IJBPAS, January, 2016, 5(1): 257-270

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTI-INFLAMMATORY OF SLOW-RELEASE CORTICOSTEROIDON CARRAGEENAN INDUCED PAW EDEMA IN WISTAR RATS

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ABSTRACT

The aim of the present study was to evaluate the anti-inflammatory ability of liposomal prednisolone in a carrageenan induced acute inflammatory paw edema model. Drug delivery to inflammatory tissue via liposomal nanoparticles may improve therapeutic agents' risk–benefit ratios. Prednisolone encapsulated in long-circulating liposomes can inhibit inflammation after administration. The mechanism by which liposomal prednisolone inhibits Production of inflammatory factor. Variable efficacies have been reported for prednisolone drugs as anti-inflammatory treatment.

Liposomal prednisolone was administered to male Wistar rats. Carrageenan at a concentration of 1% was injected into hind paw of rats. The thickness of paws was measured by caliper within 0, 1, 2, 3, and 4 hours after the injections to confirm the inflammation.

The prednisolone and liposomal prednisolone are efficacious in controlling the paw sizes, as indicators of inflammation; the liposomal prednisolone is remarkably more effective in controlling the inflammation in long term.

Keywords: Liposomal, long circulation, Inflammation

INTRODUCTION

Inflammation is a complex biological response of the body to cell damage and vascularized tissue, which can be classified as either acute or chronic depending on the time of onset (Ferrero - Miliani *et al*., 2007). Acute inflammation is the body's primary response to injurious stimuli, and some of the body's responses are characterized by pain, heat, redness, swelling, and loss of function (Gyurkovska et al., 2011, Nathan, 2002).

Ever since their first description by Alec Bangham more than half a century ago, liposomes have been extensively used for drug delivery applications (Bangham et al., 1965, Ismailova et al., 2006) Because of their relatively straightforward preparation, as well as their excellent biodegradability and biocompatibility, liposomal systems have progressed into one of the most extensively used and clinically most advanced drug delivery platforms (Schwendener, 2007).

Liposome consist of a number of bilayers, provides the liposome with structural stability, and enables the encapsulation of pharmacologically active agents, either in the layer itself for lipophilic compounds, or, more commonly, in the aqueous core for hydrophilic compounds (Allen and Cullis, 2013). When liposomes administered locally, the liposomal formulation allows for prolonged retention of the encapsulated drug at the injected site by limiting its diffusion and degradation ('depot' function). By limiting renal excretion and hepatic degradation, some liposome formulations, especially those with high transition-temperature saturated improve the pharmacokinetics of encapsulated drugs when administered systemically, allowing them to circulate for prolonged periods of time In addition, to the 'Enhanced Permeability and Retention' (EPR) effect (Schiffelers et al., 2006).

Using the steroidal anti-inflammatory drugs are usual for inflammatory diseases treatment and prednisolone is a steroid drug index which is a common medicine (Longo et al., 2011).

Glucocorticoids are highly potent anti-inflammatory and immunosuppressive drugs. However, even at moderate doses, systematic administration of glucocorticoids causes many side effects, such as diabetes, hypertension, Cushing syndrome, and osteoporosis (Mandell et al., 1996).

Prednisolone generates anti-inflammatory effects by binding to glucocorticoid receptors, thereby triggering signal transduction pathways(De Bosscher and Haegeman, 2009). Prednisolone improves liver function and inhibits pro-inflammatory cytokines and polymorphonuclear neutrophil activation (Taïeb et al., 2000). Prednisolone have poor pharmacokinetic profile thereby rendering it ineffective in treatment and necessitates high dosages and frequent administration which, in turn, causes an array of adverse systemic effects, including diabetes mellitus, osteoporosis and hypertension(Czock et al., 2005). In order to overcome the above mentioned problems and to improve the pharmacokinetic profile, development of the slow-release dosage forms of the drug is highly desirable (van der Valk et al., 2015, Lobatto et al., 2015, Schiffelers et al., 2005, Schmidt et al., 2003) .On the other hand, encapsulation of prednisolone in long-circulating liposomes can potentially increase drug levels at the site of the action thus improving the therapeutic efficacy(Metselaar et al., 2004, Schiffelers et al., 2006).

