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Studies on evaluation of anti-inflammatory activity of *Pterospermum acerifalium* (L.) against carrageenan induced paw edema

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Abstract

Inflammation is a process in which the body's white blood cells and chemicals help protect us from infection and foreign substances such as bacteria and viruses. In some diseases, however, the body's defense system (immune system) triggers an inflammatory response when there are no foreign substances to fight off. Ethanolic extract of *Clerodendrum serratum* Linn., leaves were evaluated for anti-inflammatory activity. Extract did not show any signs of toxicity at the dose of 1000 mg/kg per organism (p.o.). From the acute toxicity test we selected two doses (50 and 100mg/kg) for subsequent pharmacological study.

Key-Words: Inflammation, *Pterospermum acerifalium*, Inflammatory response

Introduction

Medications are used as one of the intentional designs in the prevention and management of various infirmities. Although medications are useful to *Pterospermum acerifalium wild (Sterculiaceae)* commonly known as 'Kanak champa' is shrubs distributed in tropical Asia and an ever green tree with very conspicuous presence in the lower hill forests of Darjeeling and south Sikkim. It much more available at the east of Tista river and hardly occurs in central Darjeeling. It is also found in sub-Himalayan tract and outer Himalayan valleys and hills up to 4,000 ft. The plant is commonly known as Kanak champa, Muchkund (Hindi), Muskanda (Bengali), Matsakanda (Telugu), Moragos (Assamese), Vennangu (Tamil), Mushkundo (Oriya), Karnikar (Marathi) 1.

The leaves of the plant are widely used for the treatment of diabetes and as a haemostatic in Indian proprietary medicines. The plant is documented to possess beneficial effects as antioxidant, antiulcer, anti inflammatory, analgesic, hypoglycemic and antihelmentic. It is believed to be used in inflammation, abdominal pain, ascites, cures ulcers, leprosy, constipation, urinary discharges and tumours.

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A scrutiny of literature revealed some notable pharmacological activities of the plant such as antidiabetic (showed the leaves extract lower the glucose level, in type 2 diabetic models in rats), antimicrobial, haemostatic, free radical scavenging and anti-inflammatory 2.

Hill people use the white tomentum from the under surface of the leaf of *Pterospermum acerifolium* to stop bleeding 4. Flowers and bark: charred and mixed with kamala applied in suppurating small-pox as a general tonic 5. Flowers also impart a pleasant perfume and keep away insects and also used as disinfectant and made into paste with rice water used as application for hemicranias 6, 7. A good tonic is prepared from the flowers which is also a cure for inflammation, ulcers, tumours, blood troubles and leprosy 8. Dried flower powder is mixed with coconut oil (*Cocos nucifera*) and is applied on head for killing of hair lice 9. The extracts of these leaves are used in traditional medicine because of their antibacterial and antifungal activity 10. In ayurvedic anticancer treatment flowers are mixed with sugar and applied locally10.

Leaves are simple, alternate, has stipules and palmately ribbed. Length- 5 to 10 cm, Breadth- 4 to 8 cm, leaf blade nearly orbicular or oblong, sometimes lobed, 24-34 × 14-29 cm, leathery, basically densely yellowish

and gray stellate velutinous, axially sparsely hairy or glabrous, base cordate, margin entire or crenate, apextruncate, nearly rounded, or pointed; juvenile leaves palmately lobed, palate. Flowers are in axillary fascicles. Sepals are wooly. Yellowish in colour consisting five sepals and five petals 11.

The word inflammation comes from the Latin *"inflammo"*, meaning *"I set alight, I ignite"*. **"**Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.'' Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each 12.

