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Anti-inflammatory and Anti-peroxidative effect of Hamelia patens leaf extract on carrageenan induced rat paw edema.

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ABSTRACT

The present study investigates the anti-inflammatory activity of *Hamelia patens* leaf extracts using carrageenan induced paw edema in albino rats. This anti-inflammatory medicinal value of the Hamelia patens leaf has been mentioned in ancient literature. Dried leaves of Hamelia patens were crushed and the extract was prepared with ethanol using shaker. Indomethacin and *Hamelia patens* are compared in terms of their anti-inflammatory properties. Test groups were given standard Indomethacin (10mg/kg) and *Hamelia patens* leaf extract in 200mg/kg and 400mg/kg; after 1% Carrageenan (0.1 ml) injection. *Hamelia patens* leaf extract showed higher reduction in paw edema and potent anti-inflammatory activity in comparison with indomethacin. It also showed decrease in lipid peroxidation due to its anti-oxidative nature, leaving a large prospect for future anticancer research.

Introduction

Disclaimer: None

Herbal preparations are increasingly being used to counteract inflammatory conditions (Bagul; H. Srinivasa, 2005). Due to the recent advancement in medicine, the mean age of survival is increasing with increasing incidences of inflammatory disease conditions. One of the reasons can be damage of cells due to accumulation of reactive oxygen species and lipid per-oxidation in the ageing population. The overall inflammatory process in relation to its cellular effectors is the favorable environment for causation of cancers (A. Mantovani, 2008). Even though we are not totally aware of the physiological mechanism, we have been using different herbal products for different disease process since ages (A. Helen, 2007). Natural products in general and medicinal plants with secondary metabolites in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy (EK. Afolabi, 2009).

Inflammation is associated with various disease processes. Many anticancer drugs are found to be natural products or their derivatives. Based on physiological regulation, there is a suggestion for the five-point criteria for selecting Herb having anti-cancer activity (Christine M. Kaefer and John A. Milner, 2008). These criteria are: i) regulate inflammation and immune-competence, ii) decrease free radical formation, iii) suppress cell division, iv) promote apoptosis in cancerous cell, and v) carcinogen bio activation. These herbal entities should fulfill the criteria if they really possess anti-cancer properties. This observation initiates us to perform the identification of anti-inflammatory and anti-oxidative activity of the natural products.

Ethno-pharmacological information suggests that *Hamelia patens* may exhibit anti-inflammatory and anti-oxidative activity. But there are no studies to this date to focus systematically, and evaluate its anti-inflammatory property (Ahmad, *et al*, 2012). Different parts of *Hamelia patens* are still used traditionally for its anti-infective and analgesic properties. Present research has been undertaken with the ethanolic leaf extract of Hamelia patens to investigate its anti-inflammatory and anti-oxidative activity.

Hamelia patens

Hamelia patens is commonly known as scarlet bush, firebush or humming bird bush. It can be a large perennial shrub to small tree. Firebush is a bright green, colorful, fast growing shrub, capable of growing up to 4.6 m. It usually has three whorled leaves at each node. These leaves are 15 cm long, with reddish veins which reflexes upward. Recently there is a discovery of many active phytochemicals from the extract of *Hamelia*. These extract was found to have bacteria and fungus inhibiting properties.

Reactive oxygen species (ROS):

ROS play a vital role in normal cell functions as well in pathologic disease processes. ROS in the cell are produced from different enzymes (e.g. NADPH oxidase, xanthine oxidase, lipoxygenases, prostaglandin synthase, myeloperoxidase, cytochrome oxidase). They are checked in balance by antioxidant enzymes in the body; notably superoxide dismutase. catalase, glutathione peroxidase.

