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STUDY OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF POMEGRANATE SEED ARILS EXTRACT ON DIFFERENT EXPERIMENTAL MODELS OF ACUTE INFLAMMATION

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ABSTRACT

Dietary supplements containing pomegranate arils seed extract are popular *Ayurvedic medicine* for the treatment and prevention of a number of inflammatory diseases as arthritis and other inflammatory painful diseases due to its components of polyphenols, which have potential antioxidant, exerting anti-inflammatory comparable and similar to that of NSAIDs; those are associated with many side effects. Objective: Pomegranate seed arils extract chosen for testing its anti-inflammatory activities on formalin-induced inflammatory paw edema model, on acute pleurisy model induced by carrageenan and also, for testing its analgesic activities on acute visceral pain induced by acetic acid in experimental animals. The inflammatory paw edema induced by formalin in hind paw of rats, acute pleurisy induced by pleural injection of carrageenan in rats and acute pain model of writhing test induced by acetic acid in mice. The anti-inflammatory activity was assessed by measurement hind paw edema volume by water displacement method, volume of pleural exudate and its total leukocyte count were determined and numbers of writhes counted in writhing test and by comparing with control group and other groups taken a reference anti-inflammatory analgesic drug as Diclofenac sodium. There were a significant reduction in inflammatory paw edema volume, a significant reduction in inflammatory pleural exudate volume and its total content of leukocytes in rat groups received pomegranate seed extract and also, there was a significant reduction in numbers of writhing movements in mice groups received pomegranate seed extract ($p<0.05$) compared to others without treatment with pomegranate extract and when compared to reference animal groups treated by Diclofenac sodium. The results demonstrated that pomegranate arils seed extracts possess potential anti-inflammatory and analgesic properties that can signify the basis for traditional uses of pomegranate extracts for acute and chronic inflammatory painful disorders as arthritis and pleuritis.

Keywords: *Punica granatum L*, Pomegranate arils seed extract, Anti-inflammatory, Analgesic, Formalin, Carrageenan, Acetic acid, Diclofenac sodium.

INTRODUCTION

Punica granatum L. (Pomegranate), is a common small fruit tree, belonging to the Punicaceae family which grown worldwide, Mediterranean area and in Far East countries. Both the juice and peel of pomegranate are rich in polyphenols [1]. The inside of a pomegranate is hundreds of arils, which are the seed pods inside a pomegranate separated by cream-colored membranes. These arils consist of juicy, brilliant-red fruit surrounding tiny, crisp, edible seeds which have many health benefits. Pomegranate seed juice is a good source of fructose, sucrose, and glucose that have been become increasingly popular because of the attribution to the plant of important biological actions including antioxidant activity and cardiovascular protection [2].

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Pomegranate and its seed arils extracts are essential compounds of anthocyanin production that cause the red color of juice with strong antioxidant and anti-inflammatory activity and with inhibition of inflammatory markers such as tumor necrosis factor alpha (TNFα**)** [3].The dried flowers of Pomegranate and its seed arils juice are used in hematuria, hemorrhoids, hemoptysis and dysentery and powdered flower buds are used in bronchitis, and Pomegranate juice is recommended as a gargle for sore throat, in leucorrhea, hemorrhages and ulcers of the uterus and rectum [4, 5]. Pomegranate's uses now are for its biological activities as antibacterial, antifungal, anthelmintic and antifertility as well as in the treatment of acquired immune deficiency syndrome (AIDS) [6].

Various extracts from different parts of the plant have antihepatotoxicity, anti-tumour and anti-inflammatory activities, and also, all pomegranate flavonoids show antioxidant activity with indirect inhibition of inflammatory markers such as tumor necrosis factor alpha (TNFα) [7, 8]. We aim for investigating potential anti-inflammatory and analgesic activities of Pomegranate seed arils extract on inflammatory paw edema induced by formalin, on carrageenan-induced pleurisy in rats and acute visceral pain induced by acetic acid in mice.

MATERIALS AND METHODS

Extraction method for Preparation of the pomegranate seed arils extract

The whole fresh pomegranate arils seed materials fruits were grounded into powder using a blender. Then the powder was dried in an oven at 40 °C for 24 h. The powder was sieved through a 24-mesh filter. The resultant powder of more than one kilogram was extracted with 2500 ml of 80 % ethanol in water at room temperature for 24 h in a shaking water bath and then was filtered. The ethanol was removed by using rotary evaporator and the resultant residue was crude ethanolic extract; approximately 110 g of crude ethanol extract and were used for study according to methods of [9].

