anti-inflammatory activity was calculated by using the relation T, Thickness of paw in control group; T0, Thickness of paw edemain the test compound treated group

$$\% \text{ of inhibition of edema} = \frac{T - T0}{T} \times 100$$

Statistical analysis

Results of anti-inflammatory activity were expressed as Mean± SD. Results were analyzed using oneway ANOVA. Differences were considered as statistically significant $p<0.05$ are compared to control.

RESULTS

From the screening of *in vitro* anti-inflammatory activity (Table 1), it was observed that Ag NPCGs showed significant HRBC membrane stabilizing activity when compared to standard anti-inflammatory drug, diclofenac sodium. The percentage of stabilization of diclofenac sodium, Ag NPCGs at 500µg ml^{-1} were found to be 88.28% and 75.28% respectively.

Table 1: The human red blood cell (HRBC) membrane stabilization activity of synthesized silver nanoparticles using latex extracts of *Calotropisgigantea* (Ag NPCGs).

Concentration (µg ml^{-1})	HRBC membrane stabilizing activity (%)	
	Diclofenac sodium	AgNPCGs
50	75.61±1.08	58.15 ±0.98
100	80.2 ±1.72	62.27 ±0.88
150	83.45 ±1.45	65.78 ±1.05
200	85.18 ±1.8	70.12 ±1.23
500	88.28 ±1.2	75.28 ±1.12

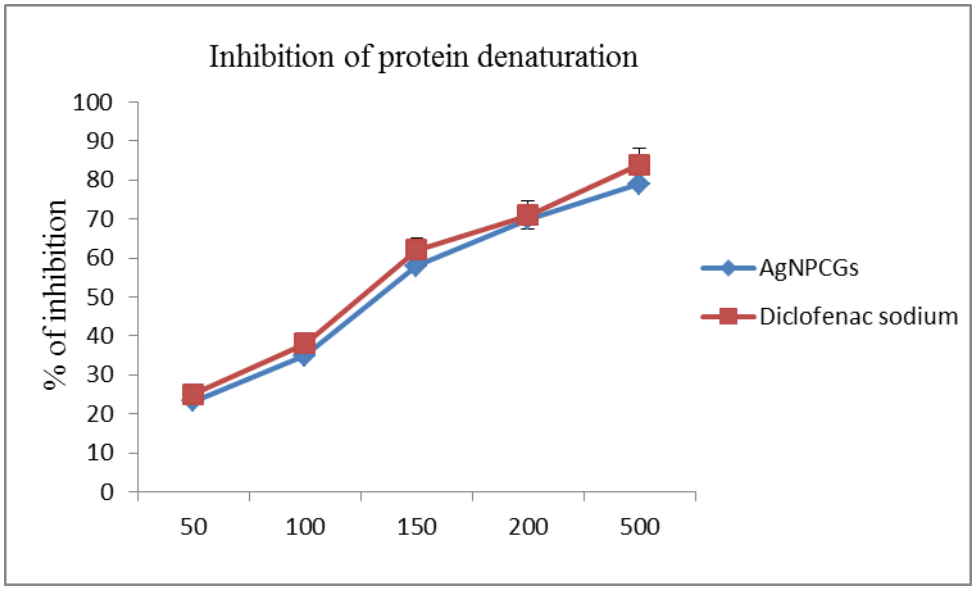

Figure 1: Shows the percentage of inhibition of protein denaturation by AgNPCGs and diclofenac sodium. Values are expressed as mean ± SEM.

Table 2: Effect of AgNPCGs on proteinase inhibitory action.

Concentration (µg ml^{-1})	Inhibition of proteinase action (%)	
	Indomethacin	Ag NPCGs
50	60.21±0.14	55.12 ±0.23
100	62±0.72	60.32±0.78
150	65.5±1.14	65.24±1.05
200	75 ±1.2	71.23±1.13
500	79±1.18	74.32±1.02

Each value represents the Mean ± SEM, Experimental groups were compared with control **p<0.01, considered extremely significant; *p<0.05.

