Clinical Pharmacology & Biopharmaceutics
ISSN: 2167-065X

Thopate, Clin Pharmacol Biopharm 2022, 11:1

Review Article Open Access

Evaluation of Anti-Inflammatory & Immunosuppressant Activity of Dexamethasone

Rutuja Thopate*

Shivajirao Pawar College of Pharmacy Pachegaon, Tal-Newasa, Ahmednagar, Maharashtra, India

Abstract

Glucocorticoids are powerful anti-inflammatory compounds and widely used in the therapy of allergic diseases. This study aimed to determine the effect of dexamethasone (Dex) on the immunomodulatory function in vitro and in vivo. The development of in vitro methods is important in drug discovery process due to the restriction of animal use in the pharmacological screening process. Corticosteroids are routinely utilized to alleviate edema in patients with intracranial lesions and are first-line agents to combat immune-related adverse events (irAEs) that arise with immune checkpoint blockade treatment. However, it is not known if or when corticosteroids can be administered without abrogating the efforts of immunotherapy. The purpose of this study was to evaluate the impact of dexamethasone on lymphocyte activation and proliferation during checkpoint blockade to provide guidance for corticosteroid use while immunotherapy is being implemented as a cancer treatment.

Keywords: Dexamethasone; Anti-inflammation; Immunosuppression

Introduction

Inflammation is the process in which a series of reactions occur as a part of body's defense mechanism against various endogenous and exogenous stimuli. According to its length, inflammation is classified as acute and chronic. Healing, a process which is not completed without inflammation is an immune response of the body; to remove foreign body from the body, it may be living organism, chemical or physical stimuli. The main symptoms of inflammation are pain, swelling, redness, heat, and immobility. The major components of inflammation are categorized as vascular and cellular changes. Vascular changes involve increased blood flow, momentary vasoconstriction of blood vessels, and vasodilation of arterioles and venules; increased permeability leads to release of chemical mediators, swelling, and increased viscosity. Cellular changes involve migration of leukocyte from circulation to the degradation of bacteria. These changes could be monitored for the investigation of anti-inflammatory activity of a compound [1,2].

The major signs of the inflammation are pain, swelling, and fever. Large varieties of anti-inflammatory drugs are available in the market. However, none of these drugs are free from side effects. The development of less gastric irritation producing drugs is important. In addition to in vivo method, some standard primary assays are available to investigate the anti-inflammatory activity in drug discovery process. The development of in vitro methods is important in drug discovery process due to the restriction of animal use in the pharmacological screening process. In vitro assays are simple and not complex like in vivo methods. Most of these methods mimic in vivo response and are now widely used for the monitoring and development of new agents against inflammation. Glucocorticoids (GCs) inhibit the expression of inflammatory mediators by macrophages and other cells and are used in the treatment of many immune-mediated inflammatory diseases [3]. Anti-inflammatory actions of GCs are widely thought to be mediated by transrepression, in which the ligand-activated GR interferes with the capacity of NF-κB and activator protein 1 to induce the transcription of inflammatory mediators.

Immunotherapy is emerging as a promising anti-cancer treatment and is now part of the standard of care for certain advanced cancers including melanoma and non-small cell lung carcinoma. Encouraging results from recent studies suggest that intracranial lesions located beyond the blood-brain barrier may also be targeted by the immune system. However, patients with intracranial lesions are frequently provided corticosteroids before commencing immunotherapy to combat cerebral edema and reduce symptom burden. Corticosteroids are also first-line agents against immune-related adverse events (irAEs) that may develop during or following immunotherapy, particularly checkpoint blockade.

Dexamethasone demonstrates anti-inflammatory as well as immunosuppression activity. As an anti-inflammatory medication Dexamethasone relieves inflammation in various parts of the body. It is used specifically to decrease swelling (edema), associated with tumors of the spine and brain, and to treat eye inflammation. To treat or prevent allergic reactions.

Dexametasone

Pharmacokinetics

Dexamethasone is 6-hydroxylated by CYP3A4 to 6α- and 6β-hydroxydexamethasone. Dexamethasone is reversibly metabolized to 11-dehydrodexamethasone by corticosteroid 11-beta-dehydrogenase isozyme 2 and can also be converted back to dexamethasone by Corticosteroid 11-beta-dehydrogenase isozyme 1.