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The endogenous and exogenous antioxidant also plays a significant role in reaching to equilibrium in the antioxidant-prooxidant balance. Some of these compounds are vitamins C and E, endogenous chelators and glutathione. Synthesis of these molecules occurs in the cells in response to normal physiological stimuli. But they can as well be harmful when their concentrations increase above a certain level in our body. In many inflammatory pathological conditions, this equilibrium is broken. This disruption in normal homeostasis will produce an increase in ROS, which can lead to tissue injury, by damaging macromolecules and lipid per-oxidation of the membranes. In addition, ROS could possibly act as a second messenger to activate and increase in formation of other mediators primarily involved in the inflammatory disease process (Miesel, 1995; Polla *et al.*, 1995; Haanen and Vermes, 1995).

Materials and methods

Preparation of Hamelia patens leaf extract

The extracts were prepared with leaves of *Hamelia patens* obtained from Loyola College, Vijayawada, Andhra Pradesh, India. We started with 25gm of the plant powder in a sterile beaker. 125 ml of ethanol (1:5) was added to it and mixed well with the help of shaker for 24 hours. After mixing properly, it was filtered through 'What Mann no: 1 filter paper'. This solution so obtained was used for experimental purposes.

Chemicals used

Carrageenan, Indomethacin (as standard anti-inflammatory drug), *Hamelia patens* ethanol leaf extracts.

Experimental Animals

Male Albino rats weighing 200-250 g were used for animal studies. These animals were grouped in polyacrylic cages and housed in standard conditions in the lab (temperature 25±2 ^{0}C) and relative humidity (50 ± 5%) with dark and light cycle (14/10hrs). The standardized continuous diets for these rats were dry pellet (Hindustan Lever, Kolkata, India) with water ad libitum. The rats were kept in the laboratory for at least 14 days before beginning the experiment. All the experiments were performed as per the guidelines of the Institutional Animal Ethics Committee, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, West Godavari Dist., A.P. These rats were also given access to Gold Mohar commercial feed (Hindustan Lever Limited, Bangalore.)

Albino Wristar rats were supplied with only water and libitium starting 16 hr. prior to the experiments. Edema in these rats can be induced by injecting 0.1 ml of carrageenan (1% in physiological solution) into the right posterior paw sole of the rats (Conforti *et al.*, 2007). For this purpose, carrageenan (Sigma) was homogenized by the use of potter, dissolved and stored at +4°C in the dark. The suspension of carrageenan was injected subcutaneously into the right hind paw 1 hr. after the test solution THC had been administered orally.

These albino rats were initially divided into 5 groups (n=6). Plethysmometer was used to measure the volume of hind-paw, before the administration of test solution (Winter and Poster, 1957). These different groups were treated with Hamelia patens leaf extract (HpLEt) (62.5, 125 and 250 mg/kg, p.o.), and 10 mg/kg body weight of Indomethacin as a standard drug at 1 hr. before eliciting paw edema. Subsequently, 0.1 ml carrageenan (1%) was given sub plantar in normal saline in the right paw of the rats to induce acute inflammation. The paw volume was measured up to 4 hr. at intervals of 1 hr. after carrageenan injection using plethysmometer. This was followed by test does of *Hamelia* leaf extracts and Indomethacin. The ratio of anti-inflammatory effect of HpLEt to control was calculated by using the equation: anti-inflammatory activity (%)=(1-D/C x100, where D represents the percentage difference in paw volume after HpLEt was administered to the rats, and C represents the percentage difference of volume in the control groups (Wintrobe *et al.* 1961).

It is well established that non-steroidal anti-inflammatory drugs, like indomethacin are potent inhibitors of prostaglandin biosynthesis. Inhibition of these drugs on prostaglandin biosynthesis played a prominent part in their anti-inflammatory effect.

Estimation of Lipid peroxidation

Lipid peroxidation in plasma was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides (in mmoles/dl) using the methods described by Fraga *et al*. (1988) and Jiang *et al*. (1992), respectively. In brief, 0.5 ml of plasma was treated with 2 ml of TBA-trichloroacetic acid (TCA)-HCl reagent (0.37% TBA, 0.25 N HCl and 15% TCA, 1:1:1 ratio), placed 15 min in a water bath followed by cooling and centrifugation for 10 min (1000 rpm) at room temperature. The clear supernatant was measured at 535 nm against a reference bank.