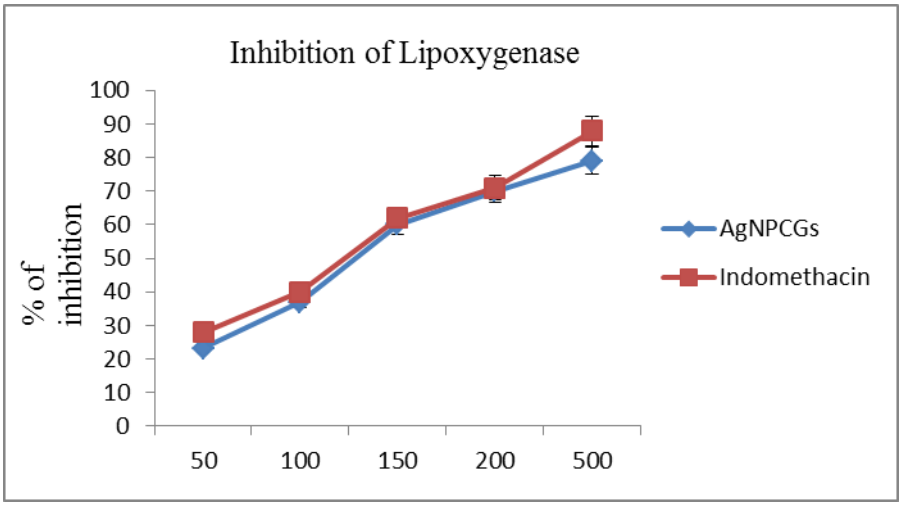

Figure 2: Shows the percentage of inhibition of lipoxynase by AgNPCGs and indomethacin. Values are expressed as mean ± SEM.

Table 3: Effect of Ag NPCGs and indomethacin on carrageenan induced paw edema.

Groups	0 hr	30 min	1hr	2hr	3hr	4hr
Carrageenan	1.6±0.02	3.42±0.08	4.02±0.1	4.02±0.1	3.95±0.08	4±0.1
Carrageenan + Indomethacin (10mg/kg bwt)	1.61±0.02	3.02±0.06	2.98±0.05	2.67±0.05	2.2±0.01**	1.81±0.01**
Carrageenan + AgNPCGs (1mg/kg bwt)	1.65±0.02	3.5±0.05	3.38±0.043	3.2±0.1	3±0.12	2.5±0.25*
Carrageenan + AgNPCGs(5 mg/kg bwt)	1.56±0.01	3.3±0.06	3.3±0.025	3.1±0.12	2.85±0.12*	2.82±0.2*
Carrageenan + AgNPCGs (10mg/kg bwt)	1.6±0.03	3.3±0.052	3.2±0.06	3±0.1	2.5±0.13*	2.32±0.25**

Values are expressed as mean ± SEM. * denotes p< 0.05-significant compared to carrageenan treated group. ** denotes p< 0.01-significant compared to carrageenan treated group.

The present findings exhibited inhibition of protein denaturation by AgNPCGs at the concentration range of 50-500 µgml^{-1}(Fig 1). Diclofenac sodium (at the concentration range of 50-500 µg ml^{-1}) was used as the standard drug, which also exhibited concentration dependent inhibition of protein denaturation (Fig 1). AgNPCGs exhibited 79% of inhibition of protein denaturation at 500 µg ml^{-1} and the effect may be compared with the standard anti-inflammatory drug, diclofenac sodium which showed 84% inhibition at the same concentration.

Ag NPCGs exhibited significant antiproteinase activity at different concentrations as shown in Table 2. It showed maximum inhibition of 74% at 500µg ml^{-1}. Indomethacin showed the maximum inhibition (79%) at 500µg ml^{-1}.

Ag NPCGs showed 23, 37, 60, 70, 79% anti-lipoxygenase inhibitory activity at 50, 100,150,200,500 µg ml^{-1} respectively. From these result, the highest inhibition was obtained at the concentration of 500µg ml^{-1}. The standard drug indomethacin showed an 88% inhibition at a concentration of 500µg ml^{-1} (Fig 2).

The anti-inflammatory effect of Ag NPCGs and the standard drug indomethacin in carrageenan induced paw edema model in mice are demonstrated in table 2. After administration ofcarrageenan, micepaw edema reached in a peak value at 3 h. AgNPCGs

exhibited maximum percent inhibition of edema at 10 mg kg^{-1} body weight concentration and the effect is almost comparable to that of the standard drug indomethacin.

