The Haryana Veterinarian (September, 2023) **62(SI-2),** 18-21 ISSN 0033-4359 (P) **Research Article**

ANTI-INFLAMMATORY ACTIVITY OF ELLAGIC ACID IN CARRAGEENAN-INDUCED PAW EDEMA IN RATS

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ABSTRACT

The present study was planned to investigate the *in vivo* anti-inflammatory activity of ellagic acid following intramuscular administration @ 75 and 150 mg/kg in male albino rats by using carrageenan-induced paw edema model. Thirty rats were divided randomly into 5 groups and each group consists of six male albino rats. All the animals were treated with Lambda carrageenan solution (1%) prepared in 0.9% normal saline subcutaneously into sub plantar region of left hind paw as a local acute edema inducer after 30 min subsequent to intramuscular administration of ellagic acid. Rats of carrageenan control groups were kept untreated and vehicle control group treated with N-Methylpyrrolidine: Triethanolamine (9:1). Rats of standard control group were treated intramuscularly with indomethacin@ 5 mg/kg body weight as a reference standard drug. Rats of other two treatment groups were treated with ellagic acid @ 75 and 150 mg/kg body weight, respectively. Edema was expressed as the increase in paw volume in ml and measured up to the tibiotarsal articulation. Volume of edematous paw was measured at 0 h (before treatment), 1, 2, 3, 4 and 5 h after treatments. Increase in paw thickness was measured by using digital plethysmometer and percent inhibition was calculated. The anti-inflammatory effect of ellagic was highest at 4 h (58.02 %) at the dose of 150 mg/kg and (45.47 %) at the dose of 75 mg/kg. The anti-inflammatory effect of standard drug indomethacin (63.14 %) was higher than ellagic acid at 4 h. Ellagic acid @ 75 and 150 mg/kg post intramuscular administration gave higher anti-inflammatory effect at 4 h in rats. Ellagic acid showed dose dependent anti-inflammatory activity in male albino rats.

Keywords: Ellagic acid, Anti-inflammatory, Paw edema

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Pain management is most critical criteria in the management of veterinary patient. Inflammation is the reaction of vascularized living tissue to local injury and consists of many interdependent cellular and humoral events, which serves to destroy, dilute or isolate the injurious agent and repair the damaged tissues (Misraulia *et al.*, 2013). Numerous reports have provided confirmation that inflammation is involved in the pathogenesis of many diseases including aging, cancer, cardiovascular dysfunction and other life-threatening and debilitating disorders (Mansouri *et al.*, 2015). NSAIDs is one of the mostcommonly prescribed and used drugs for prevalence of degenerative and inflammatory disorders because of its three major activities like anti-inflammatory, anti-pyretic and analgesic activity. Long term usage and overdose of NSAIDs causes cardiovascular risks, blood clotting, renal problems and GI tract related problems including ulceration, obstruction and hemorrhage. These are intolerable side effects of NSAIDs. Thus, the discovery of new anti-inflammatory compounds is still on great demand by scientists in academia and industry.

Natural plant products and their secondary metabolites are also of great interest in the drug discovery process. Among the various medicinal plant compounds, polyphenols play an important role for the development of new therapeutic agents. Ellagic acid (EA) is a polyphenol compound found at high concentrations in a number of vegetables and fruits, such as persimmon, raspberries, blackberries, and strawberries, in addition to nuts (Derosa *et al.*, 2016). Ellagic acid has exhibited antioxidant, anticancer, antiallergic and anti-inflammatory activities (Favarin *et al.*, 2013). Very few studies have investigated the anti-inflammatory effects of ellagic acid. In veterinary clinical medicine intramuscular route is most important route of administration in domestic animal. No information is available about investigation on the anti-inflammatory effect of ellagic acid after intramuscular administration in rats. Based on the mentioned above facts, this study was aimed to assess the *in-vivo* anti-inflammatory effect of ellagic acid following intramuscular administration in carrageenan-induced paw edema in albino rats.

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MATERIALS AND METHODS

Experimental animals:

The study was conducted on male albino wistar rats weighing between 300 to 400 grams. The animals were obtained from Jai Research foundation, Vapi, Gujarat and maintained at the laboratory animal house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari. They were kept under constant observation for two weeks prior to commencement of the experiment. The animals were divided into groups and kept in cages. Standard ration and water was provided ad libitum. All necessary managemental procedures were

adopted to keep the animals free from stress. The experimental protocol and use of animals for conducting the present study was approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and chemicals:

Pure Ellagic acid, Indomethacin was obtained from Calbiochem, Lambda (λ) carrageenan and N-Methylpyrrolidine, Triethanolamine were obtained from Sigma-Aldrich and Normal Saline (NS) was purchased from Merck Specialities Private Limited, Mumbai.