The aim of this article was to prepare and evaluate the slow-release nano-suspant of prednisolone and investigate its physicochemical properties addition to its stability.

MATERIALS AND METHODS

Animals

60 Male Wistar rats (6 – 8 weeks and the mean weight of 250±20 gr; Razi Institute, Karaj, Iran) were housed under control conditions (12 hour light-dark cycles, 22°C, and 60% humidity) with free access to food and water on recycled paper pellet bedding. The animals were cared for in accordance with the guidelines of Research branch, Islamic Azad University, Tehran, Iran.

Grouping of animals

60 rats were kept under standard conditions, and were divided in 10 groupsn=6 (A-J) and 6 series as explained in the following:

Group A: Healthy control: intramuscular (IM) injection of Dimethyl sulfoxide(DMSO) plus 100 µL SC injection of sterile distilled water (SDW) in paw;

Group B: Healthy control: intramuscular (IM) injection of SDW plus 100 µL subcutaneous (SC) injection of SDW in paw;

Group C: Inflammation with no treatment: IM injection of DMSO plus 100 µL SC

injection of carrageenan (0.1% in saline) in paw;

Group D: Inflammation with no treatment: IM injection of SDW plus 100 µL SC injection of carrageenan (0.1% in saline) in paw;

Group E: Inflammation with low-dose Prednisolone: IM injection of 2.5 mg/kg prednisolone solution in DMSO plus 100 µL SC injection of carrageenan (0.1% in saline) in paw;

Group F: Inflammation with mid-dose Prednisolone: IM injection of 5 mg/kg Prednisolone solution in DMSO plus 100 µL SC injection of carrageenan (0.1% in saline) in paw

Group G: Inflammation with high-dose Prednisolone: IM injection of 10 mg/kg Prednisolone solution in DMSO plus 100 µL SC injection of carrageenan (0.1% in saline) in paw

Group H: Inflammation with low-dose nano-formulation: IM injection of the nano-formulation equivalent to 2.5 mg/kg Prednisolone dispersed in SDW plus 100 µL SC injection of carrageenan (0.1% in saline) in paw;

Group I: Inflammation with mid-dose nano formulation: IM injection of the nano formulation equivalent to 5 mg/kg Prednisolone dispersed in SDW plus 100 µL SC injection of carrageenan (0.1% in saline) in paw;

Group J: Inflammation with high-dose nano formulation: IM injection of the nano formulation equivalent to 10 mg/kg Prednisolone dispersed in SDW plus 100 µL SC injection of carrageenan (0.1% in saline) in paw.

Inflammatory Stimulus

Carrageenan induced inflammatory model was used to assessthe anti-inflammatory of prednisolon and nano-formulation(Solanki et al., 2015, Morris, 2003).100 µl of freshly prepared carrageenan solution (1%) was Subcutaneous injected into the Left hind paw of each rat excluding the control group (A) which was maintained as vehicle control. To gauge the extent of inflammation, paw thickness was measured before and after injection of edematogenic agent at 0, 1, 2, 3, 4h with the help of digital vernier caliper (Mitotoyu, Series 500, Japan).

Assessment of Anti-inflammatory Efficacy

The drug was injected IM two times: once 12 hours, and then one hour before induced inflammation in three doses 2.5, 5 and 10 mg/kg. Prednisolone was dissolved in DMSO and nano-formulation dispersing in sterile deionized water.

Measurement of paw edema

The dorsal–ventral thickness of rat left hind paw at the central plantar surface was determined with a digital vernier caliper (Mitotoyu, Series 500, Japan) following the subcutaneous injection of Carrageenan. The paw edema was presented as the increase of thickness from the baseline values measured before the injection of the Carrageenan. For each time point, three times were measured and then averaged(Bai et al., 2006).

Statistical analysis

Descriptive data were expressed as mean±SEM. Comparison of different groups was carried out by one-way ANOVA followed by the post hoc Tukey test. All analyses were performed using IBM SPSS Statistics Software (version 22 for Windows; SPSS Inc., Chicago, IL, USA). In all comparisons $p<0.05$ was considered significant.