Parmacodynamics

Corticosteroids bind to the glucocorticoid receptor, inhibiting pro-inflammatory signals, and promoting anti-inflammatory signals. Dexamethasone's duration of action varies depending on the route. Corticosteroids have a wide therapeutic window as patients may require doses that are multiples of what the body naturally produces. Patients taking corticosteroids should be counseled regarding the risk

***Corresponding author:** Rutuja Thopate, Shivajirao Pawar College of Pharmacy Pachegaon, Tal-Newasa, Ahmednagar, Maharashtra, India; E-mail: rutujathopate6419@gmail.com

Received: 19-Jan-2022, Manuscript No. CPB-22-51900; **Editor assigned:** 21-Jan-2022, PreQC No. CPB-22-51900(PQ); **Reviewed:** 25-Jan-2022, QC No. CPB-22-51900; **Revised:** 29-Jan-2022, Manuscript No. CPB-22-51900(R); **Published:** 05-Feb-2022, DOI: 10.4172/2167-065X.1000248

Citation: Thopate R (2022) Evaluation of Anti-Inflammatory & Immunosuppressant Activity of Dexamethasone. Clin Pharmacol Biopharm 11: 248.

of hypothalamic-pituitary-adrenal axis suppression and increased susceptibility to infections**.**

Mechanism of action

The short term effects of corticosteroid are decrease vasodilation and permeability of capillaries as well as decrease leukocyte migration to site of inflammation. Corticosteroid binding to glucocorticoid receptor mediates changes in general expression that lead to multiple downstream effect over hours to days. Glucocorticoid inhibits neutrophils apoptosis and demigrination; they inhibit phospholipase A2 which decrease the formation of arachidonic acid derivatives they inhibit NF Kappa B and other inflammatory transcription factors, they promote anti-inflammatory genes like interleukin. Lower dose of corticosteroids provide an anti-inflammatory effect while higher dose are immunosuppressive. High dose of glucocorticoid for an extent period bind to the mineralo corticoid receptor raising sodium levels and decreasing potassium levels.

Brand name

Baycadron, Ciprodex, Decadron, Dexamethasone Intensol, Dextenza, Dioptrol, Hexadrol, Hidex 6-day Taper, Maxidex, Maxitrol, Neofordex, Ozurdex, Taperdex 12 Day Taper, Taperdex 6 Day Taper, Taperdex 7-day Taper, Tobradex, Zcort 7 Day Taper.

Uses

Dexamethasone is used to treat many different conditions such as allergic disorders, skin conditions, ulcerative colitis, arthritis, lupus, psoriasis, breathing disorders, eye conditions, blood cell disorders, leukemia, multiple sclerosis, inflammation.

In Vivo Screening Methods of Dexamethasone For Anti-Inflammatory Agents

In this screening methods, the potency of anti-inflammatory agents are measured, by inducing the inflammation in the experimental animal like rats, mice, monkeys, dog or either sex can be selected. Before starting any in vivo assays, it is important to study the oral acute toxicity study, for the selection of test dose in this maximum tolerated dose and minimum toxic dose are calculated, by injecting the test dose at an interval of 2 hrs in 10.50.100, 200, 300.... 2000mg/kg pattern.

Acute phase: The methods that include acute phase are as follows

- Carrageenan induced paw edema in rats
- Croton-oil induced ear edema
- Oxazolone induced ear edema
- UV erythema in guinea pigs
- Pleurisy in rats
- Granuloma air pouch technique
- Vascular permeability

Chronic phase: The methods that include this chronic phase are

- Cotton wool granuloma
- Glass rod granuloma
- Sponge implantation technique

Carrageenan induced paw odema in rats

Methodology: Male or female sprague-dawley rats with a body weight between 150 and 170g are used the animals are starved for overnight. The animal should be grouped; this grouping is based on of test compounds. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of lateral malleolus and immersed in the mercury up to this mark.

Evaluation: The paw volume was measured using plenthysmometer at 3 and 6 hour, eventually at 24 hour immediately after the injection. The increase in paw volume at 3 and 6 hours is calculated. The percentage increase in paw volume was measured by comparing the difference of the average values between treated group animals and control group animals is calculated at each interval time. A dose-response curve is run for the active drugs and ED50 values can be determined. Generally the treated group animal show much less edema than control group animal.

