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ANTI-INFLAMMATORY ACTIVITY OF ALCOHOLIC EXTRACT OF *Elaeagnus conferta* Roxb. SEEDS

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ABSTRACT

The aim of the present investigation was to evaluate the anti-inflammatory activity of the methanolic extract of *Elaeagnus conferta* Roxb. (MEC) in male Wistar rats. The efficacy of MEC at a dose of 400 and 800 mg/kg was evaluated in animals treated with 1% carrageenan solution against indomethacin (50 mg/kg). Paw thickness, paw volume, and body weight were measured before the start of the study i.e. at t=0 and thereafter at t=3, 6, and 24h. The level of inflammatory mediators (IL-6, TNF-α) were accessed at t= 24h. MEC exhibited a statistically significant decrease in paw thickness (22.71%) and paw volume (63.89%) and an increase in body weight (100.7%) in a dose-dependent manner ($p< 0.001$). Pre-treatment with MEC is effective in reducing inflammation by suppressing levels of inflammatory cytokines such as TNF-α and IL-6 and results were found to be extremely significant compared with carrageenan-controlled animals. Furthermore, results suggested that *E. conferta* Roxb. significantly decreased inflammation, which may be attributed primarily to the presence of quercetin and ascorbic acid. Therefore, it can be explored for the management of inflammation-mediated disorders.

Keywords: Carrageenan; *Elaeagnus conferta* Roxb.; Inflammation; IL-6; TNF-α

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INTRODUCTION

Inflammation, as a process of the immune system, is characterized by its ability to protect the host from harmful pathogens, radiations, foreign stimuli, and toxic compounds. Inflammasome, an immune cell sensor macromolecule recognizes various noxious signals leading to activation of inflammatory caspase-1 and secretion of inflammatory cytokines.[1-4] Inflammation, therefore, has become a global concern as it is considered one of the most significant indicators of any disease, infection, or damage of the tissue. A large number of synthetic drugs are available for treating inflammation but may possess undesirable side effects. In this regard, herbs can serve as lead molecules as these have been practiced for treating various diseases with no or minimal side effects.[5] In addition, these herbal bioactives are cost-effective, abundantly available in nature, and are also recommended as dietary supplements.[6,7] *Elaeagnus conferta* Roxb. of family *Elaeaganaceae* is a scandent shrub and has been well explored as a folk medicine by the local community for its efficacy against diabetes, inflammation, ulcer, pain, cancer, muscle spasm, oxidative stress, and pulmonary disorders.[8,9] Although several studies have indicated the nutritional importance of *E. conferta* Roxb. seeds, however, its anti-inflammatory effect against carrageenan-induced inflammation has not been investigated to date. Hence, the current experiment was performed to investigate the anti-inflammatory effect of seeds of *E. conferta* Roxb. and also to elucidate the possible mechanism of exhibiting anti-inflammatory potential.

EXPERIMENTAL

Sample Collection and Authentication

The *E. conferta* Roxb. seeds were collected after their ripening from the altitude region of Manali, Himachal Pradesh (India) and authentication was performed by a senior scientist at Govind Ballabh Pant's National

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Institute of Himalayan Environment, Himachal Pradesh (India) with registration number (GBPNIHESD/SIC/358).

Drugs and Chemicals

Indomethacin was received *ex gratis* from Kwality Pharmaceuticals Ltd., Amritsar, India. The other chemicals including carboxymethylcellulose (CMC), methanol, *n*-hexane, and carrageenan were procured from Loba Chemie Private Limited, India. The rat Elisa kits (IL-6 and TNF-α) were procured from Ray Biotech, USA.

Instruments

Digital vernier caliper (Swastik Scientific Instrumentation Pvt. Ltd., Mumbai, India), ELISA reader (Micro lab instruments), homogenizer (Noble Procetech Engineers, India), plethysmometer (VJDP-01) (VJ Instruments, Maharashtra, India), and rota evaporator (RV8, IKA, (RV-8, IKA India Pvt. Ltd, Karnataka, India) were used in the study.

Animals

Wistar albino rats of either sex (200-300 g) were housed in standard cages under laboratory conditions of 25 ± 2^0C, relative humidity of 70-80% with normal light and dark cycle. The animals were allowed to access water and libitum for a half-day-night cycle and were given free access to food and water. All the animals were grouped as per the protocol approved by the Institutional Animal Ethical Committee of LPU, Punjab, India, and were registered with code LPU/IAEC/2018/40. Further, the animals were handled in accordance with directions documented by CPSCEA.

Solvent Extraction and Extract Preparation

The *E. conferta* Roxb. seeds were dried and powdered material was macerated with *n*-hexane for 7h for defatting and then filtered using Whatman No. 1 filter paper. The pressed marc was dried, and the residual plant material was extracted thrice successively by cold maceration using methanol for 48h. The methanolic extract was air dried to obtain a methanolic extract of *E. conferta* Roxb. (MEC) and was kept in freeze until use.[10]

Carrageenan-Induced Paw Edema Model

Establishment of Animal Experimental Design

To estimate, the anti-inflammatory effect, a group sample size of six animals was randomly selected and was divided into five groups. The food was withdrawn from all animals 24 hours before the start of the experiment; however, animals were allowed for free intake of sterilized water.[11] Group I was used as normal control. Grouping according to different treatments is given in Table-1.