RESULTS

Effects of prednisolone on carrageenan-induced rat paw edema

Carrageenan-induced paw edema is a routine, useful model to assess the contribution of mediators involved in vascular changes associated with acute inflammation.

Paw volume was used as an indicator of the anti-inflammatory efficacy of the prednisolone-encapsulated liposomes formulation, at 0 hours, 4 hours. A comparison between the inflammations occurred in different groups of rats in different times following the drug administration is made in (Fig.1, 2 and 3 and Table 1).According these data, firstly, the inflammation has occurred in all test groups, secondly both the free and nano-liposomal drugs were efficacious in controlling the paw sizes, as indicators of inflammation, thirdly and most importantly, the Nano-formulation was remarkably more effective in controlling the inflammation both in short-term and long-term, and fourthly, increasing the drug dose was advantageous for anti-inflammatory effect until the dose of 10 mg/kg in both free and liposomal drug.

As shown in Fig. 1, paw thickness in the group D compared with Group B. The paw in 4 time 1 hour after inflammation (T1), 2 hour after inflammation (T2), 3 hour after inflammation (T3), 4hour after inflammation (T4) increase the paw thickness which is statistically significant ($^{**}p<0.001$). Paw thickness in the group E Compared with group D decrease in the paw thickness in 4 times T_1, T_2, T_3, T_4

statistically was not significant. paw thickness in the group F Compared with group D decrease the paw thickness in 4 time T_1, T_2, T_3, T_4 which is statistically significant ($^{\#\#}p< 0.001$). Paw thickness in the group, G compared with Group D decrease in the paw thickness in 4 time T_1, T_2, T_3, T_4which is statistically significant ($^{\#\#}p<0.001$).

As shown in Fig. 2, paw thickness in the group, C Compared with Group A The paw thickness in 4 time T_1, T_2, T_3, T_4increase in the paw thickness which is statistically significant($^{**}p<0.001$). Thickening in the group, H Compared with Group C decrease the paw thickness in 4 time T_1, T_2, T_3, T_4 which is statistically significant ($^{\#\#}p<0.001$). paw thickness in the group, I Compared with Group F (inflammation with no treatment) decrease in the paw thickness in 4 time T_1, T_2, T_3, T_4 which is statistically significant ($^{\#\#}p<0.001$). paw thickness in the group, J Compared with Group C decrease in the paw thickness in 4 time T_1, T_2, T_3, T_4which is statistically significant ($^{\#\#}p<0.001$).

As shown in Fig. 3, paw thickness in the group H compared with group, E decrease in the paw thickness in Fourth time (T_4) which is statistically significant ($^{*}p<0.05$). Paw thickness in the group I compared with Group , F decrease in the paw thickness in in 2 time T3 and T4 which is statistically significant ($^{*}p<0.05$) and ($^{**}p<0.001$). Paw thickness in the group J Compared with Group , G decrease in the paw thickness in in 2 time T3 and T4 which is statistically significant($^{*}p<0.05$) and ($^{**}p<0.001$).

DISCUSSION

Clinical applications of nanotechnology are now on the verge of becoming a reality, the promising data of experimental findings awaiting confirmation in clinical trials(Viscido et al., 2014). Although prednisolone are highly potent drugs, and although they have been proven to be useful for the treatment of many different diseases, the severe side effects associated with their prolonged and/or high-dose use have somewhat limited their broad clinical applicability (Teshima et al., 2004). Consequently, in order to improve drug efficacy and at the same time reduce toxicity, significant research efforts have focused on the development of drug delivery systems for prednisolone (Lobatto et al., 2010, Hofkens *et al*., 2011).

Recently, drug delivery systems using liposomes as drug carriers have been well studied to achieve controlled and site-

specific delivery of drugs (Fatouros and Antimisiaris, 2002).liposomes are solid, water insoluble nano and microparticles composed of a solid hydrophobic core containing a layer of a phospholipid embedded on the surface of the corea layer of a phospholipid embedded on the surface of the core. The hydrophobic core is made of solid triglycerides or fatty acid esters containing the active agent (Teshima et al., 2004).

