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EFFECT OF POLY HERBAL FORMULATION ON FREUND'S ADJUVANT INDUCED PAW EDEMA IN ALBINO RATS

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ABSTRACT

Inflammation is a pathophysiological response of living vascularised tissue to an injury. The natural products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to human and environment. Number of herbal medicines are recommended for the treatment of inflammation that has no side effects. The present study was aimed to evaluate the anti inflammatory activity of poly herbal formulation(PHF) on freund's adjuvant induced paw edema in albino rats. A poly herbal formulation was prepared by using three common plants like *Calendulo officinalis* L. (Asteraceae family), *Lantana camara* L. (Verbanaceae family), and *Desmodium gangeticum* Linn. (Fabaceae family). In present study, wistar strains of albino rats were used and the animals were divided into six groups and treated accordingly: Normal control, Freund's adjuvant treated group (0.1ml/kg bw in sub plantar region), Freund's adjuvant + standard drug indomethacin (10mg/bw orally), Freund's adjuvant + PHF (100, 200 and 300mg/kg bw). After the experimental period, the tissue samples were collected for analysing various biochemical parameters. The administration of freund's adjuvant to the experimental animals produced reduction in the levels of SOD, GSH, GPX and catalase. A significant increase in the length of paw thickness, SGOT, SGPT and lipid peroxide, hydroxyl proline and hexosamine, hydroxyl proline was noted in the animals induced with freund's adjuvant. The animls treated with poly herbal formulation (PHF) at dose level of 100,200 and 300kg/bw significantly normalise the above parameters. Administartion of standard drug indomethacin also showed similar results compared to PHF treated animals. The results conclude that the poly herbal formaulation has a potent anti inflammatory effect against freund's adjuvant induced paw edema in albino rats.

KEYWORDS: Inflammation, Hydroxyproline, Hexosamine, *Lantana camara* L. Indomethacin, etc.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to 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 injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation.[1]

Inflammation underlies almost all disease conditions and it is fundamentally a protective response, the ultimate goal of which is to get rid of the organism of both the initial cause of cell injury and the consequences of such injuries.[2] Inflammation symptoms occur when the human body attempts to counteract potentially injurious agents invading bacteria, viruses and other pathogens.[3] Inflammation can also be induced by biochemical and pharmacological agents from the environment, in addition to a diverse and potentially huge array of cell types and soluble mediators including cytokines.[4]

Prevalence ranges from 0.5-1.5% of the population in industrialized countries.[5] The incidence of the condition is low, with around 1.5 men and 3.6 women developing inflammation per 10,000 people per year. The overall occurrence of chronic inflammation is two to four times greater in women than in men. The peak age of incidence in the UK for both genders is the 70s, but people of all ages can develop the disease.

The therapy of inflammation deals with the drugs used to treat inflammatory and immune disorders. NSAIDs, glucocorticoids are prescribed for the treatment of rheumatoid arthritis. The limitations of these therapies are their well known toxicity and variations in clinical efficacy.[6] NSAIs represent an indispensable place in the treatment of inflammation.[7] Administration of NSAIDS to patients with congestive heart failure, reno vascular hypertension and cirrhosis of liver, activated rennin angiotensin system may cause infertility in women. Because of the substantial risks involved with the long term effect of NSIADs, there is increasing demand for the development of new agents with better pharmacological profile.[8] So Our focus was to develop less toxic drugs to control the inflammation.

Natural products have long been recognized as an important source of therapeutically effective medicine. Plants continue to serve as possible source for new drug and chemicals.

They are extremely useful as a lead structure for synthetic modification and optimization of bioactivity. The secondary metabolites available from the plant are very difficult to synthesize externally which limits the synthetic derivation of various potentially usable phytochemical. The folklore treatment of inflammation using plants is well known to the master of traditional medicinal practices. The plant products and their combinations are running well now in the market due to their lower side effects, efficacy and less cost.[9]

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.[10] *Lantana camara* L. belonging to the family Verbanaceae. It has several uses like anti microbial, fungicidal, insecticidal and anti cancer and[11] *Desmodiumgangeticum* 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.[12]

MATERIALS AND METHODS

PLANT IDENTIFICATION AND AUTHENTICATION

Plant sources selected for the present study are *Calendula officinalis* L. (Flower), *Lantana camara* L. (Leaves) and *Desmodium gangeticum* L. (Leaves). The Plants were collected, identified with the help of Flora of Presidency of Madras[13] 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 INDUCED PAW EDEMA

Inflammation was induced by administering 0.1 ml of freund's adjuvant into sub-plantar surface of rat hind paw.

