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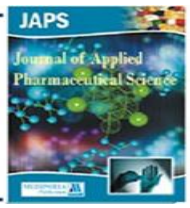

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Anti-inflammatory activity of *Talinum fruticosum* L. on formalin induced paw edema in albino rats

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ABSTRACT

Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes have serious side effects. So, number of herbal medicines is recommended for the treatment of inflammation that has no side effects. The present study is aimed to evaluate the anti inflammatory activity of *Talinum fruticosum* L on formalin induced paw edema in rats as for controlling inflammatory disorders. The objectives of the present study are to carry out phytochemical screening of selected plant drug , to prepare an aqueous extract from *Talinum fruticosum* L and to screen the in vivo anti inflammatory effect of *Talinum fruticosum* L. For phytochemical screening, the secondary metabolites like alkaloid, flavonoid, tannin, saponin, quinine were tested using qualitative spot tests. For anti inflammatory activity, wistar albino rats were used and divided into 6 groups and treated accordingly:Normal control, formalin induced group (0.1ml/kg bw), formalin+ *Talinum fruticosum* L (100mg/kg bw), formalin+ *Talinum fruticosum* L (200mg/kg bw), formalin+ *Talinum fruticosum* L (300mg/kg bw), and plant treated (300mg/kg bw).After the experimental period of 15 days, the blood and tissue samples were collected and biochemical parameter and histpathological studies were carried out.The phytochemical screening suggests th**e** standardization, identity, purity and of presence of phytochemicals like saponins, tannins, flavonoids, terpenoids and cardiac glycosides. Oral administration of formalin to the experimental animals produced reduction in the levels of SOD,GSH,GPX ,GR, serum protein and total RBC and Hb. The animals pretreated with *Talinum fruticosum* L extract at dose levels of 100,200,300mg/kg bw were significantly increased the levels of the above parameters. A significant increase in the length of the paw thickness, in the level of serum enzymes (SGOT, SGPT, ALP, CK) and Lipid peroxide (LPO), in the level of hydroxy proline, hexosamine and leucocytes was noted in the rats induced with formalin, while these levels were normalized by pretreatment with *Talinum fruticosum* L extract. The histopathological studies of edematous sections of formalin induced rat paw showed loss of cartilage, osteoblast hyperplasia. *Talinum fruticosum* L treated formalin induced rats showed moderate reduction in the cartilage and osteoblast. From the present observation, it is evidenced that *Talinum fruticosum* L would be an effective drug for the treatment of inflammatory reactions.

Keywords: *Talinum fruticosum* L., Inflammation, Flavonoid, Terpenoid, Hydroxyproline, Hexosamine

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. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotron, 2010).

Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increases vascular permeability and blood flow (Lalenti *et al*., 1995).

Eventhough various allopathic drugs like immunosuppressants,NSAIDS,corticosteroids and anti histamine are being used till now,the potential side effects give a limitations for their use.Now it is a growing concern all over for the development of new safe,potent,less toxic anti inflammatory drug.Hence ,there is a need to explore for more naturally available alternatives,so that their therapeutic values can be assessed and expanded. *Talinum fruticosum* L. is a erect,stout fleshy perennial herb.It is commonly known as pasalai keerai in south India.The leaves are used in the treatment of kidney disorders,gout and rheumatoid arthiritis. Hence, the present study is aimed to find out the possible role of *Talinum fruticosum* L against formalin induced paw edema and give a scientific rational for their use.

MATERIALS AND METHODS

Collection of Plant Materials

Plant source selected for the present study is *Talinum fruticosum* L**.** Aerial parts of the *Talinum fruticosum* L were collected from in and around Trichy, identified with the help of Flora of Presidency of Madras.The plant was authenticated with the specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's college, Trichy.

Preparation of Aqueous Plant Extract

The plant materials (leaves) were shade dried and coarsely powdered with electrical blender. 200gm of *Talinum fruticosum* L. was mixed with 1200 ml of water. Then it was boiled until it was reduced to one third and filtered. The filterate was evaporated to dryness. Paste form of the extract obtained was subjected to pre-clinical screening.