Croton oil induced ear edema in mice

Methodology: Both rats and mice are used, usually male rats (Sprague dawley) of 80-60 kg is selected, whereas male Swiss mice weighing between 18-22 g is selected. Grouping is done according to the body weight. In this methodology, a total of 15ml containing an acetonic solution of 75microg of croton oil is applied to the inner surface of the right ear of each mouse. The animals are previously, anaesthetized with diethyl ether at a rate 0.02 ml in rats and 0.01 ml in mice. Left ear remains untreated control group receives the irritant solution, while indomethacin serves as a standard (reference). Varying dose levels of test drug are applied to inner surface of the right ear of each mouse inducing inflammation. As soon as the test and standard drug are given, the animals are sacrificed by cervical dislocation, both treated and untreated ear are removed and a plug of 8 mm diameter is removed from each of the ear. The difference in weight between the two plugs is taken as the measure of edema to us response.

Evalution: This methodology is mainly used to detect anti-inflammatory activity of steroids. The % anti-inflammatory activity is calculated by, % anti-inflammatory activity = (wt of treated ear-wt of untreated ear/wt of control ear) 100

Oxazolone induced ear edema in mice

This model permits the quantitative evaluation of the topical and systemic anti-inflammatory activity of the compound following topical administration.

Methodology: The experimental animal used in this model is mice and they are divided into 12 each group. They are injected with the 10 ml of 0.5% of oxazolone in acetone in to the right ear and the same was given repeatedly 3 times per week, to the sensitized right ear. In non-sensitized animals, acetone alone was given. The mice are again injected with 0.01 ml of 2% oxazolone solution inside of the right ear, in which test or standard are resolved**.** Group of 10 to 15 animals are treated with irritant alone or with the solution of the test compound. The left ear remains untreated and actual inflammation occurs after 24 hrs. The animals are sacrificed under anesthesia, at this time and a disc of 8mm diameter is punched from both the sides. The discs are allowed to dry and are weighed on a balance. The weight difference is an indicator of inflammatory edema.

Evalution: Average values of the increase of weight are calculated for each treated group and compared statistically with control group.

UV erythema in guniea pigs

Methodology: Albino guinea pigs of both the sexes weighing about 350g are used. Four animals are used each for treatment and the control, 18 hrs prior to the experiment; the animals are shaved on both the flanks and on the back, chemically by means of a depilatory cream or using a suspension of barium chloride. The next day the test compound is dissolved in the vehicle and half of it is administered to the animal by gavage, 30min before the UV exposure. Control animals are treated with the vehicle alone. The guinea pigs are placed in a leather cuff with a hole of 1.5x2.5cm size punched in it, allowing the UV radiation to reach only this area. During this time, the remaining half of the test compound is administered. Generally the erythema is scored after 2 and 4 hr of exposure.

Evalution: The degree of erythema is evaluated in a double blinded manner. The following scores are given:

- no erythema
- weak erythema
- strong erythema
- very strong erythema

Animals with a score of 0 or 1 are said to be protected. The scoring after 2 and 4 hr generally indicates some duration of the effect and ED50 values can be calculated.

Pleurisy test

Methodology: The animal used in pleurisy test was mouse. A single dose of 0.1 ml carrageenan was injected intravenously. After 4 hr the animal was killed with an over dose of ether and the pleural cavity was washed with 1.0 ml of sterile PBS, containing heparin (20 IU per ml). Samples of the pleural lavage were collected for determination of exudation myeloperoxidase, adenosine deaminase activities, and nitric oxide levels, as well as for determination of total and differential leukocyte counts [4]. Total leukocyte counts were performed in a Neuberger Chamber. The serum level of the C-reactive protein was also analyzed. In another set of experiment animals were treated 30 min before carrageenan with a solution of Evans blue dye (25 mg/kg, IV) in order to evaluate the degree of exudation in the pleural space. The amount of dye was estimated by colorimetry using an Elisa plate reader at 600nm, by interpolation from a standard curve of Evans blue dye in the range of 0.01 to 50 g/ml [5] .

Evalution: The values of each experimental group are averaged and compared with that control. ED50 values can be calculated using various doses.