0.5 ml of plasma was treated with 0.9 ml of Fox reagent and incubated for 30 min at the temperature of 37°C. Fox reagent is obtained by mixing BHT-88mg, xylenol orange-7.6mg and ammonium iron sulphate-0.8mg, with methanol (90 ml) and 250 mmoles sulphuric acid(10 ml). The development of color was read at 560 nm.

Determination of Non-Enzymatic Antioxidants

estimation of ascorbic acid (Vitamin C)

Vitamin C was estimated by the method described of Omaye *et al*. (1979). First supernatant (0.5 ml) and TCA (1.5 ml, 6%) were centrifuged for 20 minutes. Similarly 0.5 ml of DNPH reagent was added to 0.5 ml supernatant and mixed well. It was allowed to stand at room temperature for an additional 3 hours, after which 2.5 ml of 85% sulphuric acid was added and allowed to stand for 30 minutes. Ascorbic acid (10-50μg) were taken as a set of standards and followed with similar process as a blank. These values were all read at 530 nm. and expressed as mg/dl.

estimation of Vitamin E

Vitamin E was determined by the method of Baker *et al*. (1951). First 1.5ml ethanol and 2.0 ml of petroleum ether were added to 0.1 ml of plasma, followed by mixing and centrifugation. This supernatant was evaporated to dryness at 80°C. 0.2 ml of 2, 2′ -dipyridyl solutions and 0.2 ml of ferric chloride solution added to supernatant. This mixture was kept in the dark and 2 ml of butanol was added after 5 min. The development of intense red Colour developed was read at 520 nm. Standard tocopherol (10-100μg) were taken thereafter and treated in with reference to blank having only reagent. The amount of α-tocopherol was expressed as mg/dl plasma. Protein was determined by the method given by Lowry *et al*. (1951).

Estimation of reduced glutathione

Reduced glutathione (GSH) was determined by the method postulated by Ellman (1959). An aliquot (1.0 ml) of the supernatant was treated with 0.5 ml Ellman's reagent (5, 5'-dithiobis-(2-nitrobenzoic acid) or DTNB) and 3.0 ml phosphate buffer (0.2M, pH 8.0) and the absorbance was read

at 412 nm. Glutathione peroxidase activity was expressed as μg GSH consumed/ min/mg protein and GSH.

Determination of Enzymatic Antioxidants

Estimation of Superoxide Dismutase (SOD) Activity in Serum

SOD was determined using the method of Oyanagui *et al.* (1984). First plasma was 100-fold diluted with potassium phosphate buffer and 100ml of the diluted solution was added into a test tube. This was followed by addition of 200ml of solution A (3mM hydroxylamine/hypoxanthine) and 200ml of solution B (7.5 mU/ml xanthine oxidase with 0.1mM EDTA-2Na). The mixture was added with 500ml of distilled water thereafter, mixed properly with the vortex and then stood in water for 40 min, maintained at 37 °C. The final solution obtained was added with 2 ml of solution C (300 mg of sulphanilic acid/5.0 mg *N*-1-naphtyl-ethylenediamine in 500 ml of 16.7% acetic acid) and it was allowed to stand at room temperature for 20 min. The test solution absorbance was read at 550nm and the standard curve was used to calculate the serum SOD activity.

Statistics

All data was presented as a mean ± SEM (n = 6). Induced edema in paw and granuloma weights of the groups were compared using one-way ANOVA followed by Duncan multiple range tests ($p<0.05$ was accepted as statistically significant).

