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Research Article

ANTI INFLAMMATORY EFFECT OF POLY HERBAL FORMULATION ON CARRAGEENAN INDUCED ACUTE INFLAMMATION IN ALBINO RATS

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ABSTARCT

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agents as well as to remove the consequent necrosed cells and tissue and it is manifestation of the body's response to tissue damage and infection. Most currently used drugs for the treatment of inflammation-related diseases are the steroidal and non-steroidal drugs. In addition, these drugs are known to have several adverse effects, and this has encouraged the use of medicinal plants with very little side effects to substitute for this chemical therapeutics. The present study has been undertaken to evaluate the anti inflammatory potentials of poly herbal formulation on carrageenan induced paw enema in albino rats. The polyherbal formulation was prepared from *Calendula officinalis* L, *Lantana camara* L. and *Desmodium gangeticum* Linn. were selected under study and it can be used for the preparation of poly herbal formulation. The anti inflammatory activity was studied by using carrageenan induced paw edema model. The aqueous extracts of polyherbal formulation were administered orally at dose level of 100, 200 and 300mg/kg bw for 28 days. Indomethacin was used as a standard. After the experimental period, the blood and edematous tissue samples were collected for analysing antioxidant and biochemical parameters. The paw thickness, levels of lipid peroxide, interleukin – 6, C – reactive protein, total and differential leucocytes count were significantly increased and the superoxide dismutase, reduced glutathione and total protein levels were decreased in the carrageenan induced animals. Administration of poly herbal formulation restored the levels of above parameters near to normal. The present study concludes that poly herbal formulation prepared from *Calendula officinalis* L, *Lantana camara* L. and *Desmodium gangeticum* Linn. has significant anti inflammatory activity against carrageenan induced paw edema in preclinical models.

Keywords: Inflammation, Indomethacin, Carrageenan, Interleukin – 6, Polyherbal Formulation.

INTRODUCTION

Inflammation is a very complex response that occurs as a result of an injury, infection or another stimulus, in which several cell types and secreted factors elicits protective immunity, tissue repair and resolution of tissue damage[1]. Inflammation plays an important role in a wide variety of chronic human diseases such as cardiovascular diseases and cancer. It has been confirmed that pro-inflammatory cytokines, cyclooxygenase-2 (COX-2) and free radical species interact in a complex manner in an inflammatory environment[2]. The result of each inflammatory reaction may be beneficial (defence the body against agents deranging its homeostasis) or harmful (damage to surrounding tissues).

Large numbers of Non-steroidal anti-inflammatory drugs (NSAIDs) are available in the market for the treatment of inflammation with some advantages and disadvantages. Though there are standard drugs like Aspirin, Indomethacin, Phenylbutazone, etc., these drugs are not entirely free of side effects and have their own limitation. NSAIDs are the most commonly used drugs in the world today. Pain and fever are being the most common complaints associated with inflammation. The NSAIDs used in the inflammatory conditions do not cure and remove the underlying cause of the disease but they only modify the inflammatory response to the diseases. There is a market essential for orally effective molecules that can heal inflammatory disease processes, rather than the symptoms, more effectively than presently accessible drugs. Therefore the search for new Anti inflammatory agents is in process from natural sources because they are becoming popular due to toxicity and side effects of allopathic medicines[3].

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems[4]. The plant products and their combinations are running well now in the market due to their lower side effects, efficacy and less cost.

Based on the literature review, three common plants such as *Calendulo officinalis* L. (Asteraceae family), *Lantana camara* L. (Verbanaceae family), and *Desmodium gangeticum* Linn. (Fabaceae family) are selected for the preparation of poly herbal formulation and the formulation is used to screen the anti inflammatory activity on carrageenan induced acute inflammation in albino rats.