Preliminary Phytochemical Evaluation

The aqueous extract of *Talinum fruticosum* L. was evaluated for the presence of physic chemical standards and phytochemicals using standard procedure (Edeoga *et al*., 2005).

Experimental Animals

Healthy adult wistar strain of albino rats of both sexes, two to three months old and weighing 150g-200g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. Animals were housed in standard polypropylene cages. Six animals were housed per cage, so as to provide them with sufficient space, and to avoid unnecessary morbidity and mortality. Animals were maintained under standard condition of 12: 12- hours light/ dark cycle and at an ambient temperature at 23 ± 2°C, with 65 ± 5 % humidity. Animals were fed with standard rat chow pellet obtained from Sai Durga Foods and Feeds, Bangalore, India and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Assessment of *In Vivo* Anti Inflammatory Activity

Wistar albino rats were used and divided into 6 groups and treated accordingly:Normal control, formalin induced group (0.1ml/kg bw), formalin+ *Talinum fruticosum* L (100mg/kg bw), formalin+ *Talinum fruticosum* L (200mg/kg bw), formalin+ *Talinum fruticosum* L (300mg/kg bw), and plant treated (300mg/kg bw).After the experimental period of 15 days, animals were sacrificed by cervical decapitation. Blood and tissue samples were collected and serum was separated by centrifuging at 3000 rpm for 10 minutes and subjected for the determination of Serum enzymes like Alanine transaminase (ALT)**,** Alkaline phosphatase (ALP**),** Aspartate transaminase (AST**)** (King, 1965), Creatinekinase(CK**)** (Okinaka, 1961), Enzymatic and non enzymatic antioxidants like superoxide dismutase (Misra and Fridovich, 1979), Glutathione reductase (Carlberg B and Manervick, 1975), Glutathione peroxidase (Rotruck *et al.*, 1973), Reduced glutathione (Beutler *et al*., 1963), lipid peroxide (Ohkawa *et al*., 1979), haematological parameters like total leucocytes count**,** ,Total Red Blood Corpuscles (RBC)**,** Total White Blood Corpuscles (WBC)**,** Haemoglobin (Armour *et al* ., 1964), Tissue Hexosamine **(**Wanger, 1972), Tissue Hydroxy proline (Woessner, 1971), biochemical parameters like Blood glucose (Folin and Wu, 1919), Serum protein (Lowry *et al*., 1951), Paw thickness, histopathological studies (Sujai Sunetha, 1993).

Statistical Analysis

All the results were expressed as mean ± S.E . The data were statistically analyzed by one –way analysis of variance (ANOVA) and P values <0.05 were considered as significant.

RESULTS

The Preliminary Phytochemical Screening revealed the presence of tannins, proteins, terpenoids, flavonoids, quinine, starch,cardiac glycosides in the aqueous extract of *Talinum fruticosum* L. **(Table 1).**

Table. 1- Preliminary Phytochemical Screening of Water extracts.

S.No	Test	Reaction	Observation
1	Saponins	No leather formation	---
2	Proteins	Violet red colour	+++
3	Tannins	White precipitate	+++
4	Steroids	No bluish green	---
5	Terpenoids	Pink colour	+++
6	Flavonoids	Yellow colour	+++
7	Coumarins	No yellow colour	---
8	Quinone	Red colour	+++
11	Reducing Sugar	Orange colour	+++
12	Starch	Blue colour	+++
13	Carbohydrate	Violet ring	---
14	Cardiac glycosides	Pinkish colour of ammonical layer	+++
15.	Alkaloids	No orange brown	---
16.	Lignin	No pink colour	---

+++ = Present --- = Absent.