Drugs and Chemicals

Formalin 2.5 % solution, Carrageenan: Iota (Type V, C3799), acetic acid 1 %, Pomegranate seed arils extract (400 mg /kg) and Diclofenac sodium 50 mg Tablets were used.

Experimental animal

36 Albino rats weighing 100-150 grams and 18 albino mice weighing 30-50 grams; were used in this study and were purchased. Animals were housed in cages with not more than 4 animals per cage and maintained under standard laboratory conditions (temperature 25 ± 20C) and a 12/12 h dark/light cycle and received feed, water *ad libitum*.

Anti-inflammatory activity study

Acute Formalin-induced rat paw inflammatory edema

The inflammatory paw edema induced by injection of formalin (2.5%) according to method of **[8]** by subplantar injection of 0.1 ml of 2.5 % suspension of formalin in the right hind paw of the hamster. Hind Paw edema volumes were measured by water displacement method (plethysmometer) method at 1, 2, and 3 hours after the administration of the standard drug and tested pomegranate extracts. Animals subdivided into 3 groups (6 rats per group). Group A are control group received normal saline solution and the right hind paw injected irritant formalin for induction of inflammatory edema. Group B are tested group received pomegranate seed extract (400 mg /kg) by IP, one hour before the right hind paw injected irritant formalin for induction of inflammatory edema. Group C are standard reference group received Diclofenac sodium (standard anti-inflammatory agent) at a dose of 50 mg/kg body weight by IP, one hour before the right hind paw injected irritant formalin for induction of inflammatory edema.

The anti-inflammatory effect of the pomegranate seed extract was calculated by the following equation according to method of *Asif et al* **[10].**

Anti-inflammatory activity % (Relative paw edema %) = $\frac{(V2 - V1)}{V1} \times 100$

Where

V1 the animal paw volume before injection of formalin, V2 the paw volume from after the injection of formalin.

Acute pleurisy model

Carrageenan-induced pleurisy in rats

This pleurisy model is considered a screening tool anti-inflammatory drugs; where the intrapleural injection of carrageenan induced an acute inflammatory response, characterized by an increase in the volume of the pleural exudate and number of leukocytes that migrated to the cavity in 3 hours. The test was performed according to Vinegar. 18 rats were in acute pleurisy model ; where group 1 saline treated group received (0.1 ml, p.o.), group 2 pomegranate extract treated received oral pomegranate (400mg/kg, p.o.) and group 3 Diclofenac sodium (standard group) received oral dose (50 mg/kg p.o). After 30 minutes of administration of corresponding tested drugs, all animals groups received intrapleural injection of carrageenan (200 microg/pleural cavity of each rat). 4 hours later, the pleural exudate was collected, the volume was determined, and the pleural cavity was washed with 1.0 ml of saline that contained heparin. The number of migrating leukocytes in the exudate was determined with a Neubauer chamber.

Analgesic activity study

Acetic acid induced writhing test (Acute model of visceral pain)

18 mice were used and subdivided into 3 groups (6 per each); Group 1 are control group given oral gavages with saline (10ml/kg, oral dose, p.o.), Group 2 tested group taken pomegranate seed extract (400mg/kg, p.o.), and Group 3 reference group taken Diclofenac sodium (50 mg/kg, p.o.; used as standard analgesic agent. Acetic acid solution (0.6%, 10ml/kg) at a dose of 0.1 mL/10g of body weight, was injected intra-peritoneally 30 min after the 3 groups were treated by corresponding treating agents per each group. The number of writhes responses consisting of contraction of abdominal wall and pelvic rotation followed by hind limb extension produced in each animal after injection of irritant acetic acid. The number of writhes was counted for a period of 15 minutes immediately after the acetic acid administration according to method of Collier *et al* [12].

The index of pain inhibition was calculated as follows: IPI = $\frac{(X0 - Xi)}{X0}$ X 100

X0 is the number of writhes observed in control group treated by saline, **Xi** is the number of writhes in the tested groups treated by Pomegranate seed extract and Diclofenac sodium

STATISTICAL ANALYSIS

The results obtained were expressed as mean ± S.E.M. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's *t* test to determine the level of significance. A value of $P<0.05$ was considered to be significant.