Calendula officinalis L. belonging to the family Asteraceae. It is very great to help with sore, inflamed and itchy skin conditions, for burns, eczema and nappy rash, as well as sore cracked nipples. The properties of *Calendula officinalis* L are anti inflammatory, anti septic, anti hemorrhagic activity[5]. *Lantana camara* L. belonging to the family Verbanaceae. It has several uses like anti microbial, fungicidal, insecticidal and anti cancer and rheumatism[6]. *Desmodium gangeticum* L. belonging to the family Fabaceae. It is used in Indian system of medicine as a bitter tonic, febrifuges, digestive, anti emetic and inflammatory disorders[7].

MATERIALS AND METHODS

PLANT IDENTIFICATION AND AUTHENTICATION

Plant sources selected for the present study are *Calendula officinalis* L. (Flower) (Voucher number: BISH0000619230) , *Lantana camara* L. (Leaves) (Voucher number: BISH0000699120) and *Desmodium gangeticum* L. (Leaves) (Voucher number: MS001). The Plants were collected, identified with the help of Flora of Presidency of Madras[8] and were authenticated with the help of herbarium specimen deposited at RAPINAT herbarium St.Joseph's College, Trichy, Tamilnadu, India.

PREPARATION OF THE AQUEOUS POLY HERBAL FORMULATION

The flower of *Calendula officinalis* L. leaves of *Lantana camara* Land leaves of *Desmodium gangeticum* L. were shade dried and coarsely powdered with electrical blender. 200gm of each plant material was mixed with 1.2litre of water. Then it was boiled until it was reduced to one third and filtered. The filterate was evaporated to dryness until Paste form of the extract was obtained. Equal concentration of each extract was mixed to prepare poly herbal formulation and used to screen its anti inflammatory potentials against carrageenan induced paw edema.

CHEMICAL USED TO INDUCE INFLAMMATION

Inflammation was induced by administering 0.1 ml of (1%) carrageenan into sub-plantar surface of rat hind paw. Carrageenan is a linear sulphated polysaccharides that are isolated from sea weed. The carrageenan induced paw edema is a useful model to assess the contribution of mediators involved in vascular changes associated with acute inflammation.

ANIMAL'S ETHIC STATEMENT

Animals used for the experimental procedure were approved according to ethical norms and guidelines set by animal ethical committee of our institute with approval number of 790/Po/Re/S/03/CPCSEA.

EXPERIMENTAL DESIGN

Wistar strain of albino rats of either sex weighing 150-250 g were used and the animals were divided into five groups (n=6) viz. Group I: Normal control, Group II: The animals were induced with 1ml of carrageenan(1%) on 28th day from date of initiation, Group III, IV and V : The animals were treated with poly herbal formulation (100mg/kg bw), (200mg/kg bw) and (300mg/kg bw)for 28days. In 29th day 1ml of 1% carrageenan were induced to all the rats of Group III, IV and V. Group VI: The animals were treated with Standard drug (Indomethacin 20mg/kg bw) for 28 days. On 29th day 0.1% carrageenan was induced into sub plantar region of rat hind paw.

The paw thickness was measured by using vernier caliper. After the experimental period animals were sacrificed by cervical dislocation. Blood was collected and used for the enumeration of total and differential WBC count[9] and ESR. The serum was prepared by centrifuging the blood at 3000 rpm for 10 minutes. The serum was used for the determination of C- reactive protein and IL- 6[10] and total protein[11]. Edematous tissues was homogenized in 0.1M phosphate buffer, P^{H} 7.4 and used for studying Lipid peroxidation[12], Superoxide dismutase[13], reduced glutathione[14].

RESULT AND DISCUSSION

The anti inflammatory activity of polyherbal formulation on the level of the paw thickness was given in Table 1. The level of paw thickness was found to be higher in carrageenan induced rats when compared to normal rats. Pre treatment with poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw, significantly decreased in the level of the paw thickness in dose dependent manner.

