ORIGINAL ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF TARAXEROL ACETATE

Ubaid ur Rahman[1], Sobia Ali[1], Ubaidullah[1], Inamullah Khan[2], Mudassir Ahmad Khan[1], Saatea Arif[1], Saif ur Rahman Wazir[1]

[1]Department of Biochemistry, Khyber Medical College, Peshawar - Pakistan

[2]Department of Pharmacy, University of Peshawar - Pakistan

ABSTRACT

Objectives: To investigate the anti- inflammatory effect of Taraxerol acetate, a pentacyclic triterpene isolated from the aerial parts of Artemisia roxburghiana.

Material and Methods: The plant materials were shade dried and ground into powder which was extracted 3 times by soaking with 24 L of methanol for two weeks. Preliminary fractionation of the crude extract, obtained from combine extract, was done by using solvents of increasing polarity: hexane, chloroform, ethyl acetate and n-butanol. The ethyl acetate fraction was subjected to silica gel column chromatography with hexane to yield taraxerol acetate. The structure of isolated compound was confirmed by comparing their corresponding NMR and mass spectral data with those previously reported. Carregeneen induced paw edema model was used to carry out the anti- inflammatory activity of Taraxerol acetate in Wistar adult rats.

Results: TA significantly ($P < 0.05$) reduced the edema induced by carrageenan at a dose of 60 mg/Kg body weight when measured both at 3h and 5h.

Conclusion: Taraxerol acetate has a significant anti-inflammatory effect in addition to its gastroprotective effect making it a safer anti-inflammatory agent.

Key Words: Taraxerol acetate, Artemisia roxburghiana, Anti-inflammatory, paw edema model.

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INTRODUCTION

Inflammation is the response of the host to any injurious agent which involves a series of changes like vasodilatation, increased permeability and loss of fluid into the extra-vascular tissue at the site of injury. When the inflammation occurs in or just under the skin, it is characterized by swelling, redness, tenderness, and pain. Elsewhere, it is a key component of asthma, ulcerative colitis, and many other diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the whole world for the treatment of inflammation. Although the efficacy of NSAIDs is well documented, but their side effects like erosions of the mucosa of stomach and first part of duodenum can lead to ulcers and then perforation and hemorrhage, which are life threatening situations[1,2]. NSAIDs also cause damage to the kidney leading to secondary hypertension and various other cardiovascular complications[3,4]. In the last few decades a lot of researchers concentrated on medicinal plants having anti-inflammatory effects. Pentacyclic triterpene is a major sub-class of terpenoids which are known for their anti-inflammatory activitiess. The objective of the present study was to explore the anti-inflammatory activity of Taraxerol acetate (TA), a pentacyclic triterpene. Chemical structures of Taraxerol acetate is shown in Figure 1.

Dr. Ubaid ur Rahman (Corresponding Author)
Associate Professor
Department of Biochemistry,
Khyber Medical College, Peshawar - Pakistan
Cell: 0322-9950198
E-mail: ubaidnbio@gmail.com

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MATERIAL AND METHODS

The compound Taraxerol acetate was isolated from the aerial parts of Artemisia roxburghiana in Hazara University campus Mansehra, Pakistan. The powder form of this compound was donated to us by Mr. Ishtiaq Department of Chemistry, Hazara University, Mansehra, Pakistan.

Animals

For this study Wistar adult rats were used. They were of either sex, weighing 180±10g. They were kept

10 per cage with 12h light-dark cycle under the standard laboratory conditions and fed with water and rat feed. All the established ethical principles for the laboratory animals were observed. All the rats, before the commencement of experiments were acclimatized to the laboratory environment for 7 days. The anti-inflammatory activity was tested by a model called carrageenan-induced paw edema test in the rat[5].