EXPERIMENTAL DESIGN

Wistar strain of albino rats of either sex weighing 150-250 g were used and the animals were divided into six groups (n=6) viz. **Group I**: Normal control, **Group II**: The animals were induced with 0.1ml freund's adjuvant on 28th day from date of initiation, **Group VI**: The animals were treated with Standard drug (Indomethacin 10mg/kg bw) for 28 days. On 29th day 0.1ml of freund's adjuvant was induced into sub plantar region of rat hind paw.**Group IV, V and VI**: The animals were treated with poly herbal formulation (100mg/kg bw), (200mg/kg bw) and (300mg/kg bw) for 28days. In 29th day 0.1ml of freund's adjuvant were induced to all the rats of Group IV, V and VI.

After the experimental period, the paw thickness[14] was measured at before and after induction using vernear caliper and the animals sacrificed with cervical dislocation. The blood and tissue samples were collected for analysing lipid peroxide[15], superoxide dismutase[16], glutathione reductase[17], glutathione peroxidase[18] and catalase[19], mucopolysaacharides like hexosamine[20], hydroxyl proline[21] and serum enzymes such as SGOT[22], SGPT.[22]

RESULT AND DISCUSSION

Table 1: Paw thickness of experimental animals.

Groups	DAY 1 (µm)	DAY 29 (µm)
Group I	4.58±0.07	4.49±0.14
Group II	4.61±0.11*	7.79±0.14*
Group III	4.39±0.08**	5.11±0.05**
Group IV	4.47±0.12	6.03±0.18*
Group V	4.49±0.09**	5.71±0.08**
Group VI	4.30±0.10	5.33±0.02

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 III and Group IV with Group II.

The length of the paw thickness is given in Table 1. The length of paw thickness was found to be higher in freund's adjuvant induced rats when compared to normal rats. Treatment with poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw, significantly decreased in the length of the paw thickness in dose dependent manner.

The result of the freund's adjuvant induced paw inflammation reveals that poly herbal formulation (100, 200, 300 mg/kg bw) produced significant inhibition of paw thickness as compared to adjuvant induced rats. The effect of poly herbal formulation was observed between 1 to 6 h. This was revealed that the poly herbal formulation was effective in preventing a more sustained late phase regulated by neutrophilic infiltration and accumulation of edematous fluid. It is suggest that it may be involving multiple anti inflammatory factors and mediators.

Table 2: Level of LPO in experimental animals.

Groups	LPO (nM of MDA formed/g of tissue)
Group I	1.92.00±0.25
Group II	14.97±0.25*
Group III	5.49±0.24**
Group IV	11.91±0.18
Group V	10.68±0.14
Group VI	7.56±0.36**

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 III and Group IV with Group II

The level of LPO is given in Table 2. The level of LPO was found to be higher in freund's adjuvant induced rats when compared to normal rats. Treatment with poly herbal formulation significantly reduced the concentration of tissue lipid peroxide in the dose dependent manner. 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.[23] Administration of freund's adjuvants 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.[24] The inflammatory tissue damages are due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites.[25] Increased LPO level in inflammation can be due to the increased

oxidative stress which leads to the depletion of glutathione. Hence the concentration of LPO was found to be higher in adjuvant induced rats. On treatment with poly herbal formulation at the dose level of 100, 200, 300mg/kg bw significantly brought down the increased LPO level to normal. And standard drug treated animals showed a similar result compared to normal and plant treated animals.

Table 3: Levels of Enzymatic and Non Enzymatic Antioxidants in Experimental Animals.

Group	Reduced Glutathione (mg/g tissue)	Glutathione peroxidise (mg of glutathione reduced/g tissue)	SOD (mM of epinephrine oxidised/ml/min/mg protein)	Catalase (µmoles og H_2O_2 hydrolysed/g tissue/minutes)
Group I	1972.5±68.60211	86.25±1.20	1.48±0.03	43.75±0.65
Group II	3097.5±53.90965*	40.32±1.25*	0.40±0.01*	19.23±0.14*
Group III	2192±33.26**	77.25±0.60**	1.29±0.07**	39.12±0.17**
Group IV	2782.5±118.98	51.82±1.90	0.594±0.09	23.61±0.32
Group V	2439.3±70.9	63.91±2.10	0.843±0.04	31.25±0.14
Group VI	2225.36±63.7**	71.32±1.89**	1.14±0.12**	35.23±0.21**

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 III and Group IV with Group II

In freund's adjuvant induced group 2 rats, the levels of antioxidant enzymes like SOD, GPX, GSH, Catalase were decrease significantly when compared to normal rats (group 1). On treatment with the aqueous extract of poly herbal formulation at the dose level of 100,200.300 mg/bw, a significant increase in level of SOD, GPX and GSH and catalase was observed when compared with the freund's adjuvant induced rats.(Table 3).