The effect of poly herbal formulation on lipid peroxide was measured to demonstrate the oxidative damage. The significant increase in lipid peroxide level was observed in carrageenan induced animals while treated with poly herbal formulation decrease the lipid peroxide level was observed.

The level of SOD and GSH was evaluated to estimate endogenous defences against free radical formation. Table 2. Shows the changes in antioxidant levels in the experimental groups. A marked decrease in GSH concentrations was found in the carrageenan induced groups and the poly herbal formulation significantly restored the reduction in SOD and GSH level.

The changes in the level of WBC and ESR was given in the Table 3. The carrageenan induced rats showed the increased level of total WBC and ESR compared to normal rats. Administration of Poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw, a significantly decreased the total WBC and ESR in the dose dependent manner by inhibiting the migration of leukocytes in the inflamed area.

The effect of poly herbal formulation on total leucocytes is given in Table 4. The level of total leucocytes was found to be higher in carrageenan induced rats when compared to normal rats. The administration of poly herbal formulation at dose level of 100, 200, 300 mg/bw significantly decreased the total leucocyte count in carrageenan induced rats.

The levels of inflammatory markers are given in Table 5. The inflamed rats showed the increased levels of interleukins- 6 and C – reactive protein. Pre treatment with poly herbal formulation and standard drug exhibit these parameters near normal. The increased level of CRP was indicated the active inflammation caused by carrageenan and the decreased level of IL 6 in plant treated groups due to the inhibition of release of pro inflammatory cytokines by macrophages.

The level of protein was decreased significantly ($p<0.01$) in carrageenan induced Group II rats when compared to the normal rats (Group 1). On pre treatment with the aqueous extract of poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw, a significant increase in serum protein was observed as compared to edematous rats (Table.6).

TABLE 1: PAW THICKNESS OF EXPERIMENTAL ANIMALS

Groups	Paw thickness(µm)	
	DAY 1	DAY 29
I	4.58±0.07	4.59±0.07
II	4.48±0.07*	7.73±0.07*
III	4.40±0.09	5.93±0.08
IV	4.45±0.10	5.79±0.11
V	4.65±0.13**	5.56±0.11**
VI	4.40±0.09**	5.15±0.06**

µm – Micrometer, Values are mean ± SEM (n=5)
P< 0.05 statistically significant when compared to Group II with Group I
P< 0.05 statistically significant when compared to Group VI I with Group II, VII and XII.

TABLE 2: LEVELS OF LPO AND ANTIOXIDANTS IN EXPERIMENTAL ANIMALS

Groups	LPO (nM of MDA formed/g of tissue)	Reduced Glutathione (mg/g tissue)	SOD (mM of epinephrine oxidised/min/mg protein)
I	6.92.00±0.25	2.11±0.04	1.48±0.03
II	14.97±0.25*	1.23±0.08*	0.40±0.01*
III	11.91±0.18	1.52±0.06**	0.54±0.09**
IV	10.68±0.14	1.86±0.06	0.750±0.03
V	7.56±0.36**	2.03±0.05	0.843±0.04
VI	5.49±0.24**	2.08±0.07**	1.29±0.07**

nM of MDA formed/g of tissue – Nanomole of malondialdehyde formed per gram of tissue, mg/g tissue – milligram per gram tissue, mM of epinephrine oxidised/min/mg protein – Millimole of epinephrine e oxidised per minutes per milligram protein.
Values are mean ± SEM (n=5)
P< 0.05 statistically significant when compared to Group IIwith Group I
P< 0.05 statistically significant when compared to Group VI I with Group II, VII and XII.

TABLE 3: LEVELS OF TOTAL WBC AND ESR

Groups	WBC (Thousands of cells/cumm)	ESR (mm)
I	4450± 187.08	1.32±0.01
II	15600 ±260.76*	2.95±0.05*
III	11366±258.19**	1.70±0.05**
IV	9583±348.8	1.53±0.05
V	4883±108.01	1.45±0.05
VI	5583±318.85**	1.24±0.02**

Thousands of cells/cumm – Thousands of cells per cubic millimeter, mm – millimetre, Values are mean ± SEM (n=5)
P< 0.05 statistically significant when compared to Group II with Group I
P< 0.05 statistically significant when compared to Group VI I with Group II, VII and XII.