Table-1: Induction of Inflammation and Treatment Schedule

Groups	Treatment	Doses
I	Healthy control	1% solution of CMC, *per oral*
II	Carrageenan control	Carrageenan 0.1 ml, 1% CMC, *subcutaneously*
III	Standard control (Indomethacin, IND)	IND in 1% CMC (10 mg/kg), *per oral*
IV	MEC (400)	MEC in 1% CMC (400 mg/kg), *per oral*
V	MEC (800)	MEC in 1% CMC (800 mg/kg), *per oral*

Edema Measurement

Measurement of Paw Volume and Paw Thickness

Paw edema was calculated by the paw volume and thickness using an electronic water plethysmometer and digital vernier caliper respectively before injecting carrageenan, followed by measurements after 3h, 6h, and 24h.[12]

Body Weight Recording

The body weight of the animals of all groups was recorded before administration of the phlogistic agent (carrageenan) and/or treatment, thereafter at intervals of 3h, 6h, and 24h.

Estimation of Inflammation Mediators

The inflammatory mediators i.e. tumor necrosis factor (TNF)-α and interleukin (IL) -6 expressions were estimated using ELISA kits and the manufacturer's protocol was followed for estimating the level of inflammation mediators.

Statistical Analysis

The data of treated and untreated animals was evaluated by one-way ANOVA, thereafter following Bonferroni posttests through GraphPad software (Version 6.02, San Diego, CA), and was expressed in terms of standard error of the mean. The difference was considered significant if $p < 0.05$, more significant with $p < 0.01$, and highly significant having $p < 0.001$.

RESULTS AND DISCUSSION

Effect of MEC on Carrageenan-Induced Inflammation

Measurement of Paw Volume and Thickness

To estimate, insight into the efficacy of treatments in inflammation, two parameters i.e. paw volume and paw thickness have great importance. In this study, the rats were administered two different doses to induce inflammation *viz.* MEC (400) (group IV), MEC (800) (group V). After 24h, a significant difference in % increase in paw thickness and volume was observed in carrageenan control rats than control group rats ($p< 0.001$). Therefore, it indicated the induction and progression of disease. Fig.-1 reveals that MEC lowered paw volume significantly ($p< 0.001$) (63.89% in group V and 100.78% in group IV) after 24h as compared to 3h (116.61% and 174.59% in both IV and V groups respectively), though in carrageenan control animals; the decrease was noted only from 156.00% to 149.98% within same time. In the case of indomethacin-treated animals, a significant decrease (31.19%) was seen compared to diseased animals ($p< 0.001$) which may be due to the anti-inflammatory ability of this drug as it belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs). Also, MEC reduced paw thickness considerably compared to carrageenan control as observed after 24h. However, in group II, a marginal change in % increase in thickness was observed at 3h (89.21%) to 24h (98.33%) (Fig.-1).

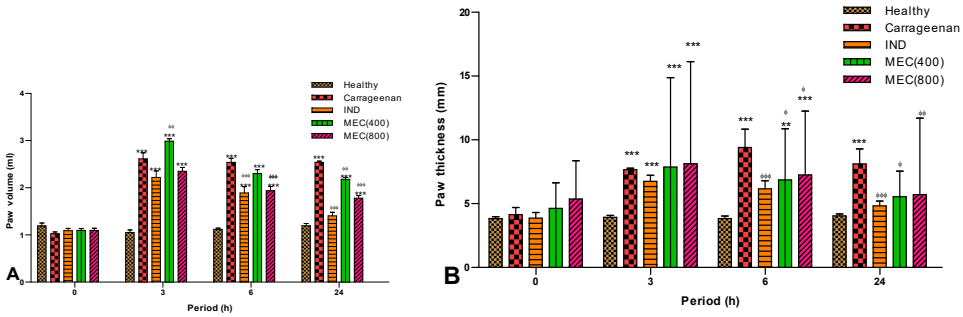

Fig.-1: Effect of Pre-Treatment with MEC on Paw Volume and Paw Thickness. The Data was Represented with Mean ± SEM having n=6. The Significance Relevance is Shown as: *= $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ than Carrageenan-Treated Animals (ANOVA(Two-way) followed by Bonferroni Posttests)

Body Weight Recording

The change in body weight in different study groups with respect to time is depicted in Fig.-2. The rats of group I, showed a slow increase in body weight (%), and a significant decrease ($p< 0.001$) in body weight of diseased group animals (group II) was observed with the progress of time till 24 h. In treatment group animals (III, IV, and V), there was an abrupt increase in body weight (%) at times 3h and 6h, however, the change seemed to be quite significant compared to healthy group animals (Group I) ($p< 0.001$). This obvious change in body weight can be attributed to the beneficial effects of indomethacin and MEC in dose dose-dependent manner. However, a minor variation in the body weight of animals (non-significant) in terms % change of the body weight in animals of group IV and V at 6h and 24h compared to that of the healthy control rats indicated the profound efficiency of MEC in attenuating the inflammatory condition which is also in concordance with the results reported in paw thickness and paw volume.