Administration of formalin in rats resulted in a significant increase in the levels of LPO, total leucocytes, total WBC, hydroxyl proline,hexosamine,serum enzymes,blood glucose and also in the length of the paw thickness while, these increase in the levels of above parameters were inhibited by the pretreatment of *Talinum fruticosum* L extract. Formalin to the experimental animals produced reduction in the levels of SOD, GSH, GPX, GR, serum protein and total RBC and Hb. The animals pretreated with *Talinum fruticosum* L extract at dose levels of 100, 200, 300mg/kg bw were significantly increased the levels of SOD, GSH, GPX and GR **(Table 2-8).**

Table 2. Paw Thickness and LPO in Experimental Animals.

Groups	Before Induction (μm)	After Induction (μm)	LPO (ng of MDA /g tissue)
Group I	0.43±0.03*	0.61±0.04*	3076.2±.13.34*
Group II	0.88±0.02*	1.35±0.03*	9265.7± 41.17*
Group III	0.80±0.03	0.93±0.02	7768.6±16.76
Group IV	0.60±0.03	0.69±0.03	6897.5±42.68
Group V	0.39±0.01*	0.44±0.03*	5566.6±18.63*
GroupVI	0.29±0.01	0.31±0.03	2255.78 ±13.17

Values are mean±S.E.M(n=6) .P<0.05*.

Table.3. Levels of Antioxidants in Experimental Animals.

Groups	GSH (mg /g tissue)	GPx (mg of glutathione reduced/g tissue)	GRase (mg of glutathione oxidized/g tissue)	SOD (mM of epinephrine oxidized/min/mg protein)
Group I	2.11±0.04*	49.51±0.04*	6.37±0.05*	10.06±0.05*
Group II	1.23±0.08*	12.50±0.04*	2.06±0.04*	5.15 ±0.10*
Group III	1.52±0.06	23.03±0.05	3.73±0.08	8.02± 0.07
Group IV	1.86±0.06	39.100.05	4.47±0.10	9.23± 0.08
Group V	2.03±0.05*	44.64±0.03*	5.73±0.08*	10.09±0.10*
GroupVI	2.08±0.03	47.70±0.03	5.76±0.06	10.10±0.14

Values are mean±S.E.M(n=6) . P<0.05*.

Table. 4. 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	315± 1.88	1225.83±18.5
Group IV	260.83± 1.60	945.20±17.4
Group V	242.50± 1.48*	914.16± 13.93*
Group VI	195.83±0.78	860±13.03

Values are mean±S.E.M(n=6). P<0.05*

Table. 5. Level of Leucocytes in Experimental Animals.

Groups	Neutrophilis %	Lymphocytes %	Eosinophils %
Group I	56.26±0.18*	46.13±0.07*	4.16±0.07*
Group II	77.5±1.87*	63.25±0.18*	8.62±0.09*
Group III	69.30± 0.25	58.29±0.20	6.20±0.07
Group IV	68.25± 0.18	55.06±0.03	5.23±0.11
Group V	63.20± 0.14*	52.20±0.14*	3.86±0.10*
GroupVI	52.35±0.30	48.12±0.06	3.20±0.06

Values are mean±S.E.M(n=6). P<0.01*.

Table. 6. Levels of Hemoglobin, Total RBC and WBC in Experimental Animals.

Groups	Hb (%)	RBC (millions of cells/mm3)	WBC (Thousands of cells/mm3)
Group I	13.9± 2.10*	5.57±0.16*	4450± 187.08*
Group II	9.56±1.35*	3.50±0.32*	15600 ±260.76*
Group III	11.45± 0.18	4.37±0.09	11366±258.19
Group IV	12.72±0.25	4.72±0.10	9583±348.8
Group V	12.89± 0.08*	5.27±0.12*	5583±318.85*
GroupVI	13.37±0.27	5.55±0.03	4883±108.01

Values are mean±S.E.M(n=6). P<0.05*.

Table.7. Levels of Serum Enzymes in Experimental Animals.

