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Anti Inflammatory Activity of Ferulic Acid against Carrageenan and Formalin Induced Paw Edema in Swiss Albino Mice

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Abstract: ***The anti-inflammatory activity of ferulic acid (FA) was evaluated using carrageenan and formalin-induced paw edema models in Swiss albino mice. The anti-inflammatory activity was found to be dose dependent in both paw edema model. Administration of the FA showed significant ($p<0.001$) inhibition of paw edema, 28% and 37.5% on 6th hour at the doses of 100 and 200 mg/kg body weight respectively in Carrageenan induced paw edema model. Similar pattern of paw edema inhibition was seen in formalin-induced paw edema model. The maximum percentage inhibition in paw edema was 31.4 % and 46.87 % on 6th day at the doses of 100 and 200 mg/kg, respectively. The results of present study demonstrate that FA possess significant anti-inflammatory potential.***

Keywords: anti-inflammatory activity, paw edema, carrageenan, formalin

1. Introduction

Inflammation is a reaction of living tissues towards injury and it comprises systemic and local responses (Joel *et al*.,1998). The generation of free radicals, particularly reactive oxygen species and their high activity plays an important role in the progression of a great number of pathological disturbances like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson's disease, Alzheimer's disease, etc (Nickavar *et al*., 2007; Hafeez *et al*., 2013). Many present day diseases are reported to be due to the shift in balance of pro-oxidant and antioxidant homeostasis in the body (Schulz *et al*., 2000). Reactive oxygen species (ROS), which include superoxide radical, hydrogen peroxide (H_2O_2) and the hydroxyl radical (-OH) are well documented as cytotoxic intermediates. These ROS differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission (Adelman *et al*.,1988; Mohanty*a et al.,* 2015), modification of polypeptides, lipid peroxidation etc leading to cell membrane disintegration, membrane protein damage and DNA mutation.

Inflammation activates a range of inflammatory cells that induce and trigger several oxidant generating enzymes such as NADPH oxidase, nitric oxide synthase, myeloperoxidase and eosinophil peroxidise etc. These enzymes generate high concentrations of different free radicals and oxidants such as superoxide anion, nitric oxide, nitroxyl, nitrogen dioxide, hydrogen peroxide etc, which react with each other to produce more potent reactive oxygen and nitrogen species that can damage DNA, RNA, lipids and proteins and also leads to multistage carcinogenesis (Ohshima *et al*., 2003). Therefore much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit peroxidation and to protect DNA and other macromolecules from damage due to free radicals (Wu *et al*., 2017).

In spite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of world populations cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant materials. A large number of aromatic, spicy, medicinal and other plants have chemical compounds, exhibiting antioxidant properties. Well known source of natural antioxidants are mainly, plant phenolics that may seen in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Naik and Krishnamurthy, 2018). These antioxidant compounds hold anti-inflammatory, antiatherosclerotic, antitumor, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Sala *et al*., 2002). Many anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently known to have an antioxidant and/or radical scavenging mechanism as part of their activity (Lin *et al*., 2000; Repetto and Lesuy, 2002). Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolic are increasingly of interest in the food industry, because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food (Sevgi *et al*., 2015). Hence interest in natural antioxidants, especially phytochemicals has greatly increased in recent years (Kumar *et al*., 2010).

Ferulic acid (FA) or 4-Hydroxy-3-Methoxycinnamic Acid, derivative of cinnamic acid with molecular formula $C_{10}H_{10}O_4$, a ubiquitous natural phenolic phyto chemical present in seeds, leaves, its free form and covalently conjugated to the plant cell wall polysaccharides, glycoprotein, polyamines, lignin and hydroxyl fatty acids (**Figure. 1**). As an antioxidant, FA play a major role in the body's defense against carcinogenesis by inhibiting the formation of N-nitroso compounds (Dai and Mumper, 2010). Moreover, FA is a strong scavenger of free radicals and it has been approved in certain countries as food

additive to prevent lipid peroxidation (Roginsky & Lissi, 2005; Balasubashini *et al*., 2004). The present paper reports the investigation on the anti-inflammatory properties of FA.

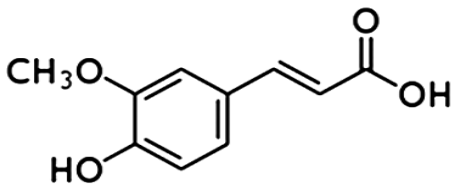

Figure 1: Ferulic acid (FA)

2. Materials and Methods

Animals

Female Swiss albino mice weighing 22- 25 g were obtained from the Small Animal Breeding Section (SABS), Mannuthy, Thrissur, Kerala. They were maintained under standard conditions of temperature and humidity in the Centre's Animal House Facility. The animals were given standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. All animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly adhering to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division of Government of India.