Progressive destruction of the tissue would compromise the survival of the organism. However, chronic inflammation can also lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). It is for that reason that inflammation is normally closely regulated by the body. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movementof plasma and leukocytes (especially granulo cytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process 13. Inflammatory abnormalities are a large group of disorders which underlie a vast variety of human diseases. The immune system is often involved with inflammatory disorders, demonstrated in both allergic reactions and some myopathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with etiological origins in a inflammatory processes include cancer, atherosclerosis, and ischaemic heart disease 14. Examples of disorders associated with inflammation include Acne vulgaris, Asthma, Autoimmune diseases, Celiac disease, Chronic prostitutes, Glomerulonephritis, Hypersensitivities, Inflammatory bowel diseases, Pelvic inflammatory disease, Reperfusion injury, Rheumatoid arthritis, Transplant rejection, Interstitial cystitis 15. Acute inflammation begins within seconds to minutes following the injury of tissues. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. The damage may be purely physical, or it may involve the activation of an immune response. Three main processes occur:

- Increased blood flow due to dilation of blood vessels (arterioles) supplying the region
- Increased permeability of the capillaries, allowing fluid and blood proteins to move into the interstitial spaces
- Migration of neutrophils (and perhaps a few macrophages) out of the capillaries and venules and into interstitial spaces 16.

Chronic inflammation, on the other hand, is a disease. The system has gotten hung up, and instead of protecting the organism (our bodies) it starts to kill the organism, slowly but surely. Today modern medicine is starting to admit that chronic inflammation is the main contributing factor to all chronic degenerative diseases, and the root cause of the two greatest killers in America: Cancer and Heart Disease. In deed, chronic inflammation might just be the root cause of all degenerative disease 17.

Material and Methods

Plant materials: The *Pterospermum acerifolium* leaf was provided from Sapience Bioanalytical research lab., Bhopal, (M.P.).

Animals: Swiss albino mice and albino rats was provided from sapience bioanlytical research lab Bhopal (M.P.).

Chemicals: Carrageenan was purchased from Himedia. Diclofenac sod. was provided as gift sample from Aristo pharma, Bhopal. All other chemicals used for this study were of analytical grade.

Extraction of anti-inflammatory extract of *pterospermum acerifolium*

The leaves were shade dried for 1 week. The dried leaves were reduced to powder. The powder was extracted with petroleum ether by using soxhlation. The liquid filtrates were concentrated and evaporated to dryness at room temp. The yield of extract was

weighed. The dry extract was stored in a refrigerator at 4°C until use for the proposal experiment.

Phytochemical investigation

The plant extract screened for the presence of various phytochemical constituents i.e. alkaloids, carbohydrates, glycosides, gums and mucilage, proteins, tannins, phenolic compounds and flavonoids, etc by employing standard screening tests.

Anti -inflammatory activity of *Pterospermum acerifolium*

Animal care and handling

The experiment was carried out on Swiss albino mice of 4 months, of both sexes, weighing 30gm and Wistar albino rats of 4 months, of both sexes, weighing between 150 to 180 gm. They were provided from Sapience Bioanalytical research lab., Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1413/PO/a/11/CPCSEA).

Drug preparation: 10mg diclofenac sodium was prepared by suspending in 5ml of 0.5% CMC solution.300mg extract was suspended in 10ml of 0.5% CMC solution.

Acute toxicity study of extract

Acute toxicity studies were performed according to the OECD guidelines (425).Three mice were administered single dose of extract (1000 mg/kg). Animals were observed for any sign of toxicity 3 hours after dose administration and also after 24 hour.

Carrageenan-induced paw edema

Acute inflammation was caused by injecting 0.1 ml of 1 % (w/v) carrageenan in saline into the sub-plantar region of the right hind paw of each rats. The paw volume was measured

plethysmometrically at 0 hr, 1 h, 2h and 3 h after the carrageenan injection. Edema was expressed as mean increase in paw volume relative to control animals.18

Experimental Design

In the experiment, a total of 8 rats were used. The rats were divided into 4 groups comprising of 2 animals in each group as follows:

Group I: Control, 0.1 ml carrageenan injected in right hind paw

Group II: Standard, rats received Diclofenac sod. (10 mg/kg p.o.) + 0.S1 ml carrageenan injected in right hind paw.