Granuloma pouch technique

Methodology: Male or female Sprague-Dawley rats with a body weight between 150 and 200 g are used. Ten animals are taken for controls and for test groups. The back of the animals is shaved and disinfected. With a very thin needle a pneumoderma is made in the middle of the dorsal skin by injection of 20ml of air under ether anesthesia, which results in oval air pouch.0.5 ml of a 1% solution of Croton oil in sesame oil is injected, avoiding any leakage of air [6]. The air pouch is removed after 48hrs.

The test compound is injected directly in to the air sac at the same time as the irritant. On the 4th or 5th day animals are sacrificed under anesthesia. The pouch is opened and the exudate is collected in glass cylinders. Total no. of leukocytes migrated in to the pouch are evaluated after staining with Erythrosine B [7].

Evalution: The average value of the exudates of the controls and the test groups is calculated. Comparison is made by statistical.

Vascular permeability

Methodology: Albino Wistar are used each group containing 4 rats. Control group will receive distilled water 1% w/v 1 ml/100 g by oral route and other group will receive test compound by oral route and standard group will receive diclofenac 10 ml/kg by intraperitoneal route. After 1hr of the administration, rats are injected with 0.25ml of0.6% v/v solution of acetic acid intraperitoneally. Immediately, 10 ml/kg of 10% w/ Evans blue is injected intravenously via tail vain. After 30 min, the animals are anesthetized with ether anesthesia and sacrificed. The abdomen is cut open and exposed viscera [8]. The animals are held by a flap of abdominal wall over a Petri dish. The peritoneal fluid (exudates) collected, filtered and made up the volume to 10 ml using normal saline solution and centrifuged at 3000 rpm for 15 min. The absorbance (A) of the supernatant is measured at 590 nm using spectrophotometer.

Evalution: Decreased concentration of dye with respect to absorbance indicates reduction in permeability.

Cotton wool induced granuloma

Methodology: Male rats weighing about 180-200g are used. The test drugs were administered orally once on a dosage regimen for 7 days and the control group received the vehicle [9]. Two sterilized pellets of cotton wool were implanted subcutaneously, one on each side of abdomen of the animal, under the light ether anaesthesia and sterile technique. The rats were sacrificed on the 8th day, the implanted pellet was dissected out and the wet weight was recorded. Thymuses were also dissected out. Both of these were dried at 60°c for 18 hrs and the dry weight was recorded.

Evalution: The weight of transudate and the granuloma as well as the percent granuloma inhibition of the test drugs were calculated. The body weight gain was also recorded.

Glass rod granuloma technique

Methodology: In this technique, glass rod with a diameter of 6mm and 40 mm length are selected. Male sprague-dawley rats with an initial weight of 130g are selected. These rats are anaesthetized with ether and their back skins were shaved and disinfected. From an incision in the caudal region a subcutaneous tunnel is formed in the cranial direction with a closed blunted forceps. The glass rod is introduced in to this tunnel which lies on the back of the animal. The incision wound is closed by sutures [10]. The animals are kept in separate cages; the rods remain insitu for 20 or 40 days. At the end, the animals are sacrificed under CO_2 anesthesia. The glass rod is prepared with surrounding connective tissue which forms a tube around the glass rod. Wet weight of the granuloma tissue is recorded, finally it is dried and the dry weight is also recorded.

Evalution: Granuloma weight reduced by the test compound is compared with that of the standard.

Sponge implantation technique

Methodology: Sponges used for implantation are prepared from poly vinyl foam sheets (thickness) 5mm discs are punched out to

standard size and weight(10.0 ± 0.02 mg) using a 13 mm cork borer. The sponges are then soaked in 76%v/v ethanol for 30 min, and then heated at 80 for 2 hr. Prior to implantation in the animal, the sponges are soaked in sterile 0.9% saline in which drugs, antigens (or) irritants have been suspended. Sponges are implanted in female wistar rats weighing about 150-200g under ether anaesthesia.

A 20 mm dorsal incision is made and the dermis is separated from the underlying muscle layer by insertion of blunt forcepsto form separate cavities in to which the sponges are inserted [11].

Up to 8 sponges may be implanted per rat the insertion is closed with micheal clips and the animals are maintainted at a constant temperature of 24.