RESULTS

Effects of pomegranate seed extract on acute inflammatory paw edema volume

There was strong inhibition of the inflammatory hind paw edema in group B received pomegranate seed extract where the % inhibition of inflammatory paw edema was 38.35% in pomegranate treated group B compared to Diclofenac treated group C (45.22%) (Table 1 & Figure 1, Images 1 & 2).

Effect of Pomegranate extract on carrageenan-induced pleurisy in rats

Oral treatment with Pomegranate seed arils extract (400 mg/kg) in rats of group 2, significantly reduced the intensity of the pleural inflammatory response with marked reduction in total leukocyte migration, and significantly reduce pleural exudate volume by 52 % compared with rats treated only with saline. Group 3 treated with Diclofenac sodium (50 mg/kg, p.o) as the standard drug exhibited a significantly reduction in both of inflammatory exudate, and leukocyte migration to the pleural cavity compared with rats treated only with saline Table 2.

Analgesic activity of pomegranate seed extract on Acetic acid induced writhing test

Group 2 of mice taken Pomegranate seed extract showed a significant decrease in the number of writhes compared to controlled untreated group 1 saline treated (Table 1 and Table 2). In the acetic acid induced writhing, the index of pain inhibition (IPI), by pomegranate arils seed extract in group 2 exhibited very significant analgesic potency (was 52.25% inhibition) compared to group 3 treated by Diclofenac sodium at dose of 50 mg/kg b.w. (was 65.00% inhibition) (Tables 3, 4 and Figure 2, Images 3, 4, 5).

Table 1. Effects of pomegranate seed extract on formalin induced inflammatory paw edema

Rat groups	Hind Paw edema volume (mL)		
	1 h	**2 h**	**3 h**
Group A (Control saline)	78.93 ± 2.15	91.42 ± 2.33	106.11 ± 2.10
Group B (Pomegranate-formalin)	60.96 ± 1.89* (21.27%)	63.10 ± 1.51*(32.12%)	66.14 ± 2.38*(38.35%)
Group C (Diclofenac–formalin)	53.58 ± 1.29*(32.70%)	59.13 ± 1.15*(36.16 %)	60.66 ± 1.40*(45.22%)

Values are mean ± SEM. (n=6).

*****$p<0.001$ (One-way ANOVA and Dunnett's t test), significantly different from control.

Figures in parentheses are the % inhibition of paw edema in both groups of pomegranate treated and Diclofenac treated groups.

Table 2. Effect of pomegranate seed arils extract treatment on exudate volume and leukocytes number 3 hours after carrageenan injection in pleural cavity in rats at dose of (200 microg /pleural cavity).

Parameters measured / Rat groups	Exudate volume (mL)	Inhibition (%) of pleural exudate	Total leukocytes count (cell /mm^3) X 10^3
Group 1(Control saline treated)	0.72 ± 0.15	-	62045 ± 7210
Group 2 (Pomegranate extract treated)	0.31 ± 0.05*	52	47865 ± 8446
Group 3 (Diclofenac sodium treated)	0.24 ± 0.1*	65	52389 ± 6218

Data are mean ± SEM of 6 rats per group

*P < 0.05 compared to control group (ANOVA, Dunnett's t test).

Table 3. Effect of pomegranate arils seed extract on number of writhes in different mice groups

Drugs / Animal groups	Mean of Number of writhes count for 15 minutes after acetic acid
	Mean of number of Writhing per group
Group 1 Control saline	40
Group 2 Pomegranate-acetic	19
Group 3 Diclofenac–acetic	14

Table 4. Effect of pomegranate arils seed extract on index of pain inhibition (IPI) of acetic acid induced writhes in mice

Drugs / Animal groups	Parameters measured		
	Dose (mg/Kg)	Writhing	% of inhibition (IPI)
Group 1 Control saline	10 mL/Kg	39.1 ± 1.61*	-----
Group 2 Pomegranate-acetic	400	18.2 ±1.33*	52.25
Group 3 Diclofenac–acetic	50	13.5 ± 1.20*	65.00

Writhes values represent mean ± SEM (n=6).
*p<0.001(One -way ANOVA and Dunnett's t test, significantly different from control).