Researchers have shown that the processing methods for liposome preparation have significant effects on its properties such as size and efficiency (Maestrelli et al., 2006, Elgart et al., 2012).The prednisolone and liposomal prednisolone are efficacious in controlling the paw sizes, as indicators of inflammation; the liposomal prednisolone is remarkably more effective in controlling the inflammation in long term (Metselaar et al., 2003, Metselaar et al., 2004, Yamazaki et al., 2013, Banciu et al., 2008, Banciu et al., 2006).

Using nano-liposome structure and designing prednisolone nano-formulation lead to an increase in drug delivery and additionally, it leads to slow-release in comparison with the ordinary prednisolone. Using the slow release form eventually leads to an increase in the medication distances, reduces the value of medicine, and finally reduces the concentration of the drug in body and drug presence in target tissue for longer times (Metselaar et al., 2003). It also leads to an increase in the therapeutic index of drug, i.e., drug usage become easier and its side effects will be reduced and it becomes economically affordable.

ACKNOWLEDGMENT

The authors would like to thank Behdashtkar pharmaceutical laboratories (Rasht, Iran) for their kindly donation of prednisolone.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

ALLEN, T. M. & CULLIS, P. R. 2013. Liposomal drug delivery systems: from concept to clinical applications. *Advanced drug delivery reviews,* 65**,** 36-48.

BAI, Z.-T., LIU, T., CHAI, Z.-F., PANG, X.-Y. & JI, Y.-H. 2006. Rat pain-related responses induced by experimental scorpion BmK sting. *European journal of pharmacology*, 552**,** 67-77.

BANCIU, M., METSELAAR, J. M., SCHIFFELERS, R. M. & STORM, G. 2008. Liposomal glucocorticoids as tumor-targeted anti-angiogenic nanomedicine in B16 melanoma-bearing mice. *The Journal of steroid biochemistry and molecular biology,* 111**,** 101-110.

BANCIU, M., SCHIFFELERS, R. M., FENS, M. H., METSELAAR, J. M. & STORM, G. 2006. Anti-angiogenic effects of liposomal prednisolone phosphate on B16 melanoma in mice. *Journal of controlled release,* 113**,** 1-8.

BANGHAM, A., STANDISH, M. M. & WATKINS, J. 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of molecular biology,* 13**,** 238-IN27.

CZOCK, D., KELLER, F., RASCHE, F. M. & HäUSSLER, U. 2005. Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clinical pharmacokinetics,* 44**,** 61-98.

DE BOSSCHER, K. & HAEGEMAN, G. 2009. Minireview: latest perspectives on antiinflammatory actions of glucocorticoids. *Molecular endocrinology,* 23**,** 281-291.

ELGART, A., CHERNIAKOV, I., ALDOUBY, Y., DOMB, A. J. & HOFFMAN, A. 2012. Lipospheres and pro-nano lipospheres for delivery of poorly water soluble compounds. *Chemistry and physics of lipids,* 165**,** 438-453.

FATOUROS, D. G. & ANTIMISIARIS, S. G. 2002. Effect of amphiphilic drugs on the stability and zeta-potential of their liposome formulations: a study with prednisolone, diazepam, and griseofulvin. *Journal of colloid and interface science,* 251**,** 271-277.

FERRERO -MILIANI, L., NIELSEN, O., ANDERSEN, P. & GIRARDIN, S. 2007. Chronic inflammation: importance of NOD2 and NALP3 ininterleukin1β generation. *Clinical & Experimental Immunology,* 147**,** 227-235.

GYURKOVSKA, V., ALIPIEVA, K., MACIUK, A., DIMITROVA, P., IVANOVSKA, N., HAAS, C., BLEY, T. & GEORGIEV, M.

2011. Anti-inflammatory activity of Devil's claw in vitro systems and their active constituents. *Food Chemistry*.

HOFKENS, W., VAN DEN HOVEN, J. M., PESMAN, G. J., NABBE, K. C., SWEEP, F. C., STORM, G., VAN DEN BERG, W. B. & VAN LENT, P. L. 2011. Safety of glucocorticoids can be improved by lower yet still effective dosages of liposomal steroid formulations in murine antigen-induced arthritis: comparison of prednisolone with budesonide. *International journal of pharmaceutics,* 416**,** 493-498.