***In vivo* anti-inflammatory:**

The carrageenan-induced rat paw edema model was used with minor modification as described (Suebsasana *et al.*, 2009). Ellagic acid (75 mg/kg and 150 mg/kg) was prepared in 1ml of N-Methylpyrrolidine: Triethanolamine (9:1) and Indomethacin (5 mg/kg) was prepared in DMSO. Experiment animals (n=30) were divided into 5 groups with 6 animals in each group. A mark on the left hind paw was made in each animal and initial volume was measured by immersing in the plethysmometer persplex tube. Group -I animals were kept as Carrageenan control, Group-II animals were given N-Methylpyrrolidine: Triethanolamine (9:1) (Vehicle Control), Group-III animals were treated with Indomethacin (5 mg/kg, IM), Group-IV animals were treated with Ellagic acid (75 mg/kg, IM) and Group-V animals were treated with Ellagic acid (150 mg/kg, IM). All the animals were treated with Lambda carrageenan solution (1%) prepared in 0.9% normal saline subcutaneously into sub plantar region of left hind paw. Half an hour before the carrageenan administration, vehicle, test drug and positive control drug were injected via intramuscular route in respective animal groups. Inflammation in the form of edema was measured in paw volume (ml) before carageenan administration and at 1, 2, 3, 4 and 5 h after carrageenan administration and expressed as percent edema formation in relation to initial paw volume before carrageenan injection for each animal. The paw volume data for test drug and positive control drug were analyzed and expressed as percent inhibition of edema formation in comparison to carrageenan control group.

Statistical analysis:

All the data have been presented as mean ± SE. Statistical comparison of the mean values in different groups was made using one-way analysis of variance (ANOVA), using software SPSS 20. Significant differences ($p<0.01$) between different experimental groups were determined by Duncan's test.

RESULTS AND DISCUSSION

In the present experiment of *in vivo* anti-inflammatory activity of ellagic acid @ 75 and 150 mg/kg body weight following intramuscular administration no any clinical signs of toxicity were observed in experimental rats. Carrageenan-induced paw edema model is widely used to assess the anti-inflammatory activity of several natural and synthetic compounds (Panthong *et al.*, 2007). The carageenan-induced rat paw edema is used as a distinct acute inflammatory model. Carrageenan dilates postcapillary venules that result in exudation of inflammatory fluid and cells. This process involves the release of several proinflammatory mediators. These events represent the early exudative inflammatory phase and its inhibition terminates the inflammatory process (Uwjejua *et al.*, 2002). Another feature of the inflammatory process is infiltration of polymorphonuclear cells into the tissue (Zabihi *et al.*, 2016).

The result of anti-inflammatory effect is presented as change in paw edema volume (ml) in Table 1 and percentage values of inhibition of inflammation by different treatments at different time intervals are presented in Table 2 and graphically depicted in Fig. 1. The paw edema volume (ml) in the carrageenan group was significantly ($p<0.01$) higher as compared to other treatment groups upto 5h of observation period. As compared to carrageenan group, the paw edema volume of vehicle treated group was non-significantly differed upto 5h of observation period. The paw edema volume in the test drug groups (EA 75 mg/kg and EA 150 mg/kg) were significantly ($p<0.01$) differed as compared to carrageenan and vehicle treated group. The paw edema volume in positive control (Indomethacin) group was non-significantly differed with ellagic acid doses @75mg/kg and @150 mg/kg groups at 3h. In this experiment the peak inflammation was observed at 3h in standard drug Indomethacin, ellagic acid at the dose rate of 75 mg/kg and 150 mg/kg treated groups and then it was subsided the edema volume of these three groups at 4h and to increase again at 5h.

Edema formation in the rat paw is a triphasic event with involvement of several inflammatory mediators. The initial phase (during the first 2h after carrageenan injection) is attributed to the release of chemical mediators such as histamine and serotonin. The intermediate phase (2-2.5 h) of edema is due to the release of kinin, protease, and lysosome. The last phase (2.5-6h) just begins after the intermediate phase and is subsequent to the emancipation of bradykinin and prostaglandins such as PGE2 in tissue (Suba *et al.*, 2005). Increase in paw volume as an index of inflammation reaches a peak of 4h following carrageenan injection and is modulated by some inhibitory molecules of the inflammatory cascade (Di Rosa *et al.*, 1971).