Fig 1. Control group with right hind paw inflammatory edema induced with formalin

Fig 2. Pomegranate treated group with marked reduction in inflammatory paw edema of right hind paw

Fig 3. Injection of acetic acid I.P

Fig 4. Showing writhing movement : torsion of body

Fig 5. Showing writhing movement : extension of hind limb and tail

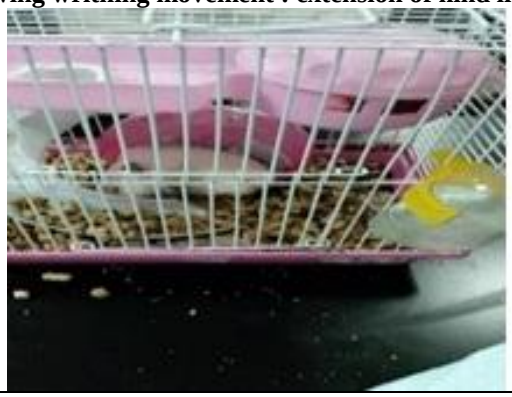


Fig 6. Demonstrating effects of Pomegranate extract on acute inflammatory paw edema volume

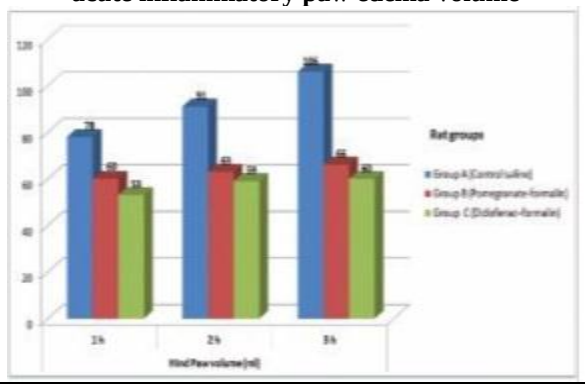
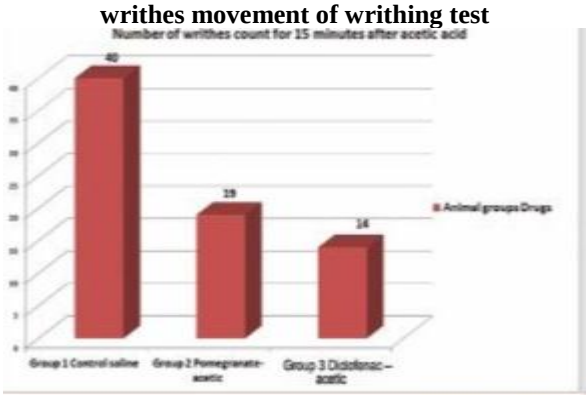


Fig 7. Demonstrating effects of Pomegranate extract on writhes movement of writhing test



DISCUSSION

Inflammation is associated with the pathophysiology of various clinical conditions such as arthritis and oesarthritis; where acute inflammation is a beneficial host defensive response to tissue damage or any injurious stimuli [13]. NSAIDs used for treatment of acute and chronic inflammatory conditions, but have gastrointestinal irritation therefore, the use of plants that having anti-inflammatory effects without side effects can be good replacement for this drug class. In the present study, the anti-inflammatory and analgesic activities of pomegranate seed extract was investigated, using acute models of inflammation induced by formalin and by acetic acid writhing test ; showed that the pomegranate seed extract possess a significant anti-inflammatory, anti-edematogenic and analgesic effects on rats with acute inflammatory paw edema and mice injected i.p acetic acid Therefore, the results study are an indication that pomegranate seed extracts can be effective in acute inflammatory painful disorders most probably via inhibition of soluble proinflammatory mediators TNF-α, interleukins (e.g. IL-6 and IL-8), bioactive lipids such as eicosanoids (e.g. prostaglandin E2 and lipoxygenase derived products, which are strongly implicated in pathophysiology of acute and chronic painful inflammatory conditions [14].

The formalin-induced rat paw edema is very similar to carrageen-induced rat paw edema which is a biphasic process. The release of histamine or serotonin occurs in the first phase and the second phase is associated with the production of bradykinin, protease, prostaglandin, and lysosome [15]. Therefore, the inhibition of formalin-induced inflammation and reduction of writhes movements by the extract of pomegranate could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis, which was supported by results of Ahmed et [16] who reported that standardized extracts of pomegranate fruit have been showed to possess dose dependent anti-inflammatory, analgesic and cartilage sparing effects on rat paw edema. Anti-inflammatory and analgesic effects of pomegranate extract could be attributed to its high content of flavonoids that have ability to inhibit pain perception and exerting anti-inflammatory properties due to their inhibitory effects on COX1 and COX2 enzymes involved in the production of the chemical mediator of inflammation [15-17].

