Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

IN VIVO AND *IN VITRO* ANTIINFLAMMATORY ACTIVITY OF LEAVES OF *IPOMOEA STAPHYLINA*

FIRDOUS SM[1*], RAJU KONERI[1]

[1]Department of Pharmaceutical Science, NIMS University, Shobha Nagar, Delhi Highway, Jaipur 303121, Rajasthan, India.
Email: firdous_cology@rediffmail.com

Received: 04 Aug 2012, Revised and Accepted: 17 Sep 2012

ABSTRACT

The present investigation was carried out to find the effect of ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions for antiinflammatory activity in rodents. The extract and its fractions (200 mg/kg, p.o.) showed significant antiinflammatory activity in carrageenan induced paw oedema. In cotton-pellet induced granuloma model the extract and its fractions (200 mg/kg, p.o.) significantly (P<0.001) decreased the granuloma formation. The inhibitory effects of the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions on the production inflammatory cytokines tumor necrosis factor-alpha (TNF-α) was studied in a lipopolysacchride (LPS) activated RAW 264.7 cell line. The ethanolic extract its fractions potently inhibited TNF-α. The viability of cells at different concentration (0-300 µg/ml) was unaffected as determined by the MTT cytotoxicity assay.

Keywords: *Ipomoea staphylina*, Anti-inflammatory, Carrageenan, Granuloma, Tumor necrosis factor-alpha (TNF-α), Lipopolysacchride, Cytotoxicity.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation[1].

The treatment of inflammation is depends on nonsteroidal or steroidal anti-inflammatory agents[2]. Nonsteroidal antiinflammatory drugs (NSAID) reduce the pain and inflammation by blocking the cyclooxygenase enzyme (COX), and thus the production of prostaglandin[3], but long-term administration of NSAID may induce gastrointestinal ulcers and renal disorders due to their non-selective inhibition of both isoforms of COX enzyme, the constitutive (COX-1) and the inducible (COX-2) isoforms[4-6]. On other hand COX-2 inhibitors have been associated with cardiovascular side effects[7]. Therefore, developing new agents with more potential antiinflammatory activities and with lesser side effects is, at present, of great interest.

Ipomoea staphylina is an extensive climber belonging to the family convolvulaceae. A literature review reveals antiulcer property[8] of *Ipomoea staphylina.* Other species of genus Ipomoea like *Ipomoea pes-caprae*, *Ipomoea imperati*, *Ipomoea involucrate* and *Ipomoea asarifolia* has been reported for antiinflammatory activity[9-12]. So, the present study was carried out to evaluate the antiinflammatory activity of ethanolic extract and its ethyl acetate and n-butanol fractions of leaves of *Ipomoea staphylina.*

MATERIALS AND METHODS

Plant Material

Leaves of *Ipomoea staphylina* were collected from forest area of Karnataka near to Bangalore. The *Ipomoea staphylina* plant taxonomically identified and authenticated by Dr. K. Karthigeyan at Central National Herbarium, Botanic Garden, Howrah, where the voucher specimen is conserved under the reference number SMF-01. The leaves of *Ipomoea staphylina* were cleaned and dried under shade at room temperature for several days and powdered. The powder was defatted with petroleum ether (60-80 GR) for 72 h and then the dried powder was extracted with ethyl alcohol to get a yield of 10.2 % w/w. The ethanolic extract was dispersed in distilled water and partitioned with ethyl acetate in a separating funnel till the colourless ethyl acetate fraction is obtained. Then the aqueous part is then partitioned with n-butanol to get the butanol fraction. Ethyl acetate and butanol fraction so obtained was concentrated by keeping in boiling water bath to get the solid residue. The dried extracts were stored in airtight container and placed in refrigerator.

Drugs and chemicals

Carrageenan and lipopolyscharide were purchased from Sigma Chemical Co. TNF-α assay kit from Amersham Bioscience, MTT Assay powder from SRL and Diclofenac was purchased from Micro Labs. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental animals

In-breed wistar rats (150-200 g) of either sex were maintained under controlled conditions of temperature (23± 2°C) and humidity (50 ± 5%) and a 12-hour light–dark cycle, were used for the experiment. They were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

Mice were kept overnight fasting prior to drug administration. Animals were received a single oral dose (2000 mg/kg, b.w.) of ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions. After the administration of *Ipomoea staphylina* leaves extract and its different fractions food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks[13].