Groups	SGPT(IU/L)	SGOT(IU/L)	ALP(IU/L)	Ck (IU/L)
Group I	38.23±1.37*	33.76±1.47*	96.98±1.56*	272.75±1.84*
Group II	87.67±1.87*	78.89±1.95*	222.98±1.54*	487.65±1.90*
Group III	78.89±1.55	69.87±1.72	193.68±1.84	395.66± 1.86
Group IV	67.84±1.58	52.68±1.85	175.60±1.86	320.05±1.46
Group V	56.96±1.44*	43.73±1.80*	140.63±1.88*	274.75±1.75*
GroupVI	37.83±1.48	31.30±1.43	118.60±1.82	232.94±1.64

Values are mean±S.E.M(n=6). P<0.05*.

Table .8: Levels of Blood Glucose and Serum Protein in Experimental Animals.

Groups	Blood glucose (mg/dl)	Serum Protein (g/dl)
Group I	92.65±1.94*	6.72±0.09*
Group II	228.60± 2.00*	4.67±0.16*
Group III	181.8±1.98	5.30± 0.14
Group IV	162± 1.90	5.87± 0.10
Group V	144.25±1.28*	6.58± 0.17*
GroupVI	112.78±0.84	6.68±0.16

Values are mean±S.E.M(n=6). P<0.05*.

DISCUSSION

Formalin induced paw edema in rats is one of the most suitable test procedure to screen the acute inflammation and it is believed to be a biphasic event.

Among the various phytoconstituents flavonoids have beneficial effects in the inflammatory conditions and that the anti inflammatory activity is a common property of many terpenoids (Muthaiah *et al*., 1993. Flavonoids are particulary reported for significant antioxidant, vasculoprotector, anti hepato toxic, anti allergic, anti inflammatory and anti tumor activity (Singh and Gambhir , 1998). The anti inflammatory effects of triterpenes have been attributed to various mechanisms including inhibition of lipoxygenase and cycloxygenase activities (Andrikopoulos *et al*., 2003).

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 level was increased during inflammation (Bonata *et al*., 1980). Administration of formalin 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 (Telang *et al*., 1990). The inflammatory tissue damages could be due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (Conner and Grisham, 1996). Hence, in the present study, the concentration of LPO was found to be higher in formalin induced rats. On pretreatment with the *Talinum fruticosum* L at the dose level of 100,200,300mg/kg bw, the LPO level was significantly brought down to normal.

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 (Gillham *et al*., 1997) 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**.** 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 (Lewis. 1989). Pretreatment of *Talinum fruticosum* L at the dose level of 100, 200, 300 mg/bw in the formalin induced inflammatory rats do not allow activities of these enzymes that substantially reflecting the anti oxidant potency of *Talinum fruticosum* L.

Formalin 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 (Houck and Jacob . 1969). Hence the levels of hexosamine and hydroxyproline were found to be higher in formalin induced rats. Pretreatment of *Talinum fruticosum* L. inhibited the accmulation of hydyoxy proline and hexosamine in edematous tissue of formalin induced rats.

Leucocytes play a major role in the development and propagation of inflammation. Neutrophils play a crucial role in the development and manifestation of inflammation and they are the major source of free radicals at the site of inflammtion. Neutrophil derived free radical is known to because of inflammation and cytokines produced by neutrophils are also responsible for inflammtion. The reduction in the population of neutrophils after the pre treatment of *T*alinum *fruticosum*.L shows its involvement in suppressing inflammation (Goel *et al*., 2001). Eosinophils are granule containing leucocytes that differentiate from stem cell precursors. It synthesize and release lipid derived mediators which stimulate responses in tissues**.** In addition, it produce cytokines such as interleukins(IL-3,IL-5) and granulocyte macrophage stimulating factor that contribute pro inflammatory functions. Lymphocyte are the predominant cell in chronic inflammation. It can cause permanent distortion of the tissue, interfying its function.The reduction in the population of lymphocyte and eosinophils in formalin induced rats treated with *T*alinum *fruticosum*.L shows its antiinflammatory property.