The results of the present study were in accordance to results of Bagri et al., [18] reported that the phytochemical analysis of pomegranate seed and flower extract also revealed that it contains flavonoids characterized as 5,6,7,8,2′,3′,5′-heptahydroxy- 4′-methoxyflavanone (punicaflavanol) and 5,6,7,8,2′,5′-hexahydroxy-4′- methoxyflavanone -7-*β*-D- xylopyranoside (granatumfla vanyl xyloside) and All these Flavonoids are well known for their ability to inhibit pain perception and exerting anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation [19 & 20]. Furthermore, a previous findings supporting our results were suggested that ellagic acid, a commonly used herbal supplement, may enhance the effects of ketorolac in reducing or eliminating inflammatory nociception for up to 24 hours, based on a rat model. The affordability of ellagic acid and its potential to enhance other COX inhibitors such as ketorolac has the potential to make healthcare for all persons with inflammatory pain more cost-effective and obtainable [21].

Acute pleurisy model is considered a screening tool anti-inflammatory drugs. In the present study, the oral treatment with Pomegranate extract (400 mg/kg) significantly reduced exudate accumulation in the pleural cavity and total leukocyte migration compared to standard group treated with anti-inflammatory drug, Declofenac sodium and when compared with rats treated only with saline. Our results were in agreement with results of previous studies reported that, Pomegranate peel aqueous extract exerted antioxidant and anti-inflammatory properties manifested by a significant inhibition of neutrophil myeloperoxidase (MPO) activity and attenuation of lipopolysaccharide-induced lung inflammation in mice and explained that Inhibition of MPO activity by pomegranate peel aqueous extract was attributed to its anti-inflammatory action [22-24] .

Additionally, there were other studies reported that Punicalagin and ellagic acid were shown to possess strong anti-mutagenic and anti-proliferative activities in B(a) P-induced lung cancer model [25, 26].

CONCLUSION

Results of pomegranate showed a favorable anti-inflammatory and analgesic activities could be used for prevention and treatment of several inflammatory painful conditions as osteoarthritis, rheumatoid arthritis, and lung pleurisy. Furthermore, clinical trials needed for investigating antioxidant, anti-inflammatory and analgesic effects of pomegranate to signify its therapeutic uses on human clinically.