TABLE 4: LEVELS OF DIFFERENTIAL LEUCOCYTES ON EXPERIMENTAL ANIMALS

Groups	Neutrophils(%)	Lymphocytes(%)	Monocytes(%)
I	67.5±2.581989	24.5±0.645497	5.5±0.288675
II	86.75±3.41565*	56.5±0.645497*	7.5±0.288675*
III	81±2.82**	33.5±0.645497**	5.65±0.35**
IV	74.5±2.58	26.5±0.64	4.5±0.20
V	73.5±5.291503	21.45±0.645	3.15±0.28
VI	70.25±2.516611**	25.5±0.645497**	4.4±0.288675**

% - Percentage, Values are mean ± SEM (n=5)
P< 0.05 statistically significant when compared to Group IIwith Group I
P< 0.05 statistically significant when compared to Group VI I with Group II, VII and XII.

TABLE 5: LEVELS OF INFLAMMATORY MARKERS IN EXPERIMENTAL ANIMALS

Groups	Interleukins – 6 (pg/ml)	CRP (µg/ml)
I	18±3.50	0.52±0.02
II	99.54±2.07*	4.68±0.10*
III	65.27±2.44**	2.839±0.04**
IV	42.88±1.97	1.313±0.02
V	28±0.98	0.595±0.03
VI	37.82±2.04	0.694±0.02**

pg/ml – pictogram/millilitre, µg/ml - microgram/millilitre, Values are mean ± SEM (n=5)
P< 0.05 statistically significant when compared to Group II with Group I
P< 0.05 statistically significant when compared to Group VI I with Group II, VII and XII.

TABLE 6: LEVEL OF SERUM PROTEIN IN EXPERIMENTAL ANIMALS

Groups	Protein (g/dl)
Group I	6.72±0.09
Group II	4.67±0.16*
Group III	5.30± 0.08**
Group IV	5.87± 0.10
Group V	6.68± 0.16
Group VI	6.58±0.17**

g/dl – gram per decilitre, Values are mean ± SEM (n=5)
P< 0.05 statistically significant when compared to Group II with Group I
P< 0.05 statistically significant when compared to Group VI I with Group II, VII and XII.

DISCUSSION

Inflammation is the protective mechanism of the local microcirculation to tissue injury which caused by physical trauma, noxious stimuli by chemical agents, heat, antigen-antibody reaction and microbial effect. Carrageenan induced inflammation is commonly used to assess the inflammatory responses. It can induce acute type of inflammation by the release of inflammatory cytokines and free radicals.

Pre treatment of Poly herbal formulation at the dose level of 100, 200, 300mg/kg bw was found to reduce the level of paw thickness and brought to near normal. The restoration of near normal level was due to anti inflammatory property of poly herbal formulation that could be involved in the inhibition of synthesis of kinin, prostaglandin, bradykinin, lysozyme synthesis and particularly both COX -1 and COX -2. This is in agreement with earlier study by Otari *et al*., (2010)[15].

Lipid peroxidation has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. LPO increased during inflammation[16]. Administration of carrageenan produced an elevated level of LPO, which may due to the free radicals and is responsible for damaging cell membranes there by further intensifying inflammatory damage[17]. Hence the concentration of LPO was found to be higher in carrageenan induced edematous rats. On treatment with polyherbal formulation at the dose level of 100,200 and 300mg/kg bw brought down the increased LPO level to normal and standard drug treated animals showed a similar result compared to normal and Poly herbal formulation treated animals.