Carrageenan induced paw oedema

The antiinflammatory activity of ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions on carrageenan-induced paw oedema was determined[14,15]. The animals were divided into eight groups consisting of six rats each. The control group received 2.5 ml/kg of saline, the standard group received diclofenac sodium at the dose of 10mg/kg, p.o. and the test groups received the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions at the dose of 200

mg/kg, p.o and 100 mg/kg, p.o. Thirty minutes after administration of different substances, 0.1ml of 1% of carrageenan subcutaneously into the planter region was injected to all animals in the left hind paw. The paw volume, up to the tibiotarsal articulation, was measured using plethysmometer (model 7150, Ugo Basile, Italy). The measures were determined at 0 h (before carrageenan injection) and 0.5 h, 1 h, 2 h, 3 h and 4 h later.

The percentage inhibition of inflammation was calculated using the formula:

Percentage Inhibition= (1-Vt/Vc) x 100

Where Vt- Increase in paw volume in test animal

Vc - Increase in paw volume in control group.

The results obtained as mean increase in paw volume (ml) and percentage inhibition in paw oedema.

Cotton pellet induced granuloma in Rats

In cotton pellet induced granuloma method[16] sterilized cotton pellet of 50mg were implanted beneath the abdominal skin in axilla or groin region of the rat through a single incision along the midline under anesthesia using pentobarbitone (40 mg/kg, i.p.). The animals were divided into eight groups consisting of six rats each. The control group received 2.5 ml/kg of saline, the standard group received diclofenac sodium at the dose of 10mg/kg, p.o. and the test groups received the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions at the dose of 200 mg/kg, p.o and 100 mg/kg, p.o. The drugs were administered for 7 days. On the eighth day the animals were anesthetized with ether and the implanted cotton pellet with granulation tissue were removed and freed from extraneous tissues and dried in an oven at 60^0 C for 24 hours. The dried pellets were weighed and the gain in weight in each group was calculated. The difference in granulation tissue weights was noted. The increase in the granulation tissue weight of test and standard groups were compared with control group. The percentage inhibition was calculated by using the formula:

% Inhibition = (Wc - Wd / Wc) x 100

Wd = difference in pellet weight of the drug treated group

Wc = difference in pellet weight of the control group

Cell Culture

The mouse macrophage-like cell line, RAW 264.7, was purchased from the Korean Cell Line Bank (ATCC). RAW 264.7 cells were cultured in Roswell Park Memorial Institute medium (RPMI) ontaining 10% fetal bovine serum (FBS; GIBCO Inc., NY, USA) and 100 U/ml of penicillin/streptomycin. The cells were incubated at 37°C in a humidified, 5% CO2 atmosphere. To stimulate the cells, the medium was regularly replaced with fresh RPMI, and bacterial LPS (1 µg/ml) was added. . For experimental studies, cells were grown to 80-90% confluency, harvested with 0.025% trypsin and 0.52mM EDTA in phosphate buffer saline and plated at desired density and allowed to reequilibrate for 24 h before any treatment. All experiments were conducted in RPMI with 10% FBS/FCS and 1% PSN. In the presence of DMSO (0.2% vehicle control) respectively, this concentration was non toxic for cells.

MTT Assay

Cell viability was determined by MTT [3-(4,5-dimethyl thiazole-2-yl)-2,Sdiphenyltetrazolium bromide] assay[17]. One type of cells lines – Raw 264.7 (Murine macrophage cell) were exposed to hydroxamic compounds p1 and p2 at different concentrations for 24-48 h. The assay was based on the ability of the mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of pale yellow MTT and form a purple formazan crystal which is largely impermeable to the cell membrane thus resulting accumulation within living cell. After incubation of cells with MTT reagent for 2-4 hrs, a detergent solution containing 0.04N HCL, 80% absolute isopropanol and 20% Triton X, was added to lyse the cells so that the crystals were solubilised and purple color was added. Then the samples were read at a wavelength of 570 nm. The number of surviving cells is directly proportional to the level of formazan created which is proportional to the optical density of the sample.