DISCUSSION

Ag NPCGs showed dose dependent anti-inflammatory efficacy by increasing HRBC membrane stabilizing activity. By inhibiting hypotonicity induced RBC membrane lysis, biogenic Ag NPs may show their membrane stabilization effect. The exact mechanism of the membrane stabilization by the test sample is not clear yet. Induced hemolysis may arise from shrinkage of the cell due to osmosis of intercellular fluids and electrolyte or interaction of fluid proteins (Vadivu and Lakshmi 2008; Yuan et al. 2006).

Most biological proteins when denatured lose their biological function, denaturation of proteins leads toinflammation. When the mechanism of the anti-inflammatory activity is investigated, its capability of protein denaturation is generally studied (Leelaprakash and Mohan Dass 2011).

Neutrophils are known to be a rich source of serine proteinase. It was previously reported that proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee 1995).

Ag NPCGs have shown a potential anti-inflammatory activity and inhibited the lipoxygenase enzyme activity. The plant lipoxygenase pathway is comparable to the arachidonic acid cascades in animals (Gardner 1991). For this reason, *in vitro* inhibition of lipoxygenase (LOX) play a good model for the screening of any drug or compound with anti-inflammatory potential (Abad et al. 1995). LOXs may inhibit lipid hydroperoxide formation due to scavenging of lipid peroxy- radical formed in course of enzyme peroxidation.

Carragenan induced acute inflammation is one of the most appropriate assessment procedure to screen anti-inflammtory drugs. The time course of edema development in carragenan induced edema is a biphasic process (Vinegar et al. 1969). Paw edema induced by carragenan is sensitive to cycloxygenase inhibitors and are used to assess the effect of non steroidalanti-inflammatory agents those primarily inhibit the cycloxygenase which catalyzed prostaglandin synthesis (Sarkhel 2016).

Biogenic silver nanoparticles AgNPCGs inhibited albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane and also reduced the activity of lipoxygenase. The synthesized nanoparticles can be used against deleterious effects of free radicals as well as powerful anti inflammatory agent. It may be concluded from the present findings that AgNPCGs have significant anti-inflammatory activity in both *in vitro* and *in vivo* systems.

DISCLOSURE

The authors declared that no conflict of interest exists in the submission of this manuscript.

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REFERENCES

1. Iwalewa EO, McGaw LJ, Naidoo V, Eloff J NInflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology, 2007; 6: 2868-2885.
2. Khan A, Shah RD, Pallewar SEvaluation of anti-inflammatory and analgesic activity of ethanolic extracts of *Inularacemosa* and *Albiziaamara* International Journal of Pharmacognosy and Phytochemistry Research, 2010; 3: 22-27.
3. Haddad JJMolecular Regulation of Inflammatory Pain and Hyperalgesia - Is NF-κB the Lynchpin? Focus Article and Critical Review. Experimental and Clinical Sciences, 2007; 6: 68-92.
4. Bhattacharya R and Mukherjee P Biological properties of "naked" metal nanoparticles. Advanced Drug Delivery Reviews, 2008; 60: 1289-1306.
5. Verma H, Prasad SB, Yashwant, Singh H Herbal drug delivery system: A modern era prospective. International Journal of Current Pharmaceutical Review and Research, 2013, 4:88-101.
6. Sun Y, Yin Y, Mayers BT, Herricks T, Xia Y Uniform form silver nanowires synthesis by reducing AgNO3 with ethylene glycol in presence of seeds and poly (vinyl pyrrolidone). Chemistry of Materials, 2002; 14: 4736–4745.
7. Yin B, Ma H, Wang S, Chen S Electrochemical synthesis of silver nanoparticles under protection of poly (Nvinyl pyrrolidone). Journal of Physical Chemistry B, 2003; 107: 8898–8904.