Group III rats received pet. ether Extract of *Pterospermum acerifolium* leaf, (50mg/kg p.o.) once daily for 7 days + 0.1 ml carrageenan injected in right hind paw.

Group IV rats received pet. ether Extract of *Pterospermum acerifolium* leaf, (100mg/kg p.o.) once daily for 7 days + 0.1 ml carrageenan injected in right hind paw19.

Results and Discussion

Pterospermum acerifolium extract (with petroleum ether) shown following activity against inflammation induced in Albino rats. The phytochemical analysis of ethanolic extract of *pterospermum acerifolium* leaves revealed the presence of alkaloids, carbohydrate, Glycosides, proteins, flavonoids, steroids etc. In the acute toxicity assay no deaths were observed or no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis thus the median lethal dose (LD50) was determined to be higher than the dose tested i.e. 2.0 g/ kg b.w. (Table -1). Extract did not show any signs of toxicity at the dose of 1000 mg/kg per organism (p.o.) From the acute toxicity test we selected two doses (50 and 100mg/kg) for subsequent pharmacological study.

Carrageenan-induced paw edema is the standard experimental model for acute inflammation. Carrageenan is the pholistic agent of choice for testing anti inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits high degree of reproducibility. The development of edema has been described as biphasic**.** The first phase (1h) is mediated through the release of serotonin and histamine and the second phase (over 1 h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by konin (see in table no 3)**.**

Inflammation can be caused by a number of factors that can damage cells but is broadly divided into five different categories which are given below:

1. **Physical** – can be *mechanical* as in a car accident injury or assault or environmental like severe cold and heat (burns).
2. **Chemical** – for example : Acid 'burns', drugs, venom.
3. **Infection** – bacteria, viruses, fungi and other parasites
4. **Ischemia** – lack of or restricted blood supply which may eventually lead to death of tissue (necrosis) known as an infarct.
5. **Immune** – autoimmune conditions and allergies

Conclusion

Experimental studies revels that petroleum ether extract of *Pterospermum acerifolium* (at dose 100 mg/kg) produced an anti-inflammatory action by

decreasing the paw volume in the model of carrageenan-induced paw edema in rats. Low dose (50mg/kg) was not showed anti-inflammatory action. Diclofenac sodium was used as a standard anti-inflammatory drug. Further studies are needed to isolate the active principles of the extract.