The percentage inhibition of paw edema in ellagic acid at doses of 75 and 150 mg/kg groups were 42.73% and 57.92%, respectively at 1h after the injection of carrageenan. It may be due to the inhibition of chemical mediators such

Table 1. Carrageenan-induced paw edema volume (ml) of treated group with Vehicle, Carrageenan, Indomethacin, Ellagic Acid (75 mg/kg) and Ellagic Acid (150 mg/kg) in albino rats (n=6)

Group	1 h	2 h	3 h	4 h	5 h
Carrageenan	$1.21\pm0.03_{A}^{b}$	$1.52\pm0.05_{B}^{c}$	$1.84\pm0.06_{C}^{b}$	$2.06\pm0.06_{D}^{c}$	$2.11\pm0.03_{D}^{c}$
Vehicle	$1.15\pm0.04_{A}^{b}$	$1.55\pm0.11_{B}^{c}$	$1.70\pm0.09_{C}^{b}$	$1.94\pm0.11_{D}^{c}$	$1.96\pm0.16_{D}^{c}$
Indomethacin	$0.50\pm0.09_{A}^{a}$	$0.88\pm0.09_{B}^{a}$	$1.22\pm0.04_{C}^{a}$	$0.71\pm0.10_{AB}^{a}$	$0.80\pm0.08_{B}^{a}$
Ellagic Acid (75 mg/kg)	$0.66\pm0.12_{A}^{a}$	$1.25\pm0.11_{BC}^{b}$	$1.42\pm0.16_{C}^{a}$	$1.04\pm0.08_{B}^{b}$	$1.12\pm0.11_{BC}^{b}$
Ellagic Acid (150 mg/kg)	$0.49\pm0.10_{A}^{a}$	$1.08\pm0.05_{BC}^{ab}$	$1.18\pm0.07_{C}^{a}$	$0.78\pm0.15_{AB}^{ab}$	$1.00\pm0.10_{BC}^{ab}$

a-cMeans bearing different superscripts within a column (between treatment groups) differ significantly ($p<0.01$).
A-DMeans bearing different subscripts between a column (between time interval) differ significantly ($p<0.01$).

Table 2. Percent inhibition of inflammation after Indomethacin, Ellagic Acid (75 mg/kg) and Ellagic Acid (150 mg/kg) in albino rats (n=6)

Group	Percent inhibition of inflammation					Average percentage of Inhibition
Group	1 h	2 h	3 h	4 h	5 h	
Indomethacin	55.14	41.61	26.78	63.14	57.07	48.75
EA (75 mg/kg)	42.73	17.27	15.35	45.47	39.33	32.03
EA (150 mg/kg)	57.92	29.51	29.62	58.02	45.35	47.70

Values are % of inhibition over Vehicle control group

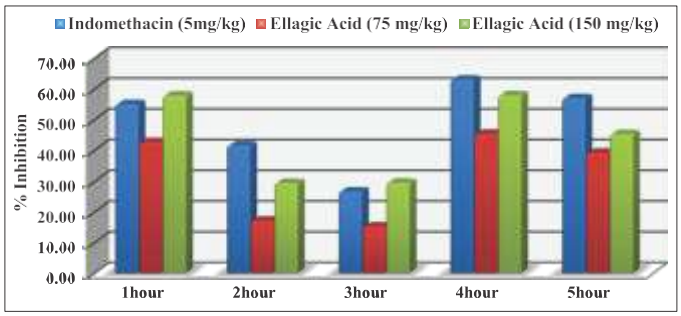

Fig. 1. Percent inhibition of inflammation in rats under different treatments

as histamine and serotonin at initial phase and they may contribute in its anti-inflammatory effects. Some studies have shown that inhibition of H1 and H2 receptors and histamine release, serotonin and muscarinic receptors can suppress the inflammation (Zabihi *et al.*, 2016). The percentage inhibition of paw edema by ellagic acid at the dose of 75 mg/kg was declined of 17.27% at 2 h and 15.35% at 3h post-carrageenan injection, whereas, at the dose of 150 mg/kg, percentage inhibition of paw edema was 29.51% at 2h and 29.62% at 3h post-carrageenan injection. The maximum percentage inhibition of paw edema was 45.47% and 58.02% at doses of 75 and 150 mg/kg, respectively after 4h of carrageenan treatment, whereas standard drug indomethacin was produced 63.14% after 4h of carrageenan inject. At 5h of observation period the percent inhibition of paw edema was 39.33% and 45.35% at doses of 75 and 150 mg/kg, respectively, whereas standard drug indomethacin was produced 57.07% after 5h of carrageenan inject. It may be due to blocking of the release of prostaglandin during late phase of inflammation.