ISMAILOVA, G., EFREMENKO, V. & SAVEL'EVA, I. 2006. Efficacy of a new magnet-driven liposomal form of prednisolone in the treatment of experimental allergic dermatitis. *Pharmaceutical Chemistry Journal,* 40**,** 177-181.

LOBATTO, M. E., CALCAGNO, C., OTTEN, M. J., MILLON, A., RAMACHANDRAN, S., PARIDAANS, M. P., VAN DER VALK, F. M., STORM, G., STROES, E. S. & FAYAD, Z. A. 2015. Pharmaceutical development and preclinical evaluation of a GMP-grade anti-inflammatory nanotherapy. *Nanomedicine: Nanotechnology, Biology and Medicine,* 11**,** 1133-1140.

LOBATTO, M. E., FAYAD, Z. A., SILVERA, S., VUCIC, E., CALCAGNO, C., MANI, V., DICKSON, S. D., NICOLAY, K., BANCIU, M. & SCHIFFELERS, R. M. 2010. Multimodal clinical imaging to longitudinally assess a nanomedical anti-inflammatory treatment in experimental atherosclerosis. *Molecular pharmaceutics,* 7**,** 2020-2029.

LONGO, D., FAUCI, A., KASPER, D. & HAUSER, S. 2011. *Harrison's Principles of Internal Medicine 18th edition*, McGraw-Hill Professional.

MAESTRELLI, F., GONZALEZ-RODRIGUEZ, M. L., RABASCO, A. M. & MURA, P. 2006. Effect of preparation technique on the properties of liposomes encapsulating ketoprofen cyclodextrin complexes aimed for transdermal delivery. *International journal of pharmaceutics,* 312**,** 53-60.

MANDELL, G., PETRI, W., HARDMAN, J., LIMBIRD, L., MOLINOFF, P. & RUDDON, R. 1996. Goodman and Gilman's the pharmacological basis of therapeutics. *McGraw-Hill, New York***,** 1073-1101.

METSELAAR, J., VAN DEN BERG, W., HOLTHUYSEN, A., WAUBEN, M., STORM, G. & VAN LENT, P. 2004. Liposomal targeting of glucocorticoids to synovial lining cells strongly increases therapeutic benefit in collagen type II arthritis. *Annals of the rheumatic diseases,* 63**,** 348-353.

METSELAAR, J. M., WAUBEN, M. H., WAGENAAR-HILBERS, J., BOERMAN, O. C. & STORM, G. 2003. Complete remission of experimental arthritis by joint targeting of glucocorticoids with long-circulating liposomes. *Arthritis & Rheumatism,* 48**,** 2059-2066.

MORRIS, C. J. 2003. Carrageenan-induced paw edema in the rat and mouse. *Inflammation protocols*. Springer.

NATHAN, C. 2002. Points of control in inflammation. *Nature,* 420**,** 846-852.

SCHIFFELERS, R. M., BANCIU, M., METSELAAR, J. M. & STORM, G. 2006. Therapeutic application of long-circulating liposomal glucocorticoids in auto-immune diseases and cancer. *Journal of liposome research,* 16**,** 185-194.

SCHIFFELERS, R. M., METSELAAR, J. M., FENS, M. H., JANSSEN, A. P., MOLEMA, G. & STORM, G. 2005. Liposome-encapsulated prednisolone phosphate inhibits growth of established tumors in mice. *Neoplasia,* 7**,** 118-127.

SCHMIDT, J., METSELAAR, J. M., WAUBEN, M. H., TOYKA, K. V., STORM, G. & GOLD, R. 2003. Drug targeting by long-circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis. *Brain,* 126**,** 1895-1904.

SCHWENDENER, R. A. 2007. Liposomes in biology and medicine. *Bio-Applications of Nanoparticles*. Springer.

SOLANKI, H. K., SHAH, D. A., MAHERIYA, P. M. & PATEL, C.

A. 2015. Evaluation of anti-inflammatory activity of probiotic on carrageenan-induced paw edema in Wistar rats. *International journal of biological macromolecules,* 72**,** 1277-1282.