Results and Discussion

Hamelia patens has been used traditionally for treating many common disease conditions. In this context, experimental study was performed to elicit the anti-inflammatory and anti-oxidative activity of *Hamelia*. *Hamelia patens* Leaf Extract (HpLEt) was used for the anti-inflammatory activity in experimental animal models and the results are tabulated. HpLEt exhibited significant anti-inflammatory activity at the tested doses in a dose dependent manner. HpLEt showed maximum inhibition of 92.73 % at the dose of 250 mg/kg when compared with the standard drug at the time of 4 hrs (Table-1).

Table-1. Effect of HpLEt on carrageenan induced pedal oedema

GROUP	Paw volume and percentage inhibition	
	Paw volume	Percentage of Inhibition
Control	0.732 ± 0.068a	-
HpLEt 62.5mg/kg/body weight	0.469 ± 0.032b	45.93
HpLEt 125mg/kg/body weight	0.421 ± 0.024c	82.49
HpLEt 250mg/kg/body weight	0.346 ± 0.025d	92.73
Standard drug (indomethacin) 10 mg/kg body weight	1.18 ±0.26e	85.59

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common (a-e) letter differ significantly at $p<0.05$ (DMRT).

In order to understand the mechanisms of the anti-inflammatory effect we also investigated whether it attenuates the levels of TBARS, lipid hydroperoxides, glutathione, vitamin C, vitamin E and SOD activity caused by carrageenan. Rats treated with carrageenan exhibited a substantial increase in levels of TBARS and lipid hydroperoxides. Similarly they show decreased levels of glutathione content, vitamin C, vitamin E in plasma and SOD activity in serum. Pre-treatments of rats with HpLEt (Table-2 and Table-3) attenuated the increase in the levels of TBARS, lipid hydroperoxides, glutathione and SOD activity caused by carrageenan in the serum.

Table 2. Changes in the levels of plasma TBARS and Hydroperoxides in normal and in experimental rats.

Group	Plasma levels			
	Control	Standard drug (Indomethacin) 10mg/kg body weight	HpLEt 250mg/kg/body weight	Inference
TBARS (mmoles/dl)	0.421 ± 0.024a	176.2 ± 12.5e (Decreased)	0.221± 0.025b (Decreased)	Enhanced Anti-inflammatory activity
Hydroperoxides(x 10^{-5}M/100ml)	27.54 ± 1.35a	1.80 ± 0.06e (Decreased)	12.14 ± 0.85b (Decreased)	Enhanced Anti-inflammatory activity

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common (a-e) letter differ significantly at $p<0.05$ (DMRT).

Table 3. Changes in the levels of vitamin C, vitamin E, reduced glutathione (GSH), Superoxide dismutase (SOD) in plasma of control and experimental rats.

Group	Plasma levels(mg/dl)			
	Control	Standard drug (Indomethacin) 10mg/kg body weight	HpLEt 250mg/kg/body weight	Inference
Vitamin C (mg/dl)	0.81 ± 0.05a	0.28 ± 0.03^{e} (Increased)	1.59 ± 0.12b (Increased)	Enhanced Anti-inflammatory activity
Vitamin E (mg/dl)	0.65 ± 0.04a	0.20 ± 0.03^{e} (Increased)	1.45 ± 0.06b (Increased)	Enhanced Anti-inflammatory activity
Reduced Glutathione (GSH) (mg/dl)	13.02 ± 0.78a	6.72 ± 0.09^{e} (Increased)	3.08 ± 1.58b (Increased)	Enhanced Anti-inflammatory activity
Superoxide dismutase (SOD) (mg/dl)	1.95 ± 0.08a	176.2 ± 7.9^{e} (Increased)	2.89 ± 0.12b (Increased)	Enhanced Anti-inflammatory activity

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common (a-e) letter differ significantly at $p<0.05$ (DMRT).