Evaluation: For estimation of fluid phase of sponge exudates eg: protein content enzyme levels and biological mediators such as prostaglandins as well as for leucocyte migration, sponges removed after 9 hrs.

In Vitro Screening Methods of Dexamethasone For Anti-Inflammatory Agents

- Mast cell degranulation
- Adhesion assay
- Lipopolysaccharide induced response assay
- Cyclooxygenase assay

Mast cell degranulation

During the inflammation and allergy, the mast cells are degranulated. The degree of this degranulation is a significant criterion in the pharmacological screening process of therapeutic agents against inflammation. The mast cell degranulation models are widely utilized for this study. In addition to degranulation, histamine and beta-hexosaminidase are also released, which stimulate the metabolic process of arachidonic acid. The measurement of such substances is also helpful in the pharmacological screening process for new anti-inflammatory agents. There are several methods employed to detect degranulation of mast cells and release of mediators. They include enzyme-linked immunosorbent assays (ELISAs) or colorimetric assays [12]. Colorimetric assay to measure the inflammatory mediators is rapid and sensitive. Another reported method is based on the particle analysis of granules in RBL-2H3 cells. Fluorometric assay of histamine and flow cytometric Annexin-V binding assay are also available for this purpose. The percentage release of inflammatory mediator is the index of anti-inflammatory activity.

Adhesion assay

Adhesion of leukocytes is an important cellular stage during the inflammation process. Adhesion assay involves the estimation of vascular proteins vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and E-selectin in primary cells derived from human umbilical vein endothelial cells and human microvascular endothelial cell line [13-15]. Human dermal microvascular endothelial cells are the most suitable to predict in vivo situations in the best manner. In the adhesion assay, tumor necrosis factor (TNF)-alpha stimulated cell lines are incubated with test drug. The procedure involves labeling of neutrophil with appropriate fluorescent materials and allowing adhering. Finally, the measurement of fluorescence is carried out at 485 nm in an ELISA reader [16].

Platelet-neutrophil adhesion assay

In this method, neutrophils are mixed with thrombin-activated platelets, which are previously incubated with drugs for 10 min. If the test drug inhibits the adhesion of neutrophils, it indicates an anti-inflammatory activity of the tested drug. A control is also carried out along with the test sample [17].

Lipopolysaccharide (LPS) induced response assay

Expression of inducible NO synthase (iNOS) protein, NO production, TNF-alpha expression, and p38 MAP Kinase (MAPK) by LPS in mouse macrophages can be utilized for screening anti-inflammatory activity. When cell lines are treated with LPS, it induces the production of iNOS proteins, NO, TNF-alpha expression, and MAPK protein expression; these can be measured by western blot method [18]. This should be performed with and without the presence of the test drug. In the presence of existing anti-inflammatory drugs, a reduction in the production of these substances will be noted. The degree of inhibition could be compared with standard drugs [19].

Cyclooxygenase (COX) assay

The COX enzymes convert archidonic acid to prostaglandins [20]. COX has two forms, COX-1 and COX-2 [21]. Here, COX-1 is constitutive, which is important in physiological functions, whereas COX-2 is inducible and involved in pathological function [22]. In the inflammatory process, this enzyme has an important role. Hence, the estimation of the end product such as prostaglandin concentration will be helpful in the pharmacological screening of anti-inflammatory agents. The various vitro methods are employed for this purpose.

COX-1 assay

In this assay, the sample solution was mixed with L-adrenaline-D-hydrogen tartrate and hematin. Then, the incubation of the mixture is carried out with COX-1. Then, the arachidonic acid is added and incubated for 20 min at 37°C. Addition of formic acid to the mixture stops the incubation. Using enzyme immunoassay, measure the concentration of prostaglandin 2.

COX-2 assay

The basis of this assay is that N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) gets oxidized when the reduction of prostaglandin-G2 to prostaglandin-H2 occurs []. The oxidation velocity of TMPD is measured spectrophotometrically. The sample is introduced to the mixture of sodium phosphate buffer, gelatin, hematin, and purified COX-2. This mixture is pre-incubated with arachidonic acid before the addition of TMPD. The percentage inhibition is determined by measuring the absorbance at 610 nm.