TAIEB, J., MATHURIN, P., ELBIM, C., CLUZEL, P., ARCE-VICIOSO, M., BERNARD, B., OPOLON, P., GOUGEROT-POCIDALO, M. A., POYNARD, T. & CHOLLET-MARTIN, S. 2000. Blood neutrophil functions and cytokine release in severe alcoholic hepatitis: effect of corticosteroids. *Journal of hepatology,* 32**,** 579-586.

TESHIMA, M., KAWAKAMI, S., NISHIDA, K., NAKAMURA, J., SAKAEDA, T., TERAZONO, H., KITAHARA, T., NAKASHIMA, M. & SASAKI, H. 2004. Prednisolone retention in integrated liposomes by chemical approach and pharmaceutical approach. *Journal of controlled release,* 97**,** 211-218.

VAN DER VALK, F. M., VAN WIJK, D. F., LOBATTO, M. E., VERBERNE, H. J., STORM, G., WILLEMS, M. C., LEGEMATE, D. A., NEDERVEEN, A. J., CALCAGNO, C. & MANI, V. 2015. Prednisolone-containing liposomes accumulate in human atherosclerotic macrophages upon intravenous administration. *Nanomedicine: Nanotechnology, Biology and Medicine,* 11**,** 1039-1046.

VISCIDO, A., CAPANNOLO, A., LATELLA, G., CAPRILLI, R. & FRIERI, G. 2014. Nanotechnology in the treatment of inflammatory bowel diseases. *Journal of Crohn's and Colitis,* 8**,** 903-918.

YAMAZAKI, Y., YASUDA, K., MATSUYAMA, T., ISHIHARA, T., HIGA, R., SAWAIRI, T., YAMAGUCHI, M., EGI, M., AKAI, S. & MIYASE, T. 2013. A Penicillium sp. F33 metabolite and its synthetic derivatives inhibit acetyl-CoA: 1-O-alkyl-sn-glycero-3-phosphocholine acetyltransferase (a key enzyme in platelet-activating factor biosynthesis) and carrageenan-induced paw edema in mice. *Biochemical pharmacology,* 86**,** 632-644.