It is important to point that ROS have an important role in inflammation since free radicals activate arachidonic acid metabolism (especially the inducible cyclooxygenase COX-2), pro-inflammatory cytokines (i.e. interleukins IL-1 and IL-6. and tumor necrosis factor), heat shock proteins and the apoptotic process (Miesel, 1995; Polla *et al.*, 1995; Haanen and Vermes, 1995; Dinchuk *et al.*, 1995). It is well recognized that ROS such as hydroxyl radicals, hydrogen peroxide and HOCl are involved in rheumatoid arthritis (Mieseland Zuber, 1993). In accordance with it, some antioxidant compounds (Kroger *et al.*, 1997; Chikanza *et al.*, 1998) have provided protective effects in some arthritis models, ascribed to its antioxidant properties. It is also well known that ROS are involved in a variety of important disease processes in medicine including: neurodegenerative, atherosclerosis, cancer and reperfusion injury, apart from well established effect of

inflammation (Kehrer, 1993).

ROS mediated lipid per-oxidation is an important mediator of cell membrane destruction as it is capable of modifying the structural integrity of cell membrane by transforming fatty acids into lipid peroxides (Niki, 1987). The anti-oxidative effect is best studied in vivo by inducing edema in experimental rat's paw with carrageenan (Boughton Smith *et al.*, 1993). These authors have provided evidence that carrageenan paw oedema can be induced by ROS. The local and systemic inflammatory response is associated with the production of ROS which includes superoxide anions, hydrogen peroxide and peroxynitrite (Cuzzocrea *et al*., 2001). In disease process linked directly to inflammation and oxidative stress, ROS induces the cell damage by combination of different mechanism: (i) initiation of lipid peroxidation, (ii) inactivation of a variety of enzymes and (iii) depletion of glutathione (Cuzzocrea *et al*., 1998).

Under normal circumstances, formation of $O_2^{\bullet-}$ (the one electron reduction product of oxygen) is kept under tight control by SOD enzymes. These checks are provided by Mn dependent SOD2 located in mitochondria or Cu/Zn dependent SOD1 located in cytosol or SOD3 in the extracellular surfaces. SOD2 is more important in cellular function in comparison with SOD1 and SOD3, which can be highlighted by the fact that its removal in mice can be lethal (Melov *et al*., 1999, Reaume *et al*., 1996, Carlsson *et al*., 1995). In acute and chronic inflammation, the production of $O_2^{\bullet-}$is increased at a rate that overwhelms the capacity of the endogenous SOD enzyme defense system to neutralize them. The imbalance will eventually result in $O_2^{\bullet-}$mediated cellular and organ damage.

Conclusion

Present study shows that Hamelia patens leaf extracts were very effective against carrageenan-induced rat paw edema owing to their anti-inflammatory and anti-oxidant activity. Based on this analysis and comparison with the standard drug, Hamelia patens leaf extract can be an alternative to standard anti-inflammatory therapy in the future. Similarly its antioxidant property to control the free radical induced tissue and organ damage, leaves a wider space for beneficial pharmacological probability, including anti-cancer activity.