8. Dimitrijevic NM, Bartels DM, Jonah CD, Takahashi K, Rajh T Radiolytically induced formation and optical absorption spectra of colloidal silver nanoparticles in supercritical ethane. Journal of Physical Chemistry B, 2001; 105: 954–959.
9. Callegari A, Tonti D, Chergui M Photochemically grown silver nanoparticles with wavelength-controlled size and shape. Nano Letters, 2003; 3: 1565–1568.
10. Naik RR, Stringer SJ, Agarwal G, Jones S, Stone MO Biomimetic synthesis and patterning of silvernanoparticles. Nature Materials, 2002; 1: 169–172
11. SanyalS, Maity P, Pradhan A, Bepari M, MaitiChoudhury S *Calotropis gigantean* linn. Latex: phytochemical screening, antimitotic potential in *Allium cepa* root tip cells and in-vitro cytotoxicity against sarcoma-180. World Journal of Pharmacy and Pharmaceutical Sciences, 2016; 5(11): 1180-1193.
12. Kumar P, Govindaraju M, Senthamilselvi S and Premkumar K Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from *Ulva lactuca*. Colloids Surf. B: Biointerfaces, 2012; 103(2013): 658–661.
13. Maity P, Bepari M, Pradhan A, Baral R, Roy S and MaitiChoudhury S Synthesis and characterization of biogenic metal nanoparticles and its cytotoxicity and anti-neoplasticity through the induction of oxidative stress, mitochondrial dysfunction and apoptosis. Colloids and Surfaces B, 2017; 161: 111-120.
14. Kamalutheen M, Gopalkrishnan S, Ismail TS Anti-inflammatory and anti-arthritic activities of *Merremiatridentata*. E- Journal of Chemistry, 2009; 6(4): 943-948.
15. DeshpandeV, Varsha MJ, Kadam VJ *In-vitro* anti-arthritic activity of *Abutilon indicum*(Linn.) Sweet. Journal of Pharmacy Research, 2009; 2(4): 644-645.
16. Sakat S, Juvekar AR, Gambhire MN *In vitro* antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. International Journal of Pharma and Pharmacological Sciences, 2010; 2(1): 146-155.
17. Shinde UA, Kulkarni KR, Phadke AS, Nair AM, Dikshit VJ, Mungantiwar, Saraf MN Mast cell stabilizing and lipoxygenase inhibitory activity of Cedrusdeodara (Roxb.) Loud. Wood Oil. Indian Journal of Experimental Biology, 1999; 37(3): 258-261.
18. Winter CA, Risley EA, Nuss GW. Carrageenan-induced oedemain the hind paw of rat as an assay for anti-inflammatory activity. Proceedings of the Society for Experimental Biology and Medicine, 1962; 111: 544-7.
19. Vadivu R, Lakshmi KS. *In-vitro* and *in-vivo* anti-inflammatory activity of leaves of *Symplocoscochin chinensis* (lour) Moore spp Laurina. Bangladesh Journal of Pharmacology, 2008; 3(2): 121-124.

20. Yuan G, Wahlqvist ML, He G, Yang M, Li D Natural products and anti-inflammatory activity. Asia Pacific Journal of Clinical Nutrition, 2006; 15(2): 143-152.
21. Leelaprakash G, Mohan Dass S "In vitro Anti-Inflammatory activity of Methanol extract of *Enicostemma Axillare*". International Journal of Drug Delivery and Research, 2011; 3(3): 189-196.
22. Das SN and Chatterjee S. Long term toxicity study of ART-400. Indian Journal of Medical Research, 1995; 16 (2): 117-123.
23. Gardner HW. Recent investigations into the lipoxygenase pathway of plants. Biochimica Biophysica Acta, 1991; 1084: 221–239.
24. Abad MJ, Bermejo P, Villar A The activity of flavonoids extracted from Tanacetummicrophyllum DC. (Compositae) on soybean lipoxygenase and prostaglandin synthetase. General Pharmacology, 1995; 26: 815–819.
25. Hanaa Mohamed El-Rafie, Manal Abdel-Aziz Hamed Antioxidant and anti-inflammatory activities of silver nanoparticles biosynthesized from aqueous leaves extracts of four Terminalia species. Advances in Natural Sciences: Nanoscience and Nanotechnology, 2014; 5: 035008.
26. Vinegar R, Schreiber W, Hugo R Biphasic development of carrageenan in rats. Journal of Pharmacology and Experimental Therapeutics, 1969; 166: 96–103.
27. Sarkhel S Evaluation of the anti-inflammatory activities of *Quillaja saponaria* Mol. saponin extract in mice. Toxicology Reports, 2016; 3: 1–3.