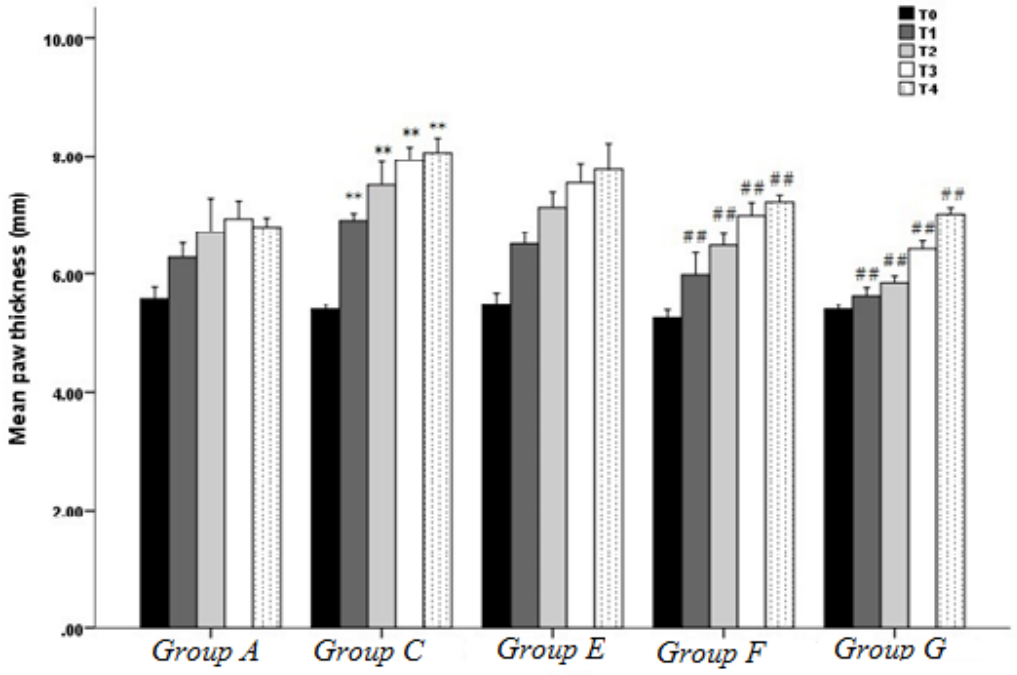

Fig.1: The comparison the paw thickness averages between control, inflammation and doses prednisolone in 5 different times. (The data is presented as mean +SD, n = 6). Group A: DMSO control, Group, Group C: DMSO, carrageenan control, Group E: 2.5 mg/kg prednisolone , Group F: 5 mg/kg Prednisolone, Group G: 10 mg/kg Prednisolone, . T_0: carrageenan injection time. T_1:1 hour after inflammation, T_2: 2 hour after inflammation, T_3: 3 hour after inflammation, T_4: 4hour after inflammation.

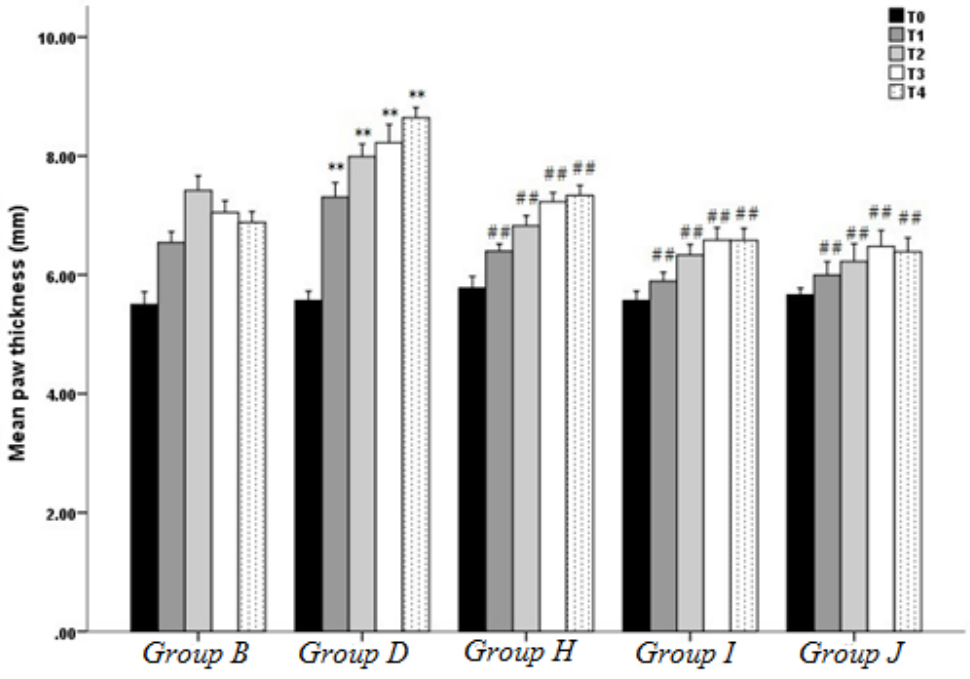

Fig.2: The comparison of the paw thickness in different groups Control and nano-formulations (The data is presented as mean +SD, n = 6). Group B: SDW control, Group D: SDW, carrageenan control, Group H: nano-formulation equivalent to 2.5 mg/kg Prednisolone, Group I: nano formulation equivalent to 5 mg/kg Prednisolone, Group J: nano formulation equivalent to 10 mg/kg Prednisolone,. T0: carrageenan injection time. T1:1 hour after inflammation, T2: 2 hour after inflammation, T3: 3 hour after inflammation, T4: 4hour after inflammation.