Superoxide dismutase is the most significant mitochondrial antioxidant enzymes and it imparts resistance against SO_2^-. The phagocytes activation and SO_2^- production during inflammatory condition[18] can destruct contiguous tissue either by a powerful direct oxidizing action or indirectly as with hydrogen peroxide and hydroxyl radicals formed from ROS, which initiate LPO resulting in membrane destruction. The membrane destruction then provokes inflammatory response by the production of mediators and chemostatic factors[19]. In the present study, the level of SOD was decreased in carrageenan induced edematous rats whereas the treatment with polyherbal formulation the level was reached near normal.

Glutathione is an essential endogeneous antioxidant, which play a vital role in guarding cells against oxidative stress through glutathione redox system. The depletion of glutathione appears to be liable for the generation of lipid peroxide[20]. Reduced glutathione is a extremely sensitive indicator of cell functionality and visibility. In the presence study, it was found that carrageenan induced inflammation had reduced the levels of superoxide dismutase and reduced glutathione whereas in rats treated with poly herbal formulation these levels reached near normal due to antioxidant role of selected plant sources which indicates the protection of oxidative stress at the inflamed site.

The increase in level of WBC count during inflammation caused by carrageenan may be due to the release of interleukins, responsible for the production of both granulocytes and macrophage colony stimulating factor. Pre-treatment with the poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw significantly decreases the WBC count which indicates the significant recovery from the inflammatory process. The protective effects of poly herbal formulation are due to the destruction of leucocytes towards inflamed area, maintenance of reticulo-endothelial system and inhibition of release of inflammatory mediators.

ESR is an estimate of the suspension stability of RBC's in plasma, related to the number of size of red cells and to the relative concentration of plasma proteins especially fibrinogen and the α and β globulins. Increased level of ESR is an indication of active but obscure disease processes[21]. The acute phase proteins in ESR and C-reactive protein share the property of showing elevations in the concentration in response to stress or inflammation that occurs like infection, injury, and surgery and tissue necrosis. So in inflammation condition, ESR is elevated. Pre-treatment with poly herbal formulation, these level was decreased.

Leucocytes play a key role in the growth and proliferation of inflammation. Neutrophils play a significant role in the elaboration and manifestation of inflammation and they are the chief source of free radicals at the site of inflammation[22]. The Polyherbal formulation treatment reduce the population of neutrophils which reflects in the suppressing of inflammation. Lymphocyte is the predominant cell in chronic inflammation and it cause enduring deformation of the tissue, interfering its function. Monocytes are mainly involved in inflammatory signal and release various cytokines and other mediators. The reduction in the population of lymphocyte and monocytes after treatment shows that influence the inflammation.

Cytokines are the key molecule that can inhibit or propagate inflammation by activating or deactivating the genes involved in cellular processes. The pro inflammatory cytokine like interleukins and TNF –α can stimulate the liver to CRP, which is increased several folds during acute inflammation. Persistence may lead to secondary activation of many genes involved in the pathogenesis of various diseases like cancer etc. carrageenan induced inflammation causes the releases of interleukins into circulation and mediates host inflammatory response. Pre treatment with poly herbal formulation decreased the above parameters which indicates the normal inflammatory process.

Many adverse reactions leading to extensive tissue damage formed by the generation of free radical that cause damage to lipids, proteins, DNA[23]. The previous study also noted that the

level of serum protein is lowered in rheumatoid arthritis because of free radical formation[24]. The proteins were clearly altered during the pathogenesis of inflammation which has been reported earlier[25]. Hence, the Poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw, significantly increased the protein level in serum.

CONCLUSION

The results of the present study, it can be concluded that the poly herbal formulation from *Calendula officinalis* L., *Lantana camara* L. and *Desmodium gangeticum* Linn. has notable anti-inflammatory potential against carrageenan induced paw edema due to the regulation of inflammatory mediators, lipid peroxide and white blood cells count. Further the detailed studies to be carried out to analyse the mechanism of action of various pro inflammatory cytokines and growth factors using different types of inflammatory models.

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