Carrageenan induced paw edema model for anti-inflammatory activity

This model was employed to investigate the anti-inflammatory effects of TA. Carrageenan was used to induce inflammatory edema in the animals[6]. Rats were briefly anaesthetized with ether for this experiment. Initially normal paw volume of each rat was noted by plethysmometer. Paw volume was measured up to tibiotarsal articulation. The rats were divided into four groups, each had 5 rats. To the first group known as negative control, saline was given. The treated groups were three in number. To the group I, TA was given in a dose of 30 mg/Kg body weight; to the group II, TA was administered in a dose of 60 mg/Kg body weight and to the group III, Indomethacin in a dose of 5 mg/Kg body weight was administered (positive control). After one hour acute inflammation was induced in the right hind paw of each rat in all groups by subplantar injection of 1% suspension of carrageenan (0.1 ml) using 2% gum acacia as a suspending agent in normal saline. Paw volume was again measured 3 hours after the first injection of Carrageenan. The difference between final paw volume and initial paw volume (Vt – Vc) was taken as edema value. The values were expressed as mean ± SEM. The following formula was used to calculate the percent inhibition. % edema inhibition = [1- (Vt / Vc)] X 100 Where, Vt and Vc are edema volume in the drug treated and control groups, respectively[7].

RESULTS

The in-vivo anti-inflammatory investigation indicated that TA at a dose of 60 mg/Kg body weight had significantly ($P < 0.05$) decreased the edema induced by Carrageenan, when measured both at 3h and 5h Figure 2. The activity of indomethacin, the standard compound used in this experiment, was comparatively better than the test compound, TA. In a dose of 30 mg/kg body weight TA had no significant anti-inflammatory activity.

DISCUSSION

Carrageenan induced paw edema is an important in vivo investigational model to induce acute inflammation. It is extensively used to investigate the anti-inflammatory (anti edematous) effects of the newly