Screening Methods of Dexamethasone For Immunosuppression Activity

Methods

- T-cell preparation culture and treatment
- Westeron blot analysis

T-cell preparation, culture and treatment

Healthy donor leukapheresis packs were obtained from the NIH blood bank (protocol 99-CC-0168). T cells were negatively selected using an Easy Sep Hu-man T cell isolation kit (Stem cell Technologies) and cryopreserved in 90% FBS and 10% DMSO until

use. Cells were thawed in a 37°C water bath and cultured overnight in RPMI1640 medium containing 10% feta lbovine serum, 1% penicillin-streptomycin-glutamine,1% MEM non-essential amino acids solution, 15 mm HEPES, 1 mm sodium pyruvate and 55 μM2-mercaptoethanol. Cells were plated at 1*105/200 μlin 96-well round-bottom plates with M-450 Tosylactivated beads. Dexamethasone was purchased from Sigma Aldrich (D4902) and dissolved in DMSO. Niviolumab and ipilimumab F(ab')2 were used to block PD-1 and CTLA-4, respectively. Ipilimumab F(ab')2 was created using a Pierce F(ab')2 Preparation Kit per the manufacturer's instructions (Thermo Fisher Scientific, MA, USA). Cells were incubated at 37 °C in 20% O2, and 5% CO2 for four days for proliferation analyses and two days for Western blot and qPCR analyses.

Western blot analysis

Isolated human T cells were collected after 48 h of stimulation and lysed in RIPA buffer with EDTA- free protease inhibitor cocktail set III (EMD Millipore, Billerica, Massachusetts, USA). Pierce BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA) was used to determine protein concentration. Sample swere separated by SDS-PAGE (Bio-Rad) and transferred onto 0.2 μm pore size poly vinylidene fluoridemembranes (PVDF) (In vitrogen, Carlsbad, CA, USA). The following antibodies were purchased from Cell Signaling: cleaved caspase 3 (5A1E), p27kip (2552 s),cyclin D3 (DCS22), CDK4 (D9G3E). Anti-CTLA-4(EPR1476) was purchased from Abcam. The bandswere detected by Super Signal West Pico chemiluminescence reagent (Pierce, Rockford, IL, USA). Anti-bodiesagainst β-actin (AG74) or GAPDHstandard were used as internal standards.

Conclusion

Anti-inflammatory agent the several in vitro methods are developed for the pharmacological screening of anti-inflammatory activity. Many of the method reflect In vivo performance. These methods help to understand the real mechanism of inflammation and to identify new compounds possessing the anti-inflammatory activity. It is very difficult to develop single in vitro method for anti-inflammatory activity. Even in future. The mentioned method will accelerate the anti-inflammatory drug development process. The impact of dexamethasone on T cell subsets in the setting of immunotherapy. Dexamethasone blocks naïve T cell proliferation and differentiation by attenuating CD28 co-stimulation. Because co-stimulation is essential for successful T cell priming and expansion, these data suggest that corticosteroid similar response in immunotherapy treatment-naïve patients or those with poorly antigenic tumors. However, T cells may be partially protected or rescued from the immunosuppressive effects of dexamethasone with administration of CTLA-4 blockade. Additionally, negative corticosteroid effects are diminished after developing a successful anti-tumor immune response.