References

1. Arya, V. and Arya, M. L. Review On Anti-Inflammatory Plant Barks. *International Journal Of Pharmtech Research*, 3 (2);2011: 899-908.
2. Olijide, O. A., Makinde, M. J. and Awe, S. O. Effects of the aqueous extract of Bridelia ferruginea stem bark on carrageenan-induced edema and granuloma tissue formation in rats and mice, *J of Ethnopharmacology*, 66;1999: 113-117.
3. Periyanayagam, J. B., Sharma, S. K. and Pillai, K. K. Anti-inflammatory activity Trichodesma indicumroot extract in experimental animals. *J of ethnopharmacology*, 104 (3); 2006: 410-414.
4. Yerima, M., Magaji, G., Yaro, H. and Tanko, M. Analgesic And Anti-Inflammatory Activities Of The Methanolic Leaves Extract Of *Securinega Virosa* (Euphorbiaceae). *Nigerian Journal Of Pharmaceutical Sciences*, 8; 2009: 213-218.
5. Shalini, S. and Rajesh, K. Studies on the anti-inflammatory activity of *Heliotropium indicum*. *JITPS*, 1 (1);2010: 43–46.
6. Smain, A., Martin, D., Guillemo, S. and José, L. R. Anti-inflammatory, anti-oxidant, and apoptotic activities of four plant species used in folk medicine in the Mediterranean basin. *Pak. J. Pharm. Sci.*, .25 (1); 2012: 65-72.
7. Manna, A. K. and Jena, J. Anti Inflammatory And Analgesic Activity Of Bark Extract Of *Pterospermum Acerifolium. International Journal Of Current Pharmaceutical Research*, 1(1); 2009: 56-62.
8. Senapati, A. K., Swain, S. R. And Satyanarayana, S. Toxicological Studies Of The Hydroalcoholic Extract Of *Pterospermum Acerifolium* Flowers. *Pharmacologyonlin*, 5; 2009:161-167.
9. Mohan, P. "Anti- inflammatory activity of *Sarcostemma secamone* (l) bennet whole plant against carrageenan induced paw edema". *Bioscience Discovery*, 3(3); 2012: 288-291.
10. Caius, J. F. The Medicinal and Poisonous Plants of India. *Indian Medicinal Plant*, 5(2);1990: 489-493.
11. Cotran; K. C. Robbins Pathologic Basis of Disease. Philadelphia. *Br. J. Pharmacol*, 19; 1998: 42-47.
12. Calixto, J. B., Campos, M. M., Otuki, M. F. and Santos, A. R. S. Anti-inflammatory compounds from plant origion. Part II. Modulation of Pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta medica*, 70; 2004: 93-103.
13. Abbas, A. B. and Lichtman, A. H. "Ch.2 Innate Immunity". In Saunders (Elsevier). Basic Immunology. Functions and disorders of the immune system (3rd ed.). ISBN 978-1-4160-4688-2.2009.
14. Balachandran, P. and Govindrajan, R. Cancer –An ayurvedic perspective. *Pharmacol Res*, 51; 2005: 19-30.
15. Niehaus, W. G. and Samuelson, B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry*, 6; 1968: 126-130.
16. Elias, G. and Rao, M. N. Inhibition of albumin denaturation and anti-inflammatory activity of dehydrozingerone and its analogs. *Indian Journal of Experimental Biology*, 26;1988: 540-542.
17. Bjorkman, R. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. *Acta Anaesthesiol Scand Suppl*, 103;1995: 1-44.
18. Winter, C. A., Risely, E. A. and Nussm, G. W. Carrageenin-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society of Experimental Biological Medicine111, 1962, 544-547.
19. Vinegar, R., Schreiber, W. and Hugo, R. Biphasic development of carrageenin edema in rats. *Journal of Pharmacology and Experimental Therapeutics*, 166; 1969: 96-103.

Table 1: Preliminary phytochemical tests for identification of phytoconstituents in *Pterospermum acerifolium*

EXTRACTION BY FRACTIONATION METHOD	
Tests for	**Petroleum ether**
Alkaloids	+
Carbohydrates	+
Glycosides	+
Tannins - phenolic compounds	-
Protein and amino acids	+
Gum and mucilage	-
Flavones and flavonoids	+
Saponins	+
Steroids and sterols	+

Table 2: Observation of acute oral toxicity study of petroleum ether extract of *Pterospermum acerifolium* leaf in mice

Animals	Dose per organism (mg/kg)	Observation
1	1000	animal survived
2	1000	animal survived
3	1000	animal survived

Table 3: Effect of petroleum ether extract of *Pterospermum acerifolium* leaf on carrageenan induced paw edema in rats

Groups	Animals	Paw volume (mm)							
		0 h	Mean	1h	Mean	2h	Mean	3h	Mean
Group I (Control)	1	**0.1**	0.15	**0.1**	0.15	**0.3**	0.3	**0.3**	0.3
	2	**0.2**		**0.2**		**0.3**		**0.3**	
Group II (Diclofenac sod.)	1	**0.2**	0.15	**0.1**	0.1	**0.2**	0.15	**0.2**	0.15
	2	**0.1**		**0.1**		**0.1**		**0.1**	
Group III (Pet.E PA,50mg/kg)	1	**0.1**	0.15	**0.2**	0.15	**0.2**	0.2	**0.3**	0.3
	2	**0.2**		**0.1**		**0.2**		**0.3**	
Group III (Pet.E PA,100mg/kg)	1	**0.2**	0.15	**0.3**	0.2	**0.2**	0.15	**0.2**	0.15
	2	**0.1**		**0.1**		**0.1**		**0.1**	