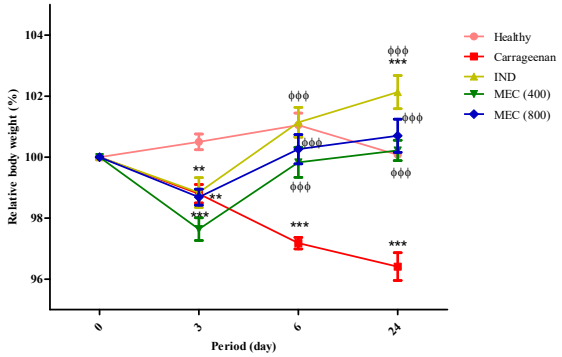

Fig.-2: Effect of Pre-Treatment with MEC on Body Weight. The Data was Represented with Mean ± SEM having n=6. The Significance Relevance is shown as: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ than Carrageenan-Treated Animals (Two-way ANOVA followed by Bonferroni Posttests

Inflammatory Mediators

The influence of MEC on serum level of inflammatory mediators can be clearly seen as pre-treatment with MEC exhibited a dose-dependent anti-inflammatory effect *via* reducing inflammation by suppressing expression of TNF-α and IL-6 and were found to be extremely significant compared with carrageenan-controlled animals (Fig.-3). The genus Elaeagnus contains many species, that have been reported to exhibit diverse therapeutic benefits against various pathological conditions such as asthma, ulcers, bacterial infection, pain, oxidative stress, cancer, and ulcers.[13,14] The important regulators of inflammation include different cytokines such as IL-1β, IL-6, TNF-α, and PGE2.[15] The anti-inflammatory effect of MEC is mediated through oxidative stress regulation and could be related to the presence of different bioactive phytoconstituents including ascorbic acid and quercetin that behave as reducing agent by trapping nascent oxygen. In this experimental design, MEC exhibited comparable anti-inflammatory activity in terms of a significant lowering in paw thickness, paw volume, and an increase in body weight of groups IV and V in a dose-related manner. The study indicated that treatment with MEC had comparable results to that of standard drug indomethacin. The enhanced expression of inflammatory cytokines such as TNF-α and leptin resulted in a decrease in body weight as it has been reported that TNF-α is involved in energy utilization through promoting lipid and protein catabolism and by lowering the need for energy intake *via* its anorectic effects.[16] Previously, some researchers also have reported *in vitro* anti-inflammatory activity of leaves of *E. conferta* Roxb. Compared with the control group, serum cytokine levels of IL-6 and TNF-α were significantly increased in the diseased control group ($p< 0.001$), however, it down-regulated in groups pre-treated with MEC ($p< 0.001$). The study indicates that MEC could attenuate inflammatory cytokines i.e. IL-6 and TNF-α, and thus could be proposed as an alternative strategy to treat inflammation-mediated conditions.

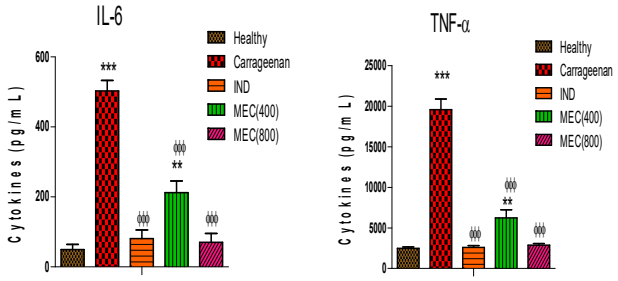

Fig.-3: Effect of Pre-Treatment with MEC on the Levels of Inflammatory Mediators in Rats. The Data was Represented with Mean ± SEM having n=6. The Significance Relevance is shown as: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ than Carrageenan-Treated Animals (ANOVA(One-way) Followed by Tukey's Post Hoc Analysis)

CONCLUSION

During the first phase of inflammation, a large number of cytokines are released and have a profound influence on the pathogenesis of many inflammatory disorders. The anti-inflammatory activity of *E. conferta* Roxb. was clearly demonstrated in carrageenan-induced inflammation which may be attributed to the down-regulation of TNF-α and IL-6. The reported anti-inflammatory effect of MEC can be due to the presence of key components of plant extract i.e. quercetin and ascorbic acid which are responsible for scavenging free radicals and also inducing anti-inflammatory activity thus offers a useful approach for the management of inflammation-mediated disorders. The above findings can be further explored for future development of plant-based, safer, and effective anti-inflammatory therapy.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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