Our hypothesis is that, fluctuating percentage of edema inhibition from the 1h to 5h with ellagic acid following intramuscular administration might be hampered by its limited bioavailability of drug and which could be depend on binding of ellagic acid to protein and metabolized products. Some studies reported that free ellagic acid either be absorbed or further metabolized by the gut microbiota to produce urolithins. These metabolited are bioavailable and have been exerting anti-inflammatory and cancer chemo-preventive effects (Sarrias *et al.*, 2015). Moreover, It may be due to effect of carrageenan begin to decrease. The increase in paw edema by carrageenan lasted only 5h to 6h and gradually decreased within 24h after injection (Di Rosa *et al.*,1971).

The high average percent of inhibition in ellagic acid at dose rate of 150 mg/kg group (47.70%) as compared to dose rate of ellagic acid @75 mg/kg group (32.03%), whereas ellagic acid @150 mg/kg, IM gave alike anti-inflammatory activity as Indomethacin (48.75%). The results of the present study opined that following intramuscular administration of ellagic acid at doses of 75 mg /kg and 150 mg/kg had exerted a dose-dependent inhibitory activity in carrageenan-induced paw inflammation in rat.

In support to our findings, similar observations were reported for the anti-inflammatory activity of *Punica granatum* (pomegranate) ethanolic whole fruit extracts and synthetic ellagic acid at 0.1 ml/10 g body weight in carrageenan induced paw edema in mice revealed 49.4% and 77.3% inhibition of inflammation, respectively (Bhandary *et al.*, 2014). Gupta *et al.* (2021) reported that *in vitro* antiinflammatory activity of ethanol and aqueous extracts of *Terminalia bellirica* plant and ellagic acid by inhibition of heat-induced albumin denaturation. Results showed that the both the extracts and EA exhibited

concentration dependent anti-inflammatory activity. Maximum inhibition of albumin denaturation by EA (86.62±0.65%) was observed at the concentration of 16.7 µg/ml while AQ and Eth extract produced 67.57% and 77.67% inhibition, respectively at the concentration of 50µg /ml. Favarin *et al.* (2013) investigated anti-Inflammatory effects of ellagic acid on acute lung injury (ALI) induced by acid in mice. Results indicated that the ellagic acid displayed anti-inflammatory properties for the resolution of ALI inflammation by decreasing the severity of HCl acid-initiated acute lung injury by reduced several inflammatory parameters like vascular permeability alterations and neutrophil recruitment to the BALF and the lung, reduced the proinflammatory cytokine IL-6 and increased the anti-inflammatory cytokine IL-10 in the BALF without down regulating the NF-, B and AP-1 signaling pathways. BenSaad *et al.* (2017) studied the Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A & B isolated from the ethyl acetate (EtOAc) fraction of *Punica granatum* and reported that isolated compounds from *P. granatum* were able to significantly inhibit the inflammatory mediators NO, PGE2, and IL-6 in LPS-induced RAW 267.4 macrophages.

Based on these reports, it can be inferred that the inhibitory effect of the ellagic acid on carrageenan induced inflammation in rat may be due to inhibition of the mediators responsible for inflammation. The present study revealed that intramuscular administration of ellagic acid showed dose-dependent *in vivo* anti-inflammatory activity against carrageenan-induced rat paw edema. The highest anti-inflammatory activity of all two doses of ellagic acid was observed at 4h post intramuscular administration in wistar rat. Thus, it is one of the best bioactive compounds have shown *in vivo* anti-inflammatory activity. However, Preliminary pharmacokinetic study of ellagic acid following intramuscular administration in rat is required because this study paves the way for further attention and research to identify the active metabolite products of ellagic acid responsible for anti-inflammatory activity. Additional analysis of cytokines and histo-pathological examination of rat paw tissue would be performed because it might be helpful to find out the actual molecular mechanism underlying inhibition of the first, intermediate and second phase edema is of interest and helpful in developing the new anti-inflammatory herbal compound.

CONCLUSION

The present results showed that Ellagic acid is one of the best bioactive compounds that have shown *in vivo* anti-inflammatory activity. However, Preliminary pharmacokinetic study of ellagic acid following intramuscular administration in rats is required because this study paves the way for further attention and research to identify the active metabolite products of ellagic acid responsible for anti-inflammatory activity. Additional analysis of cytokines and histo-pathological examination of rat paw tissue would be performed because it might be helpful to find out the actual molecular mechanism underlying inhibition of the first, intermediate and second phase edema is of interest and helpful in developing the new anti-inflammatory herbal compound.

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