Studies on anti-inflammatory activity

Carrageenan induced acute paw edema

Animals were divided into 4 groups comprising of 5 animals in each group. For all groups acute inflammation was induced by sub plantar injection of 0.02 ml freshly prepared 1% of carrageenan in normal saline in the right hind paw of mice (Winter *et al*., 1962). The administration of FA and diclofenac and induction of paw edema is described below.

Group I- Distilled water (0.1ml) + 0.02 ml of 1% carrageenan injection

Group II- Ferulic acid 100 mg/kg (in 0.1 ml distilled water *p.o*) + 0.02 ml of 1% carrageenan injection

Group III - Ferulic acid 200 mg/kg (in 0.1 ml distilled water *p.o*) + 0.02 ml of 1% carrageenan injection

Group IV- Diclofenac 10 mg/kg body weight (in 0.1 ml distilled water *p.o*) + 0.02 ml of 1% carrageenan injection

Animals which received carrageenan injection alone were kept as control. Diclofenac (10 mg/kg body weight) served as standard reference drug. FA and diclofenac were administered one hour prior to the sub plantar injection of carrageenan. The paw thickness was measured using vernier callipers at one hour intervals for 6 hours following carrageenan injection.

Formalin induced chronic paw edema

The animals were treated in the same way as in the above models; except formalin (20 µl of freshly prepared 2% formalin) was used as the edematogenic agent instead of carrageenan. The drug treatment was continued for 6 consecutive days. Diclofenac (10 mg/kg body weight) was used as the reference drug.

In all the above models, the degree of edema formation was determined as increase in paw thickness. The increase in paw thickness and percent inhibition were calculated as follows.

Increase in paw thickness in control (P_C) or treatment (P_T) = P_t-P_0,

$$\text{Percent inhibition} = \frac{P_C - P_T \times 100}{Pc}$$

Where P_t indicates paw thickness at time t, P_0 is initial paw thickness, P_C represents increase in paw thickness of the control group and P_T is the increase in paw thickness of the treatment groups.

3. Results

Carrageenan induced paw edema

The sub plantar injection of carrageenan in Swiss albino mice produced a local inflammatory response. The paw edema found to reach the peak at 2nd hour and after that it was found to reduce. Administration of the ferulic acid produced 28 % inhibition in the paw thickness at a dose of 100 mg/kg body weight at 6th hour and 37.5% inhibition in paw thickness at a dose of 200 mg/kg at 6th hour ($p<0.001$) (**Figure .2**).

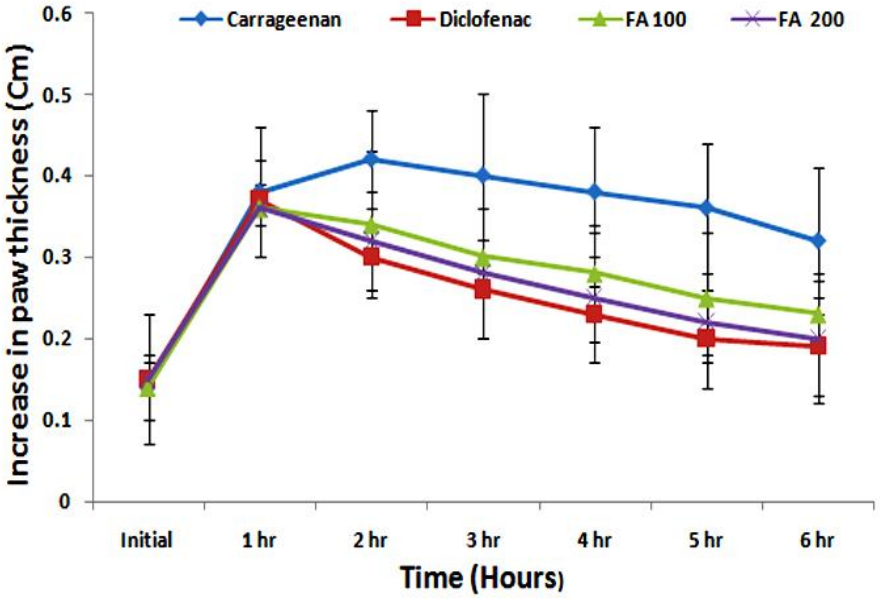

Figure 2: Effect of Ferulic acid (100mg/kg &200 mg/kg) on carrageenan induced paw edema in mice. All values expressed as Mean ± S.D, (n=5).

Formalin induced paw edema

In the formalin-induced paw edema test for chronic inflammation, the sub plantar injection of formalin in Swiss Albino mice produced a local inflammatory response which reached a maximum intensity of edema at the 3rd day. Administration of ferulic acid at doses of 100 mg/kg body weight showed 31.4 % inhibition of edema on 6th day, where as ferulic acid exhibited 46.87 % inhibition at a dose of 200 mg/kg on 6th day ($p<0.001$) (**Figure. 3**).