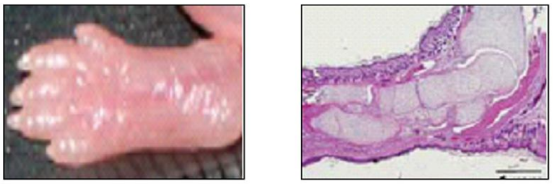
Total WBC which plays a major role in body defense mechanism. The increase in WBC count during inflammation may be due to the release of interleukins, responsible for the production of both granulocytes and macrophage colony stimulating factor (Eric and Lawrence, 1996). Hence in the present study the level of WBC was found to be higher in formalin induced inflammation. Pre treatment with the *Talinum fruticosum* L. at the dose levels 0f 100, 200, 300 mg/kg bw significantly decrease the WBC count that indicate the significant recovery from the inflammatory process.

Hemoglobin and RBC play a major role in the oxygen transport. Formalin induction causes the significant decrease in the RBC and Hb which leads to anaemia. Pretreatment with the *Talinum fruticosum L* at the dose levels 0f 100, 200, 300 mg/kg bw altered these levels to normal. The low concentration of Hb is noted in chronic inflammatory disease such as rheumatoid arthritis which is usually associated with the anorexia and weight loss. Such a decline in Hb level has been reported earlier (Swingle and Shideman, 1972). 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 (Anderson *et al*., 1971). 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 (Nair *et al*., 1998). So it can be assumed that *Talinum fruticosum* L extract might be acting by either inhibiting the lysosomal enzymes or stabilizing the membrane. Treatment with *Talinum fruticosum* L extracts 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.

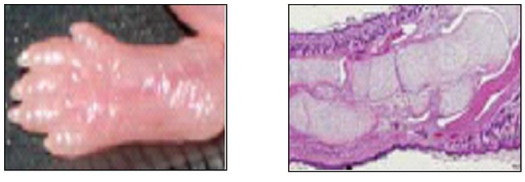
Proteins are the building block of amino acids. The propagation of free radical can bring many adverse reactions leading to extensive tissue damage. Lipids, proteins, DNA are very susceptible to attack by free radicals (Yu *et al*., 1992). The level of serum protein content is lowered in rheumatoid arthritis.The proteins were clearly changed the perception of the pathogenesis of inflammation which has been reported earlier (Weissman, 1967). Chronic inflammation is known to stimulate protein metabolism in animals (Mercier *et al*., 1921). Hence in the present study, *Talinum fruticosum* L treated rats showed no significant decline in the protein level in formalin induced rats.

Histopathological sections of normal rat paw showed normal architectural pattern of cartilage and osteoblast. The edematous tissue section of formalin induced rats showed a loss of cartilage, osteoblast hyperplasia, and accumulation of abundant mono and poly morpho nuclear cells in the joint and congestion of vessels and loss of marrow and dis array. While pretreatment with *T*alinum *fruticosum*.L extract showed moderate reduction in the cartilage, no osteoblast hyperplasia, moderate accumulation of abundant mono and poly morpho nuclear cells in the joint, healing of vessels and gain of marrow in formalin induced rats as compared to untreated rats.

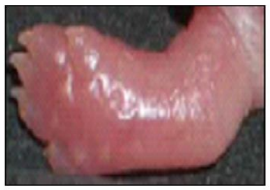
Photograph of Normal Rat Paw

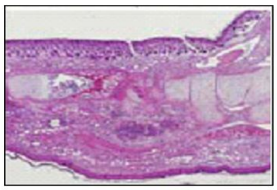


Photograph og Hind Limb of *Talinum fruticosum* L Treated rat Paw Induced with Formalin



Photograph of Hind Limb of Formalin Induced Rat





CONCLUSION

In conclusion, the present experimental findings of hematological, histopathological ,enzymatic and non enzymatic, biochemical parameters suggests that *Talinum fruticosum* L is a promising anti-inflammatory agent in the treatment of inflammation in dose dependent manner of therapeutic range and the phytochemical screening of the plant source contains flavonoids that confirms anti inflammatory potential of *Talinum fruticosum* L.

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