References

1. Bagul. MS; H Srinivasa ; NS Kanaki ; M Rajani, 2005. Ind. J .Pharm.,37,399.
2. Baker H., Frank O., De Anelis B., Freingold S, 1980. "Plasma tocopherols in man at various tissues after ingesting free or acetylated tocopherol".Nutr. Exp. Int; 21: 531-536.
3. Boughton-Smith NK, Deakin AM, Follenfant RL, Whittle BJ, Garland LG, 1993. "Role of oxygen radicals and arachidonic acid metabolites in the reverse passive Arthus reaction and carrageenan paw oedema in the rat". Br J Pharmacology; 110: 896-902.
4. Carlsson LM, Jonsson J, Edlund T and Marklund SL, 1995. "Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia". Proc .Natl. Acad. Sci. USA.; 92: 6264–6268.
5. Cuzzocrea S, Caputi AP, Zingarelli B, 1998. "Peroxynitrite-mediated DNA strand breakage activates poly (ADP-ribose) synthetase and causes cellular energy depletion in carrageenan-induced pleurisy". Immunology; 93:96-101.
6. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D, 2001."Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury". Pharmacol Rev; 53:135-59.
7. Cuzzocrea S, Zingarelli B, Gilard E, Hake P, Salzman AL, Szabó C, 1998. "Anti-inflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenaninduced models of inflammation". Free Radic Biol Med. 24: 450–459.
8. Dinchuk JE, Car BD, Foci-It RJ, Johnston JJ, Jaffee BD, Covingthon MB, Contel NR, Eng VM, Collins RJ, Czerniak PM, Gorry SA Trzaskos JM, 1995."Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II". Nature; 23: 406-409.
9. Ellman G.C, 1959. "Tissue sulfhydryl groups". Arch Biochem Biophy; 82: 70 - 77.
10. Fraga C.G., Leibovity B.F., Topeel A.L., 1988. "Lipid peroxidation measured as TBARS in tissue slices-Characterization and comparison with homogenate and microsomes". Free Rad Biol Med; 4: 155 - 161.
11. Haanen C, Vermes I, 1995. "Apoptosis and inflammation, Mediators of Inflammation", Cancer Cell 4, 1, pp. 5-15.
12. Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology. 8th ed.; pgs.161–172.
13. Helen A M Ratheesh; 2007, "Anti-inflammatory activity of Ruta graveolens Linn on carrageenan induced paw edema in Wristar male rats" J.Biotech ; 6,1209 .
14. Jiang Z.Y., Hunt J.V., Wolff S.P, 1992. "Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low-density lipoprotein". Anal Biochem; 202: 384 - 387.
15. Kehrer JP. 1993. "Free radicals as mediators of tissue injury and disease". Crit. Rev Toxicol.; 23: 21-48.
16. Kroger H., 1995. "Anti-inflammatory effects of NADPH oxidase inhibitors. Inflammation. Mol. Cell 19: 347-362.
17. Mantovani. A; P Allavena; A Sica, 2008. Cancer-related inflammation". Nature, "454:436-444.
18. Melov S, Coskun P, Patel M, Tuinstra R, Cottrell B, Jun AS, Zastawny TH, Dizdaroglu M, Goodman SI, Huang TT, Miziorko H, Epstein CJ and Wallace DC, 1999. "Mitochondrial disease in superoxide dismutase 2 mutant mice". Proc Natl. Acad. Sci. USA; 96: 846–851.
19. Miesel R, Dietrich A, Ohde M, Altrichter S, Braun C, Ockenfels H., 1997. Suppression of type II collagen-induced arthritis by N-acetyl-L-cysteine in mice. Gen Pharmacol; 29:671-674.
20. Niki E., 1987 "Antioxidants in relation to lipid peroxidation". Review. Chem Phys Lipids; 44: 227-53.
21. Obodozie; EK Afolabi, 2009. "Evaluation of anti-inflammatory activity in ethanolic extract of Coriandrum sativum L. using carrageenan induced paw oedema in albino rats". J Phram Pharmacol;3(5):259-264.
22. Omaye S.T., Turbull T.P., Sauberlich H.C., 1979. "Selected methods for determination of ascorbic acid in cells, tissues and fluids". Methods Enzymol; 6: 3 - 11.
23. Oyanagui Y., 1984. "Reevaluation of assay methods and establishment of kit for superoxide dismutase activity". Anal Biochem; 42: 290-296.
24. Reaume AG, Elliott JT, Hoffman EK, Kowall NW, Ferrante RJ, Siwek DF, Wilcox HM, Flood DG, Beal MF, Brown RH Jr, Scott RW and Snider WD., 1996. "Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury". Nat Genet; 13: 43–47.
25. Winter CA, Porter CC., 1957. "Effect of alterations in side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters". J Am Pharm Assoc Am Pharm Assoc (Baltim); 46: 515-519.