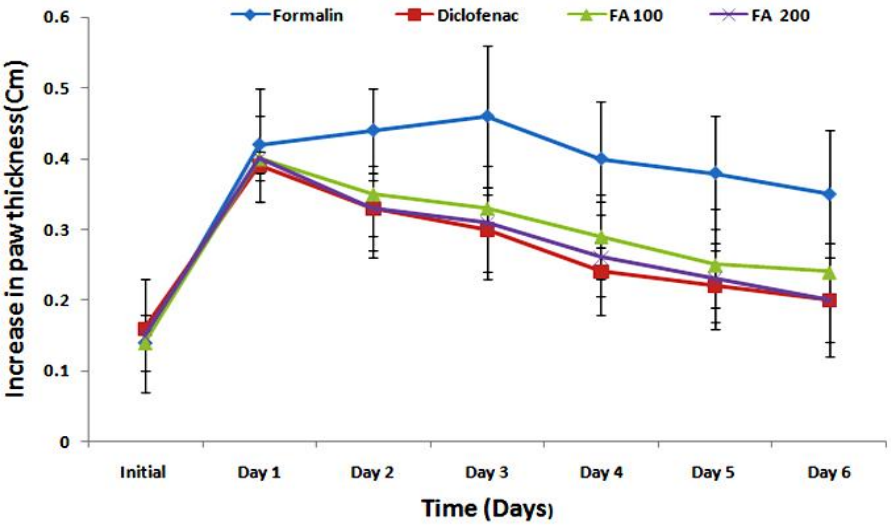

Figure 3: Effect of Ferulic acid (100mg/kg &200 mg/kg) on formalin induced paw edema in mice. All values expressed as Mean ± S.D, (n=5).

4. Discussion

Various studies suggest that the inflammatory tissue damages are due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (Cross *et al*., 1987; Winrow *et al*., 1993). In addition to this, nitric oxide is also implicated in inflammation, cancer, and other pathological conditions (Hemnani and Parihar, 1998). Interactions between superoxide and nitric oxide regulate the vascular tone or inflammation (Conner and Grisham, 1996).

Inflammation is a complex process and ROS plays an important role in the pathogenesis of inflammatory reactions (Halliwell and Gutteridge, 1985). As the inflammation is mainly produced by the oxidative burst of macrophages, antioxidants which can scavenge ROS may be effective to reduce inflammatory disorders. Inflammation, which is a pattern of response to injury, involves the accumulation of cells and exudates in irritated tissues that allows protection from further damage (Azab *et al*., 2016). Significant ameliorative activity against carrageenan and formalin induced anti-inflammation was shown by FA.

Carrageenan induced acute inflammation in animals is said to be the most suitable test procedures to monitor anti-inflammatory agents. The carrageenan induced edema is mediated by activation of platelet activating factor (PAF), prostaglandins and other inflammatory mediators (Hwang *et al*., 1986; Mansouri *et al*., 2015). Carrageenan-induced edema is a biphasic response in which the involvement of the cyclo-oxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established (Madhuri *et al*., 2016). The first phase is mediated through the release of histamine, serotonin, and kinins, whereas the second phase is related to the release of prostaglandin oxygen-derived free radicals and production of inducible cyclo-oxygenase which peak at 2-3 hours (Panthong *et al*., 2004). Carrageenan also induces a protein rich exudates containing large number of neutrophills (Lo *et al*., 1982; Naik and Krishnamurthy, 2018). The ferulic acid produced considerable inhibition of carrageenan-induced paw edema comparable in magnitude with the inhibitory action of the standard drug diclofenac.

Formalin induced paw edema is also one of the most suitable test procedure to screen chronic anti-inflammatory agents as it closely resembled human arthritis (Greenwald, 1991). The nociceptive effect of formalin is also biphasic, an early neurogenic component followed by tissue mediated response (Zhao *et al*., 2018). The ferulic acid showed significant anti-inflammatory activity against formalin induced paw edema and thus found to be effective in chronic inflammatory conditions. Ferulic acid released from wheat bran by a new strain of *A. niger* showed good anti-inflammatory activity and better antioxidant ability (Yin *et al*., 2019)

There is a strong relationship between antioxidants and inflammation (Ma and Huang, 2014). Chronic inflammation is accompanied by increased production of tissue reactive oxygen and nitrogen intermediates. Oxygen free radicals and non radical reactive oxygen intermediates released by neutrophils and other phagocytes have been increasingly implicated in inflammation/ immune disorders. Inflammation also facilitates the initiation of normal cells and their progression to malignancy through the production of inflammatory oxidants (Dhingra *et al*., 2018). Appropriate treatment of inflammation with anti-inflammatory agents, inhibitors, inhibitors of oxidant generating enzymes, and scavengers of oxidants should be explored to prevent development of human cancers associated with chronic inflammation (Ohshima *et al*, 2003).

In conclusion, the present study reveals the profound anti-inflammatory activity of ferulic acid and the effect showed by them might probably due to its significant antioxidant power.

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