Measurement of cytokine production

For the cytokine immunoassay, the cells (1×10^6/ml) were pre-incubated 1 h with MECO and further cultured 6 h with 1 µg/ml of LPS in 24-well plates. Supernatants were removed at the allotted times. TNF-*α* production was quantified by TNF assay kit (Amersham Bioscience) and following the manufacturer's instructions. The kit is specific for TNF-*α* and does not measure other cytokines.

Statistical analysis

All data were represented as mean±S.EM. Results were statistically evaluated using one way analysis of variance (ANOVA) followed by Tukey-Kramer (post tests) using INSTAT software. $P < 0.05$ was considered as statically significant.

RESULTS

In LD50 studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

The ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions were evaluated for carrageenan induced paw oedema. The result obtained indicates that the ethanolic extract of leaves of *Ipomoea staphylina* (200 mg/kg, p.o.) significantly decreased the carrageenan induced paw oedema at 0.5 h ($P<0.01$), 1 h ($P<0.01$), 2 h ($P<0.001$) 3 h ($P<0.001$) and 4 h ($P<0.001$). The ethyl acetate fraction (200 mg/kg, p.o.) significantly decreased the paw oedema at 1 h, 2 h, 3 h and 4 h ($P<0.001$). The n-butanol fraction of *Ipomoea staphylina* (200 mg/kg, p.o.) also significantly decreased the formalin induced paw oedema at 1 h ($P<0.05$), 2 h ($P<0.01$), 3 h and 4 h ($P<0.001$). Maximum inhibition of paw oedema was observed with the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions (200 mg/kg, p.o.) at 4 h. Diclofenac sodium (10 mg/kg, p.o.) and the ethanolic extract of leaves of *Ipomoea staphylina* (200 mg/kg, p.o.) inhibited paw oedema by 46.72 % and 35.24 % respectively (Table 1).

In Cotton pellet induced granuloma the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions were screened for cotton pellet induced granuloma in the rats (Table 3). The the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions at the dose of 200 mg/kg, p.o.and 100 mg/kg, p.o. significantly ($P<0.001$) decreased the granuloma formation. The the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions (200 mg/kg, p.o.) showed 53.53%, 45.79% and 33.46% of inhibition of granuloma formation respectively, whereas the standard drug diclofenac sodium (10mg/kg, p.o.) showed 47.66 % of inhibition of granuloma formation.

Table 1: Effect of *Ipomoea staphylina* extract (IS) and its fractions on carrageenan-induced paw oedema

Treatment (mg/kg)	Mean increase in paw volume (ml)						% Inhibition at 4 h
	0 h	0.5 h	1 h	2 h	3 h	4 h	
Control	0.38± 0.013	0.62± 0.022	0.72± 0.017	0.86± 0.022	1.18± 0.023	1.22± 0.027	-
Diclofenac sodium (10)	0.38± 0.018	0.45± 0.012***	0.52± 0.012***	0.61± 0.020***	0.67± 0.017***	0.65± 0.017***	46.72

IS Extract (200)	0.35± 0.016	0.52± 0.016**	0.60± 0.023**	0.68± 0.026***	0.82± 0.015***	0.79± 0.023***	35.24
IS Extract (100)	0.36± 0.023	0.59± 0.013	0.68± 0.011	0.80± 0.010	0.90± 0.019***	0.88± 0.025***	27.86
Ethyl acetate fraction of IS (200)	0.37± 0.014	0.56± 0.019	0.61± 0.014**	0.70± 0.021***	0.87± 0.024***	0.84± 0.021***	31.14
Ethyl acetate fraction of IS (100)	0.36± 0.016	0.60± 0.020	0.69± 0.014	0.79± 0.016	0.92± 0.015***	0.89± 0.018***	27.04
n-Butanol fraction of IS (200)	0.35± 0.016	0.57± 0.014	0.63± 0.018*	0.73± 0.021**	0.91± 0.024***	0.91± 0.019***	25.40
n-Butanol fraction of IS (100)	0.36± 0.017	0.61± 0.017	0.71± 0.022	0.80± 0.018	1.02± 0.023***	0.99± 0.021***	18.85