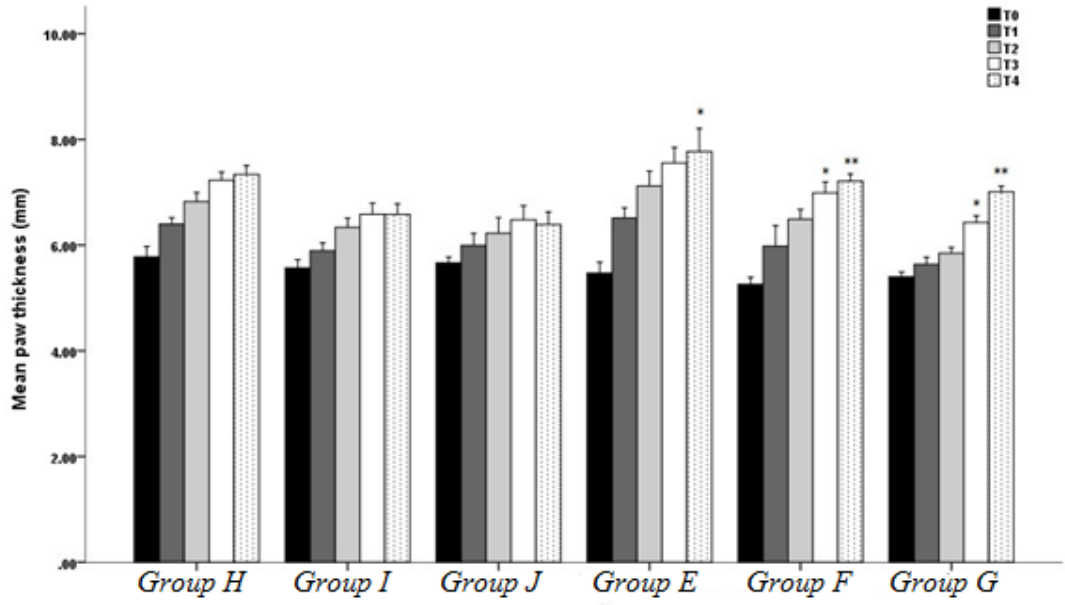

Fig.3: The comparison the paw thickness averages between, doses prednisolone and nano-formulation in 5 different times(The data is presented as mean +SD, n = 6. Group E: 2.5 mg/kg prednisolone , Group F: 5 mg/kg Prednisolone, Group G: 10 mg/kg Prednisolone, Group H: nano-formulation equivalent to 2.5 mg/kg Prednisolone, Group I: nano formulation equivalent to 5 mg/kg Prednisolone, Group J: nano formulation equivalent to 10 mg/kg Prednisolone,. T0: carrageenan injection time. T1:1 hour after inflammation, T2: 2 hour after inflammation, T3: 3 hour after inflammation, T4: 4hour after inflammation.

Table 1: The comparison between the paw thickness averages of all rats in various injection times in the groups(The data is presented as mean +SD, n = 6). Group A: DMSO control, Group B: SDW control, Group C: DMSO, carrageenan control, Group D: SDW, carrageenan control, Group E: 2.5 mg/kg prednisolone , Group F: 5 mg/kg Prednisolone, Group G: 10 mg/kg Prednisolone, Group H: nano-formulation equivalent to 2.5 mg/kg Prednisolone, Group I: nano formulation equivalent to 5 mg/kg Prednisolone, Group J: nano formulation equivalent to 10 mg/kg Prednisolone

Groups	T0 Paw thickness (mm)	T1 Paw thickness (mm)	T2 Paw thickness (mm)	T3 Paw thickness (mm)	T4 Pawthickness (mm)
Group A	5.59±0.18	6.29±0.22	6.70±0.52	6.93±0.27	6.80±0.14
Group B	5.50±0.20	6.54±0.17	7.42±0.23	7.05±0.18	6.88±0.17
Group C	5.40 ±0 .08	6.90±0.10	7.52±0.35	7.91±0.21	8.05±0.22
Group D	5.40 ±0 .08	6.90±0.10	7.52±0.35	7.91±0.21	8.05±0.22
Group E	5.47±0.19	6.51±0.18	7.11±0.26	7.55±0.28	7.77±0.41
Group F	5.25±0.12	5.98±0.37	6.49±0.17	6.98±0.19	7.20±0.13
Group G	5.40±0.08	5.63±0.12	5.84±0.10	6.42±0.12	7.00±0.10
Group H	5.77±0.18	6.39±0.11	6.82±0.16	7.22±0.14	7.33±0.16
Group I	5.56±0.15	5.89±0.14	6.33±0.16	6.58±0.20	6.58±0.19
Group J	5.66±0.10	5.99±0.21	6.22±0.28	6.48±0.25	6.38±0.22