SOD is the most important mitochondrial antioxidant enzymes and it provides defense against super oxide anions. In inflammatory condition, there is excess activation of phagocytes and production of super oxide radical[26] which can harm surrounding tissue either by a powerful direct oxidizing action or indirectly as with hydrogen peroxide and hydroxy 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.[27]

Glutathione is an important endogeneous antioxidant, which plays an important role in protecting cells against oxidative stress via glutathione redox system. Tissue glutathione depletion seems to be responsible for the induction of LPO**.** GSH, play a vital role in

protecting the cells against oxidative damage. GSH status is a highly sensitive indicator of cell functionality and visibility. In the presence study, it was found that inflammation had reduced the level of GSH whereas in rats treated with poly herbal formulation the levels reached near normal due to antioxidant role of selected plant sources.

The enzymatic antioxidant defense systems are natural protective barriers against lipid peroxidarion. SOD, GPX and catalase are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage.

In the present study, these enzymes have registered low levels in inflammatory controls indicating inflammation induced stress. Such a decline in these enzymes activities has been reported earlier.[20] Administration of poly herbal formulation at the dose level of 100, 200, 300 mg/bw and standard drug indomethacin at 10mg/kg bw to the inflammatory rats improved the activities of these enzymes that substantially reflecting the antioxidant potency of the poly herbal formulation.

Table. 3: Levels of Hydroxy Proline and Hexosamine in experimental animals.

Groups	Hydroxy proline (µg/g tissue)	Hexosamine(µg/g tissue)
Group I	223.30±0.90	925±10.50
Group II	356.60± 2.00*	1525± 19.08*
Group III	186.7± 1.88**	880±13.03**
Group IV	260.83± 1.60	1225.83±18.5
Group V	242.50± 1.48	945.20±17.4
Group VI	195.83±0.78**	914.16± 13.9**

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 III and Group IV with Group II

The level of hydroxy proline and hexosamine is given in **Table 4.** The levels of hydroxy proline and hexosamine were found to be higher in adjuvant induced rats when compared to normal rats. Treatment of poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw, significantly reduced the concentration of hydroxy proline and hexosamine in formalin induced rats.

Freund's ajuvant induction causes the changes in connective tissue metabolism, is one of the major biochemical events during the process of inflammation. These changes are effected in the alteration of relative composition of various constituents of connective tissue such as

muco polysaccharides, glyco protein, hexosamine and hydroxy proline, sialic acid.[28] Hence these levels were found to be higher in freund's adjuvant induced rats. Treatment of poly herbal formulation at the dose levels of 100, 200, 300 mg/kg bw inhibited the accmulation of hydyoxy proline and hexosamine in edematous tissue of adjuvant induced rats.

Table 5: Levels of Serum Enzymes in experimental animals.

Groups	SGPT(IU/L)	SGOT(IU/L)
Group I	38.23±1.37	33.76±1.47
Group II	87.67±1.87*	78.89±1.95*
Group III	37.83±1.48**	31.30±1.43**
Group IV	78.89±1.55	69.87±1.72
Group V	67.84±1.58	52.68±1.85
GroupVI	56.96±1.48**	43.73±1.43**

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 III and Group IV with Group II

The levels of serum enzymes is given in **Table 5.** The levels of serum enzymes were found to be higher in freund's induced rats when compared to normal rats. Treatment of poly herbal formulation at the dose levels of 100, 200, 300 mg/ kg, significantly reduced the concentration of serum enzymes in the dose dependent manner.

SGOT, SGPT, ALP are the lysosomal enzymes. There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation.[22] Most of the anti-inflammatory drugs exert their beneficial effect by inhibiting either release of lysosomal enzymes or by stabilizing lysosomal membrane which is one of the major events responsible for the inflammatory process.[29] So it can be assumed that Poly herbal formulation might be acting by either inhibiting the lysosomal enzymes or stabilizing the membrane.

Serum AST and ALT has been plays a vital role in the formation of biologically active chemical mediators such as bradykinins and kinin like substances in inflammatory process.[30] Treatment with Poly herbal formulation at dose level of 100, 200, 300 mg/ kg decreased the levels of SGOT, SGPT and may influence the formation of biologically active chemical mediators.

CONCLUSION

The above findings were concluded that the poly herbal formulation possess a significant anti inflammatory effect on paw edema induced by freund's adjuvant. Further in depth studies must be carried to analyse the clear anti inflammatory mechanism using the active constituents extracted from the selected plants under study.