All values are expressed as mean ± SEM, (n =6)

*P<0.05, ** P<0.01, *** P<0.001 as compaired to control

Table 2: Effect of *Ipomoea staphylina* extract (IS) and its fractions on changes in mean weight of cotton pellet

Treatment (mg/kg)	Weight of dry cotton pellet (mg)	% Inhibition
Control	89.16±1.97	--
Diclofenac sodium (25)	37.46±1.77***	57.98
IS Extract (200)	41.43±2.25***	53.53
IS Extract(100)	61.18±2.22***	31.38
Ethyl acetate fraction of IS (200)	48.33±1.45***	45.79
Ethyl acetate fraction of IS (100)	63.83±1.07***	28.40
n-Butanol fraction of IS (200)	59.32±1.69***	33.46
n-Butanol fraction of IS (100)	74.39±1.47***	16.56

All values are expressed as mean ± SEM, (n =6)

*** P<0.001 as compared to control

Cytotoxicity studies of ethanolic extract of leaves *Ipomoea staphylina* and its ethyl acetate fraction on RAW264.7 cells.

The MTT assay showed that up to a concentration of 300 µg/ml did not affect the cell viability at 48 h of incubation (Figure 1). Concentrations of the extract below the threshold were selected for further studies.

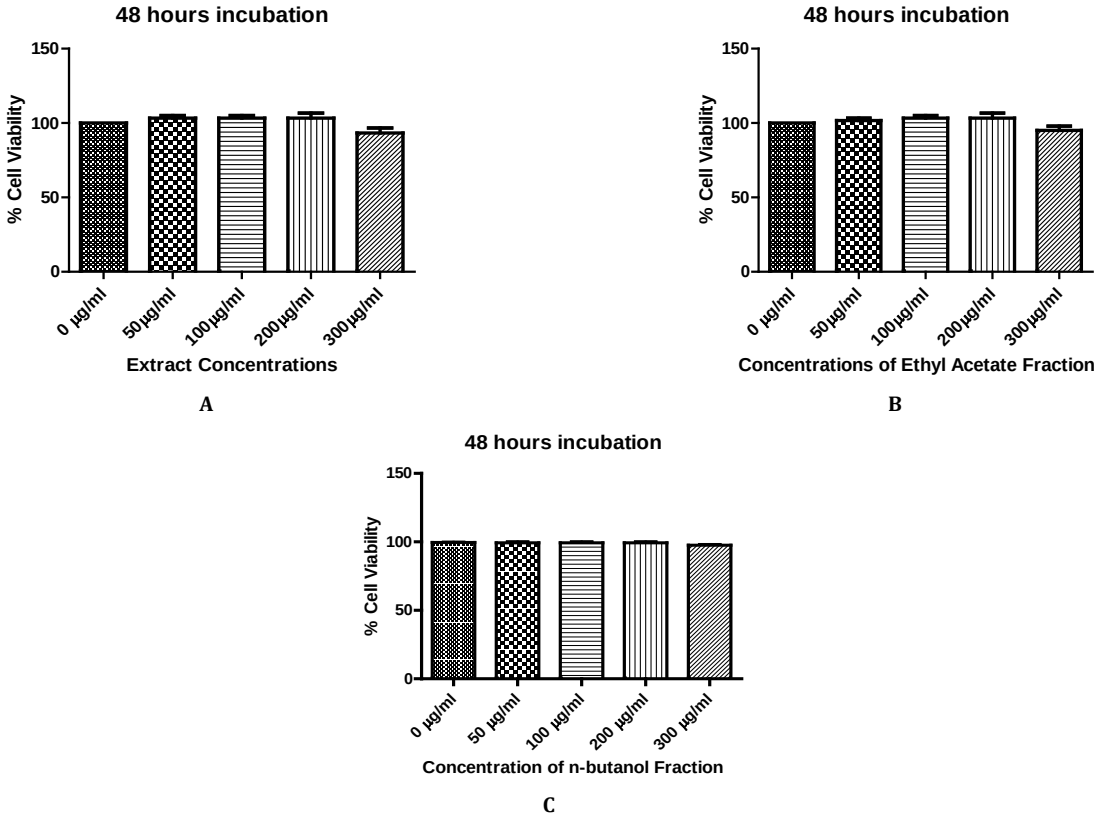

Fig. 1: MTT assay for testing cell viability of *Ipomoea staphylina* (IS) leaves extract and its fractions on RAW264.7 cells