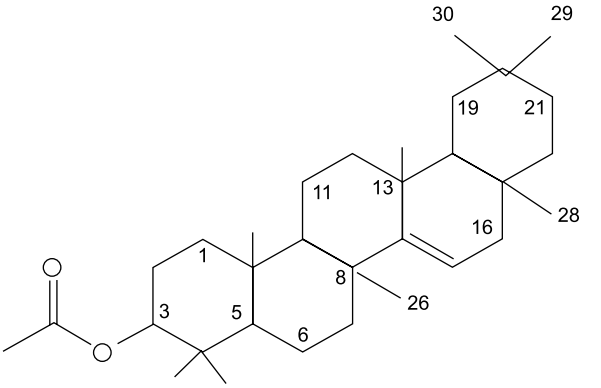

Fig 1: Chemical structure of Taraxerol acetate

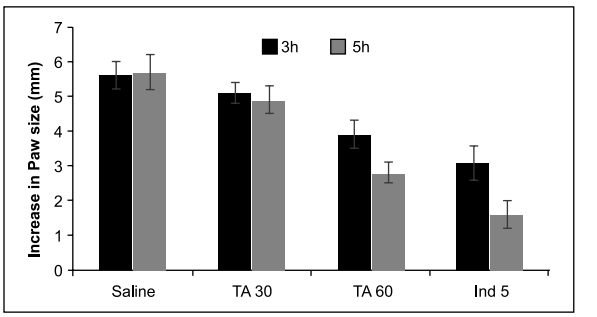

Fig 2: Effect of TA on carrageen an induced edema

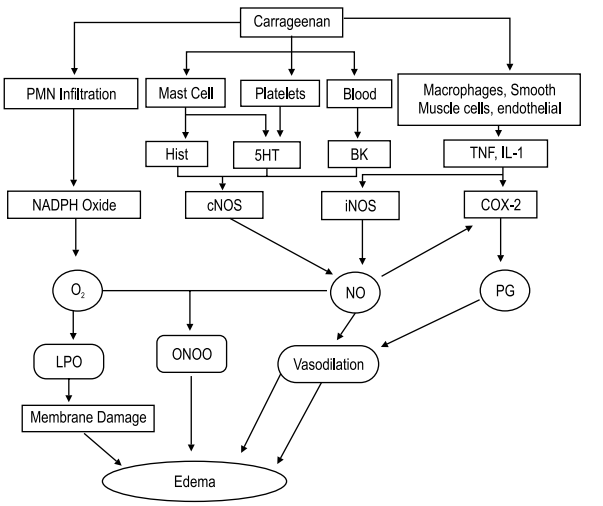

Fig 3: Mechanism of Edema Formation by Carrageenan

discovered compounds[8,9]. The injection of carrageenan induces inflammatory edema in the right hind paw in two phases. Various mediators are involved in sequence to produce this response. The initial or early phase of edema (0-1h) is caused by the release of serotonin, histamine and bradykinin[10]. In the second (later) phase (1-6h), the swelling increases and reaches to the highest volume of the hind limb and is reported to be due to the increased production of PGs[11]. Some other inflammatory mediators which are acting slowly are also involved, which are cytokines, proteases and lysosomes[8]. There is also induction of COX-2 in the later phase[12]. Infiltra-

tion of neutrophils and their activation in the local area of edema is also attributed to the 2nd phase[13], which produce oxygen derived free radicals e.g., superoxide anion ($\bar{O}2$) and hydroxyl radical (OH^{-}). Nitric oxide (NO) has been found to be another potent mediator in acute and chronic inflammation. It is produced by the oxidation of the terminal guanidino nitrogen atom of L-arginine with the help of an enzyme called nitric oxide synthase (NOS). There are three main isozymes of NOS: two of them are expressed constitutively and are called constitutive NOS isozyme (cNOS) and they are calcium/calmodulin dependent; while the 3rd one is inducible (iNOS), induced by cytokines and is independent of calcium/calmodulin[14]. NO is a potent vasodilator. It increases the vascular permeability and leads to edema by causing changes in the local blood flow. It also increases the synthesis of PG in in-vitro[15], ex-vivo[16] and in-vivo studies[17]. The NO is also involved in lipid peroxidation and cellular damage; the mechanism involved is the formation of peroxynitrate ($ONOO^{-}$) from a reaction between NO and superoxide anion. $ONOO^{-}$ being a potent oxidizing substance is capable to cause the lipid peroxidation and consequently damage the cells[18].

The interaction between NO, PG and O_2 are poorly defined and are summarized as follows: in the first hour of carrageenan injection histamine, serotonin and bradykinin are released. These agents are vasodilators and induce edema in the right hind paw. These mediators have receptors on the vascular endothelial cells and after binding with them, they activate cNOS to produce NO which is proposed to be the final and common mediator to induce the early phase of carrageenan induced inflammatory response.

As the time passes, the cytokines are produced by the macrophages, endothelial cells and smooth muscle cells. These cytokines are IL-1 and IL-2 and as well as TNF and IFN-y[19] which are responsible for the induction of iNOS to produce still larger quantities of NO to maintain the edema of later phase (sustained phase) of carrageenan induced edema. Within this time the COX-2 is also induced locally and both these pathways NOS and COX appear to amplify the inflammatory process. Mechanism of Edema Formation by Carrageenan is shown in Figure 3.

The first phase of carrageenan induced edema is prevented by antihistaminic drugs and serotonin antagonists; while second phase by the NSAID drugs inhibiting the COX to prevent PG formation. NOS inhibitors[20] cause dual inhibition of NO and PG which account for their potent and marked anti-inflammatory effects. Since TA has significantly reduced the edema caused by carrageenan injection, therefore it is believed that TA is involved in the COX inhibition (as in its analgesic activity and in the antioxidant activity to prevent formation of $\bar{O}2$) and may have a role in NOS inhibition. Further studies are suggested to investigate the actual mechanism of TA involved in the reduction of edema produced by TA.

CONCLUSION

Taraxerol acetate (TA) has anti-inflammatory activity in addition to other various important physiological effects. The gastroprotective effects and antiulcer activities of TA are quite encouraging because most commonly prescribed Non Steroid Anti-Inflammatory drugs (NSAIDs) produces gastrointestinal side effects. Therefore the consumption of TA has extra benefits of its soothing effects on the gastric mucosa.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Rahman UR: Idea.
Ali S: Data collection.
Ubaidullah: Follow up.
Khan I: Data collection.
Khan MA: Overall supervision
Arif S: Critical review and final approval.
Rahman SU: Statistics.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.