ACKOWLEDGEMENT

Nil

CONFLICT OF INTEREST

No interest

REFERENCE

1. Schubert SY, Neeman I, Resnick N. A novel mechanism for the inhibition of NF-kappaB activation in vascular endothelial cells by natural antioxidants, The FASEB. *Journal*, 16, 2002, 1931–1933.
2. Aviram M, Dornfeld L, Kaplan M, Coleman R, Gaitini D, Nitecki S, Hofman A, Rosenblat M, Volkova N, Presser D, Attias J, Hayek T, Fuhrman B. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. *Drugs under Experimental and Clinical Research*, 28, 2001, 49–62.
3. Newman RA, Lansky EP, Block ML. Pomegranate The Most Medicinal Fruit. Laguna Beach, California: Basic Health Publications. *A Wealth of Phytochemicals*, 2007, 120.
4. Ross RG, Selvasubramanian S, Jayasundar S. Immuno modulatory activity of Punica granatum in rabbits—a preliminary study. *Journal of Ethnopharmacology*, 78, 2001, 85–87.
5. Ali M and Sharma N. Phytochemical investigations of the flowers of Punica granatum. *Indian Journal of chemistry*, 45B, 2006, 1681-1685.
6. Lee CJ, Chen LG, Liang WL , Wang CC. Anti-inflammatory effects of Punica granatum Linne in vitro and in vivo. *Food Chemist*, 118, 2010, 315–322.
7. Das SC, Bhadra S, Roy S, Saha SK, Islam MS, Bachar SC. Analgesic and Anti-inflammatory Activities of Ethanolic Root Extract of Swertia chirata (Gentianaceae). *Jordan Journal of Biological Sciences*, 5, 2012, 31-36.
8. Hemmati AA, Arzi A, Sistani, NK and Mikaili, P. The Hydro-Alcoholic Extract of Pomegranate Seed Has Anti-Inflammatory Effects on Formalin-Induced Inflammation in Rat Hind Paw. *Research Journal of Biological Sciences*, 5(8), 2010, 561-564.
9. BenSaad LB, Hwi KK, Quah T. Evaluation of the antinociceptive effect of the ethanolic extract of Punica granatum. *Afr J Tradit Complement and Altern Med*, 11(3), 2014, 228-233
10. Asif M and Kumar A. Anti-inflammatory activity of ethanolic extract of Dalbergia sissoo (roxb.) Bark. *Malaysian Journal of Pharmaceutical Sciences*, 7(1), 2009, 39–50.
11. Vinegar R, Truax JF, Selph JL. Some Quantitative Temporal Characteristics of Carrageenin-Induced Pleurisy in the Rat. *Proc.Soc.Exp.Biol.Med*, 3(143), 1973, 711-714.
12. Collier HO, Dinneen LC, Johnson CA. and Scheider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol Chemother*, 3, 1968, 295-31
13. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*, 109, 2000, 177–206.
14. Das SC, Bhadra S, Roy S, Saha SK, Islam MS, Bachar SC. Analgesic and Anti-inflammatory Activities of Ethanolic Root Extract of Swertia chirata (Gentianaceae). *Jordan Journal of Biological Sciences*, 5, 2007, 31-36.
15. Shukla M, Gupta K, Rasheed Z, Khan KA, Haqqi TM. Bioavailable constituents/metabolites of pomegranate (Punica granatum L) preferentially inhibit COX2 activity ex vivo and IL1betainduced PGE2 production in human chondrocytes in vitro. *J Inflamm (Lond)*, 5, 2008, 9.
16. Ahmed SN, Wang B, Bin Hafeez VK, Cheruvu and Haqqi TM. Punica granatum L. extract inhibits IL-la-induced expreSSlOn of matrix metalloproteinases by inhibiting the activation of MAP kinases and NF -SB in hwnan chondrocytes in vitro. *J. Nutr*, 135, 2005, 2096-2102.
17. Mithun S, Sreedam C, Sajal K, *et al.* Analgesic and Anti-inflammatory Activities of Flower Extracts of Punica granatum Linn.(Punicaceae). *Journal of Applied Pharmaceutical Science*, 02(04), 2012, 133-136
18. Bagri P, Ali M, Sultana S, Aeri V. New flavonoids from Punica granatum flowers. *Chemistry of Natural Compounds*, 46(2), 2010, 201- 204.
19. Owoyele VB, Oloriegbe YY, Balogun EA, Soladoye AO. Analgesic and anti-inflammatory properties of *Nelsonia canescens* leaf extract. *J. Ethnopharmacol*, 99, 2005, 153-156.

20. Mithun S, Sreedam C, Sajal K, Zobaer A and Sitesh C. Analgesic and Anti-inflammatory Activities of Flower Extracts of *Punica granatum* Linn.(Punicaceae). *Journal of Applied Pharmaceutical Science*, 02(04), 2012, 133-136.
21. Gainok J, Daniels R, Golembiowski D, Kindred P, *et al.* Investigation of the Anti-inflammatory, Antinociceptive Effect of Ellagic Acid as Measured by Digital Paw Pressure via the Randall-Selitto Meter in Male Sprague- Dawley Rats. *AANA Journal*, 79, 2011, 4.
22. Iwata M, Suzuki S, Asai Y, Inoue T, Takagi K. Involvement of Nitric Oxide in a Rat Model of Carrageenin-Induced Pleurisy. Mediators. *Inflamm*, 2011, 11.
23. Bachoual R, Talmoudi W, Boussetta T, Braut F, El-Benna J. An aqueous pomegranate peel extract inhibits neutrophil myeloperoxidase in vitro and attenuates lung inflammation in mice. *Food Chem. Toxicol*, 49, 2011, 1224–1228.
24. Husari A, Khayat A, Bitar H, Hashem Y. Antioxidant activity of pomegranate juice reduces acute lung injury secondary to hyperoxia in an animal model. *BMC Res. Notes*, 7, 2014, 664.
25. Zahin M, Ahmad I, Gupta R. Punicalagin and ellagic acid demonstrate antimutagenic activity and inhibition of benzo[a]pyrene induced DNA adducts. *Biomed. Res. Int.*, 2014, 467465.
26. Khan N, Afaq F, Kweon MH, Kim K, Mukhtar H. Oral consumption of pomegranate fruit extract inhibits growth and progression of primary lung tumors in mice. *Cancer Res*, 67, 2007, 3475–3482.