Dose and duration dependant effect of *Ipomoea staphylina* leaves ethanolic extract (Figure 1A) and its ethyl acetate fraction (Figure 1B) and n-butanol fraction (Figure 1C) on cell viability via MTT assay. Results are presented as mean ± S.E.M.

Table 3: Effect of Ipomoea staphylina leaves ethanolic extract and its ethyl acetate fraction TNF-α expression in Lipopolysaccharide (LPS) stimulated RAW 264.7 Cells:

Treatment	TNF-α (pg/ml)
Normal	0.18±0.027
LPS (1µg/ml)	1.74±0.051###
Extract (50µg/ml)+ LPS (1µg/ml)	1.27±0.018***
Extract (100µg/ml)+ LPS (1µg/ml)	0.73±0.013***
Extract (200µg/ml)+ LPS (1µg/ml)	0.45±0.023***
Ethyl Acetate Fraction (50µg/ml)+ LPS (1µg/ml)	1.20±0.026***
Ethyl Acetate Fraction (100µg/ml)+ LPS (1µg/ml)	0.81±0.03***
Ethyl Acetate Fraction (200µg/ml)+ LPS (1µg/ml)	0.37±0.040***
n-butanol Fraction (50µg/ml)+ LPS (1µg/ml)	1.55±0.038
n-butanol Fraction (100µg/ml)+ LPS (1µg/ml)	1.42±0.032**
n-butanol Fraction (200µg/ml)+ LPS (1µg/ml)	1.26±0.020***

All values are expressed as mean ± SEM, (n =6)

###P <0 .001 when compared with the normal control group

***P <0 .001 when compared with the LPS treated group

A significant increase (P<0.001) in TNF-α level was observed in RAW264.7 cells treated with LPS. The ethanolic extract of *Ipomoea staphylina* and its ethyl acetate fraction at different concentration was found to decrease the TNF-α level significantly (P<0.001) (Table 3).

DISCUSSIONS

Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and devoid of apparent systemic effect. It induce oedema is a biphasic response which is shown to be mediated by histamine and serotonin during first 1h. After which increased vascular permeability is maintained by the release of kinins upto 2.5 h, followed by the release of kinins and finally through the release of bradykinin, prostaglandin and lysosomes from 2.5 to 6 h. The later phase is reported to be sensitive to most of the clinically effective antiinflammatory agents[18]. The mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site[19].The Carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory drugs (NSAID)[20,21]. This method was chosen for this study since it is the most prominent experimental model in search for new antiinflammatory drugs and evaluation of anti-inflammatory effect of natural products[22,23]. We found that the administration of the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions at the dose of 200 mg/kg, p.o reduced significantly the carrageenan-induced paw oedema.

The injection of carrageenan produces a typical biphasic oedema associated with the production of several inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, nitric oxide, and cytokines[15,19,23] . According to the result of our study, the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions were able to effectively inhibit the oedema in the later phases, suggesting that these compounds inhibit different chemical mediators of inflammation.

The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation[24]. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. In the present study, administration of the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions (200mg/kg, p.o.) has been observed to inhibit the weight of wet cotton pellet exhibited inhibition of granuloma. It is well known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide[25]. The proliferative phase of macrophages, neutrophils, fibroblast and collagen formation which were the basic source for granuloma formation. Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of mucopolysaccharids are indicators of the antiproliferative effect by NSAIDs. In preview of this, results of present study demonstrate that the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions has potential to inhibit sub-acute inflammation probably due to interruption of the arachidonic acid metabolism or inhibition proliferative phase.

In order to validate the use of the *Ipomoea staphylina* as a antiinflammatory compound, the effect of the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions on the production of pro-inflammatory cytokine like TNF-*α* in LPS stimulated RAW 264.7 cells were investigated by in vitro. Therefore, MTT assay was carried out to examine the cytotoxicity. The assay showed that the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions up to a concentration of 300 µg/ml did not affect the cell viability at 48 h of incubation.