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REFERENCES

1. Mitchell R N, Cotron R S, In.; Robinsons Basic Pathology, ed 7. Harcourt Pvt. Ltd; New Delhi, India, 2010; 34-42.
2. Schmid-Schonbein. G.W.: Analysis in flammation, Annu. REV. Biomed. Eng, 2006; 8: 93-131.
3. Henderson B and Wilson M. Bacterial modulins: a novel class of virulence factors which host tissue pathology by inducing cytokine synthesis. *Microbial. Rev*, 1996; 60: 316-341.
4. Rouveix BC. Clinical pharmacology of cytokines. Eur. cytokine netw, 1997; 8: 291-293.
5. Sadique J, Chandra T et al. The anti inflammatory activity of the *Enicostemma littorale* and *Molluge cerviana*. *J. Ethnopharmacol*, 2000; 71(1-2): 349-52.
6. Rheumatoid arthritis: the management of Rheumatoid arthritis in adults, NICE clinical Guideline (February 2009).
7. Van Ede, A.E.Laan, R.F.Blom, H.J.De Abreu, R.A. and van de putte: Seminars in arthritis Rheum, 1998; 27: 277-292.
8. Kulkarni SK and Varghese, N.P: Indian drugs, 1998; 35: 245-258.
9. Laurence L.B and Keithy L.P: Goodman & Gilman's manual of pharmacology and therapeutics. Mc Graw Hill publication, Ne Delhi, 2008; 429-438.
10. Harris E.D: Nw Engl.J. Med, 1990; 322(18): 1277-1288.
11. Himeshsoni *et al*. A recent update of botanicals for wound healing activity, *International research journal of pharmacy*, 2012; 3(7).
12. I.A.Ross. Medicinal plants of the world. Chemical constituents, traditional, and modern medical uses, new jersey, human press inc, 1999; 179.

13. Trivedi CP. Modi NT, Sarin RK and Rao SS, Branchodilator and anti inflammatory effect of glycosidal fraction of Acacia farnesiana, Ind J Physiol Pharmacol, 1986; 30(3): 267-268.
14. Gamble JS. Flora of Presidency of Madras, Botanical survey of India, 1997; II: 1123-24.
15. Otari. K.V., Shete, R.V., Upasani, C.D., Adak, V.S., Bagade, M.Y. Evaluation of anti-inflamatory and anti-arthritic activities of ethanolic extract of *Vernonia Anthelmintica* seeds. *Journal of cell and tissue Research,* 2010; 10(2**)**: 2269-2280.
16. Ohkawa H, Ohishi N, Yagi K, "Assay of lipid peroxide in animal tissues for thiobarbituric acid reaction", Annual Biochem, 1979; 95: 351–358.
17. Misra HP, Fridovich I, The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. *J. Biol Chem*, 1979; 247: 3170-3175.
18. Rotruck JT, Pope AL, Ganther H, Swanson, Hafeman DG, Hoeksira WG. Selenium: Biochemical role as a component of glutathione peroxidase. *Science,* 1973; 588-590.
19. Beutler E, Duron C, Kelly BM, "Improved method for the determination of blood glutathione", J. *Lab. Clin. Med,* 1963; 65: 782–797.
20. Shinha AK, Colorimetric assay of catalase, J Anal Biochem, 1972; 47: 389-394.
21. Wanger Wo."A more sensitive assay disseminating galactosamine and glucosamine in mixture". Anal. Biochem, 1972; 94: 394-396.
22. Woessner JR, The determination of hydroxyl proline in tissue and protein samples containing small proportios of this amino acid, *Arch Biochem Biophys*, 1961; 93: 440.
23. King J. Practical clinical enzymology. *Philadelphia Dvan. Nostrand Co, London,* 1965; 363.
24. Bonata IL, Parnham MJ, Vincent JE and Rapt PC. Anti –Rheumatic drugs,present deadlock and new vistas in prog Med Chem, Edited by G p Ellis and G West, vol.1(Elesvier) North Holland, New York, 1980; 185.
25. Conner EM and Grisham MB. Inflammation, free radicals, and antioxidants. *Nutrition,* 1996; 12: 274-277.
26. Telang RS chatterjee S et al. Study on analgesic and anti inflammatory avtivities of *Vitex negunda* Linn. *gen pharmacol,*1990*;* 31(5): 363-366.
27. Gillham B, Papachristodoulou K and Thomas JH. In: Wills Biochemical basis of medicine, 1997; 77: 351.
28. Lewis DA. In: Anti inflammatory Drugs from plants and Marine sources.Basel: Birkhauser Verlag, 1989; 35.

29. Houck JC and Jacob RA, Non steroidal anti inflammatory drugs,edited by Garattini S.and Dukes M N G, *Medical foundation,*1969; 94.
30. Anderson AJ, Bocklehurst WE, Wills AL.Evidence for the role of lysosomes in the formation of prostaglandins during carrageenan induced inflammation in rat. *Pharmacol. Res. Comm*, 1971; 3: 13-17.
31. Nair R B, Ravishankar B, Vijayan NP, Sasikala CK and Saraswathy V N, Anti inflammatory effect of *Strbilanthus heyneanus* Leaves-A biochemical study, *J Res Ay S ID*, 1988; 9(1-2): 46.