References

1. Koh TJ, DiPietro LA (2011) Inflammation and wound healing: The role of the macrophage. Expert Rev Mol Med 13:e.
2. Nourshargh S, Alon R (2014) Leukocyte migration into inflamed tissues. Immunity 41:694-707.
3. Hughes JP, Rees S, Kalindjian SB, Philpott KL (2011) Principles of early drug discovery. Br J Pharmacol 162:19-1249.
4. Joo T, Sowndhararajan K, Hong S, Lee J, Park SY, et al. (2014) Inhibition of nitric oxide production In LPS-stimulated RAW 264.7 cells by stem bark of Ulmus pumila L. Saudi J Biol Sci 21:427-435.
5. Langer HF, Chavakis T (2009) Leukocyte-endothelial interactions in inflammation. J Cell Mol Med 13:1211-1220.
6. Wildner O, Lipkow T, Knop J (1992) Increased expression of ICAM-1 E-selectin, and VCAM-1 by Cultured human endothelial cells upon exposure to haptens. Exp Dermatol 1:191-198.
7. Schön MP, Krahn T, Schön M, Rodriguez ML, Antonicek H, et al. (2002) Efomycine M, a new Specific inhibitor of selectin, impairs leukocyte adhesion and alleviates cutaneous inflammation. Nat Med 8:366-372.
8. Håkanson R, Rönnberg AL (1974) Improved fluorometric assay of histamine: Condensation with Ophthalaldehyde at -20. Anal Biochem 60:560-567.
9. Taylor ML, Ilton MK, Misso NL, Watkins DN, Hung J, et al. (1998) The effect of aspirin on Thrombin stimulated platelet adhesion receptor expression and the role of neutrophils. Br J Clin Pharmacol 46:139-145.
10. Lee MH, Lee JM, Jun SH, Ha CG, Lee SH, et al. (2007) In-*vitro* and n-*vivo* anti-inflammatory Action of the ethanol extract of trachelospermicaulis. J Pharm Pharmacol 59:1-130.
11. Reis J, Guan XQ, Kisselev AF, Papasian CJ, Qureshi AA, et al. (2011) LPS-induced Formation of immunoproteasomes: TNF-α and nitric oxide production are regulated by altered Composition of proteasome-active sites. Cell BiochemBiophys 60:77-88.
12. Chen Y, Junger WG (2012) Measurement of oxidative burst in neutrophils. Methods Mol Biol 844:115-124.
13. Chow LW, Yip AY, Chu WP, Loo WT, Toi M. (2011) Bone metabolism and quality-of-life of Postmenopausal women with invasive breast cancer receiving neoadjuvant hormonal therapy: Sub-analyses from celecoxib anti-aromatase neoadjuvant (CAAN) trial. J Steroid Biochem Mol Biol 125:112-119.
14. Sung-Hwan E, Eun-Hye L, Kunbawui P, Ji-Young K, Poong-Ho K, et al. (2016) Eckol from Eisenia bicyclis inhibits inflammation through the Akt/NF-κb signaling in propionibacterium acnes-Induced human keratinocyte hacat cells. J Food Biochem 41:e112.
15. Mohammed NA, Abd El-Aleem SA, El-Hafiz HA, McMahon RF (2004) Distribution of constitutive (COX-1) and inducible (COX-2) cyclooxygenase In postviral human liver cirrhosis: A possible role for COX-2 in the Pathogenesis of liver cirrhosis. J Clin Pathol 57:350-354.
16. Resch M, Steigel A, Chen ZL, Bauer R (1998) 5-lipoxygenase and Cyclooxygenase-1 inhibitory active compounds from Atractylodeslancea. J Nat Prod 61:347-350.
17. Petrovic N, Murray M (2010) Using N,N,N',N'-tetramethyl-p-Phenylenediamine (TMPD) to assay cyclooxygenase activity in vitro. In: Armstrong D, editors. Advanced Protocols in Oxidative Stress II. Methods in Molecular Biology (Methods and Protocols). Totowa, New Jersey: Humana Press 594.
18. Copeland RA, Williams JM, Giannaras J, Nurnberg S, Covington M, et al. (1994) Mechanism of selective inhibition of the inducible isoform of Prostaglandin G/H synthase. Proc Natl Acad Sci USA 91:11202-11206.
19. Farber SH, Tsvankin V, Narloch JL, Kim GJ, Salama AK, et al. (2016) Embracing rejection: Immunologic trends in brain metastasis. Oncoimmunology 5:1172153.
20. Fecci PE, Ochiai H, Mitchell DA, Grossi PM, Sweeney AE, et al. (2007) Systemic CTe14 Blockade ameliorates glioma-induced changes to the CD4+T cell compartment without affecting Regulatory T-cell function. Clin Cancer Res 13:2158-2167.
21. Larkin J, Hodi FS, Wolchok JD (2015) Combined Nivolumab and Ipilimumab or monotherapy in Untreated melanoma. N Engl J Med 373:1270-1271.
22. Weber JS, Kahler KC, Hauschild A (2012) Management of immune-related adverse events and Kinetics of response with ipilimumab. J Clin Oncol 30:2691-2697.
23. Jijith US, Jayakumari S (2018) In *vitro* pharmacological screening Methods for anti-inflammatory agents. Asian J Pharma Clin Res 11.