Macrophage plays a central role in defense mechanism by the nature of their phagocytic, cytotoxic and intracellular killing capability[26]. Bacterial lipopolysaccharide (LPS) is a natural ligand for Toll-like receptor 4 (TLR4). Stimulation of macrophage by lipopolysaccharide (LPS) results in increased production of different inflammatory mediators. Bacterial lipopolysaccharide (LPS) interacts with a variety of cell types, including endothelial cells, smooth muscle cells, granulocytes, thrombocytes, and macrophages/monocytes. Lipopolysaccharide binds first to LPS bindingprotein and then to cell surface CD14[27,28] before it activates TLR4 signaling pathways, which induce the production of proinflammatory mediators such as TNF-*α*[29].

Inflammatory cytokines appear to be interlinked in a cascade, produced serially by cells during inflammatory response. Cumulative evidence indicates that an abnormality in the production or function of TNF-*α* play an essential role in inflammatory lesions (De Nardin, 2001). TNF-*α* and other inflammatory cytokines activate the inflammatory cells and cause the production of other inflammatory mediators, which in turn modulate the gene expression, DNA damage and cellular proliferation contributing to progression of various inflammatory disorder[30]. TNF-*α*, also induce the activation of iNOS gene transcriptional factor[31] and this resultant induced production of NO has been implicated for immunological and inflammatory diseases[32]. Therefore, the cellular manipulation of production of TNF-*α* and other inflammatory cytokines is of importance for regulation of inflammatory response.

The present study showed that the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions possessed that inhibitory effect on these proinflammatory cytokine. These compounds suppressed the production of macrophage derived proinflammatory cytokines (TNF-*α*) in LPS stimulated RAW 264.7 cells in dose dependent manner.

Taken together *in vivo* and *in vitro* results suggested that the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions posses anti-inflammatory activities. The

ethanolic extract and its ethyl acetate fraction of leaves of *Ipomoea staphylina* have shown more pronounce effect on inflammation than the n-butanol fraction.

REFERENCES

1. Mitchell RN, Cotran RS. In: Robinsons Basic Pathology. 7th ed. New Delhi: Harcourt Pvt. Ltd; 2000. p. 33-42.
2. Rainsford KD. Antiinflammatory drugs in the 21st century. Sub-cellular Biochem 2007; 42: 3–27.
3. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature: New Biol 1971; 231: 232–5.
4. Robert A. Antisecretory, antiulcer, cytoprotective and diarrheogenic properties of prostaglandins. Adv PG & TX Res 1976; 2: 507–20.
5. Peskar BM. On the synthesis of prostaglandins by human gastric mucosa and its modification by drugs. Biochimica et Biophysica Acta 1977; 487: 307–14.
6. Tapiero H, Ba GN, Couvreur P, Tew KD. Polyunsaturated fatty acids (PUFA) and eicosanoids inhumanhealth and pathologies. Biomed & Pharmacother 2002; 56: 215–22.
7. Dogné JM, Supuran CT, Pratico D. Adverse cardiovascular effects of the coxibs. J Med Chem 2005; 48: 2251–7.
8. Firdous M, Nitesh SC. Evaluation of antiulcer activity of *Ipomoea staphylina*. Pharmacologyonline 2009; 3:195-9.
9. Pongprayoon U, Bohlin L, Soonthornsaratune P, Wasuwat S. Anti-inflammatory activity of *Ipomoea pes-caprae* (L.) R. Br. Phytother Res 1991; 5(2): 63–6.
10. Paula AC, Hayashi LS, Freitas JC. Antiinflammatory and antispasmodic activity of *Ipomoea imperati* (Vahl) Griseb (Convolvulaceae). Braz J Med Biol Res 2003; 36(1):105-12.
11. Ijeoma UF, Aderonke SO, Ogbonna O, Augustina MA, Ifeyinwa CN. Antinociceptive and antiinflammatory activities of crude extracts of *Ipomoea involucrata* leaves in mice and rats. Asian Pacific J Trop Med 2011; 4(2): 121-124
12. Lawal U, Ibrahim H, Agunu A, Abdulahi Y. Antiinflammatory and analgesic activity of water extract from *Ipomoea asarifolia Desr* (Convolvulaceae). Afr J Biotech 2010; 9(51): 8877-80.
13. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economics co-operation, development, Paris, June, 2000.
14. Araswathi RS, Upadhyay L, Venkatakrishnan R, Meera R, Devi P. Phytochemical investigation, analgesic and antiinflammatory activity of *Abutilon Indicum* Linn. Int J Pharm Pharml Sci 2011; 3(2): 154-6.
15. Biradar S, Kangralkar VA, Mandavkar Y, Thakur M, Chougule N. Antiinflammatory, antiarthritic, analgesic and anticonvulsant activity of Cyperus essential oils. Int J Pharm Pharml Sci 2010; 2(4): 112-5.
16. Goldstein SA, Shemano L, Daweo R, Betler J. Cotton pellet ganuloma pouch method for evaluation of anti-inflammatory activity. Arch Pharmacodyamic Ther 1976; 165: 294-301.
17. Hongo T, Mizuno Y, Haraguchi S, Yoshida TO. A new anticancer drugs sensitivity test using the microplate culture and surviving tumor cell staining methods. Gan To Kagaku Ryoho1986; 13: 247–54.
18. Hernandez PM, Rabanal Gallego R. Evaluation of the anti-inflammatory and analgesic activity of *Sideritis anariensis* var. pannosa in mice. J Ethnopharmacol 2002; 81: 43-7.
19. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of acute inflammatory response induced in rats in different sites of carrageenin and turpentine. J Pathol 1971; 104:15-29.
20. Ghule BV, Ghante MH, Upaganlawar AB, Yeole PG. Analgesic and AntiInflammatory activities of *Lagenaria siceraria* Stand. fruit juice extract in rats and mice. Phcog Mag 2006; 2(8): 232-8.
21. Rao ChV, Kartik R, Ojha SK, Rao AGMM. Anti-inflammatory and antinociceptive activity of stem juice powder of *Tinospora cordifolia* Miers in experimental animals. Hamdard Medicus 2005; 48:102-6.
22. Sugishita E, Amagaya S, Ogihara Y. Antiinflammatory testing methods: comparative evaluation of mice and rats. J Pharmacobio-dynamics 1981; 4: 565–5.
23. Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, Cirino G. Carrageenan induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. Br J Pharm 2004; 142: 331–8.
24. Winter CA, Porter CC. Effect of alterations in the side chain upon antiinflammatory and liver glycogen activities of hydrocortisone esters. J Am Pharm Assoc Sci Educ 1957; 46: 515-9.
25. Smith WL, De Witt DL. Biochemistry of prostaglandins endoperoxide H synthase-1 and synthase-2 and their differential susceptibility to NSAIDs. Sem Nephrol 1995; 15:179-94.
26. Adams DO, Hamilton A. The cell biology of macrophage activation. Annu Rev Immunol 1984; 2: 282-318.
27. Schumann RR, Leong SR, Flaggs GW, Gray PW,Wright SD,Mathison JC, Tobias PS, Ulevitch RJ. Structure and function of lipopolysaccharide binding protein. Sci 1990; 249:1429 –31.
28. Tobias PS, Soldau K, Ulevitch RJ. Identification of a lipid A binding site in the acute phase reactant lipopolysaccharide binding protein. J Biol Chem 1989; 264:10867–71.
29. Cheadle WG, Hanasawa K, Gallinaro RN, Nimmanwudipong T, KodamaM, Polk HC Jr. Endotoxin filtration and immune stimulation improve survival from gram-negative sepsis. Surg 1991; 110:785– 91.
30. Dinarello CA. Proinflammatory cytokines. Chest 2001; 118: 503-8.
31. Schutze S, Wiegmann K, Machleidt T, Kronke M. TNF-α induced activation of NF-κB. Immunobiol 1995; 193,193-203.
32. Anggard E. Nitric oxide: mediator, murderer, and medicine. Lancet 1994; 343:1199-1206.