

Original Research Article

INVITRO ANTI INFLAMMATORY ACTIVITY OF ABIES WEBBIANA USING ALBUMIN DENATURATION ASSAY

Running Title: Invitro anti inflammatory activity of Abies webbiana using albumin denaturation assay

ABSTRACT

Background :

In ayurveda, many natural plant components are used to treat inflammatory conditions for centuries with less side effects. Different parts of Abies Webbiana plant are used in ayurveda for many diseases. However, very few studies have been conducted to evaluate pharmacological effects of Abies Webbiana.

Aim:

The objective of this in vitro study was to analyze and compare the anti-inflammatory properties of A. webbiana at various concentrations using albumin denaturation assay.

Methodology:

A ready made powder of A.webbiana was mixed with 100 ml of distilled water and boiled for 10 mins the extract was obtained by filtration. The anti-inflammatory activity was done using albumin denaturation assay and Diclofenac sodium was used as a positive control. Serial dilution from 10 μ l, 20 μ l, 30 μ l, 40 μ l and 50 μ l was performed for A.webbiana and for the reference drug, reaction mixture was prepared for albumin denaturation assay.All the data was analysed statistically using SPSS version 23 (IBM). Unpaired t test was used for comparison with the positive control and one way anova followed by Tukey's post hoc test were used for the comparison between different concentrations.

Results:

The current study revealed that A. webbiana extract had anti-inflammatory activity at all the tested concentrations but was significantly lesser when compared to the standard (p<0.05). It was also evident that the absorbance rate of A.webbiana significantly increased with the increase in concentration of the extract (p<0.05). The absorbance rate at 50 μ l of both standard and A.webbiana were almost similar .

Conclusion:

It can be concluded from the present findings that the leaves of *Abies webbiana* possess concentration dependent anti-inflammatory property but is comparatively lesser than Diclofenac sodium

Key words: Albumin, denaturation assay, anti inflammatory activity, *Abies Webbiana*

INTRODUCTION

Inflammation is a protective mechanism that enables the body to protect itself against infections from toxic chemical allergens or any other harmful stimuli (1,2). At the same time when it is unrestrained it can lead to damage, disease or destruction portrayed by redness, pain swelling and disturbed psychological **functions**(3). It is the physiological response to a variety of injuries or insults, including heat, chemical agents and even bacterial infection. In the acute phase of inflammation, the response is very rapid and of short duration (4). If the insult or injury is not resolved, the response becomes chronic, which is considered as a **non physiologic** or pathologic **condition**(5). In the presence of chronic inflammation, the adaptive immune response is activated with involvement of the cellular and non-cellular mechanisms of acquired immunity (6).

Knowledge of how immune mechanisms and inflammatory responses are regulated is important for understanding the pathogenesis of complex diseases, such as periodontitis (7). The pathogenesis of periodontal diseases is mediated by the inflammatory response to bacteria in the dental biofilm (8). The two common diseases affecting the periodontium are gingivitis and periodontitis (9). Gingivitis is defined as the inflammation of the gingiva in which the connective tissue attachment to the tooth remains at its original level. The disease is limited to the soft-tissue compartment of the gingival epithelium and connective **tissue**(10). Periodontitis is the inflammation of the supporting tissues of the teeth with progressive attachment loss and bone destruction (4).

Chronic inflammation and oxidative stress can have both systemic and local **effects**(11). For example, in atherosclerosis where thickening of the arteries, chronic inflammation of blood vessel walls can result in plaque buildup in the arteries, arterial or vascular blockages, and heart

disease (12). Chronic inflammation also plays a significant role in other diseases and conditions such as chronic pain, poor sleep quality, obesity, physical impairment, and overall decreased quality of life. Inflammation can often be treated with nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen and corticosteroids like cortisol. However, there are potential risks associated with chronic use of NSAIDs including **Gastrointestinal**, renal, cerebrovascular, and Central nervous system adverse effects (13). On the other hand the adverse effects of corticosteroids are extensive and can involve many organ systems. Short-term use of corticosteroids is associated with mild side effects and long-term use can result in severe adverse effects, some of which are irreversible (14). Recently, plants as a source of medicine are gaining popularity because of their natural origin, availability in local communities, cheaper to purchase, ease of administration, and perhaps have less side effects. Herbal medicine may also be useful alternative treatment in case of numerous side effects and drug resistance.(15,16),(17)

Abies Webbiana is called **thalisa pathri** in Tamil, **thalisapathra** or **patradhham** in Sanskrit and **yew** or **Himalayan silver** in English(18,19). This lofty fir is widely distributed on higher ranges of Himalayan regions from **kashmir** to Assam states in India(20,21). It is a tall evergreen coniferous tree grown up to 60m with strong brown hair. **A.Webbiana** leaf has been reported to exhibit antibacterial, antifungal, mast cell stabilizing, anxiolytic, antitumour, anti inflammatory, antitussive anti fertility, antispasmodic properties, central nervous system depressant activity and effective against hypoglycemia rheumatism (18,20,22). Bronchodilation and antiplatelet activities of **A. Webbiana** were investigated and evaluated for the antioxidant and antimicrobial activity of A. Webbiana extract (23,24). Effect of A.Webbiana leaf extract on bleeding time and inflammation was analysed(18,22,25).

Our team has extensive knowledge and research experience that has translate into high quality publications.(26–38),(39–43) (44) (45)This study was designed to determine anti-inflammatory activity of Abies Webbiana and also compare the same with a known non steroidal anti inflammatory drug at different concentrations(46) (47) (48) (49).

MATERIALS AND METHODS

COLLECTION OF PLANTS MATERIALS AND EXTRACT PREPARATION

A.webbiana powder was obtained **ready made**. It was mixed with 100 ml of distilled water in a conical flask and kept on a stirrer for 2-3 days for the extract preparation. The conical flask was then kept at room temperature for 15 minutes and later kept at 80°C for 20 minutes. The extract was filtered using a filter paper. Thereafter, it was stored in the refrigerator.

PREPARATION OF REFERENCE DRUGS (**POSITIVE CONTROL/ STD)**

NSAID (Diclofenac) was used as a reference drug. Diclofenac was crushed into fine powder. About 0.2g of Diclofenac sodium drug powder was measured using a digital analytical balance

and was added to 20 ml of distilled water respectively. The solution was mixed well using a vortex.

Sample preparation

The different concentrations of 10 μ l, 20 μ l, 30 μ l, 40 μ l and 50 μ l were prepared for *A.webbiana* and for the reference drug, reaction mixture was prepared for albumin denaturation assay.

INHIBITION OF PROTEIN DENATURATION

In this study we have used albumin denaturation assay as an indirect measure against inflammation. Reaction mixture were incubated in water bath at 37°C \pm 2°C for 15-20 minutes and kept in room temperature or 10 minutes. Absorption of reaction mixture before and after denaturation was measured for each concentration (10 μ l, 20 μ l, 30 μ l, 40 μ l and 50 μ l) at 680 nm using a colorimeter. Each test was repeated thrice and the mean absorbance was recorded

STATISTICAL ANALYSIS

All the data were analysed statistically using SPSS version 23 (IBM). Unpaired t test was done to compare the mean absorbance of the plant extract (*A. webbiana*) with the reference drug (Diclofenac sodium). One Way Anova test followed by Tukey's post hoc test were done to compare the efficacy of *A.webbiana* at different concentrations. The difference were considered to be statistically significant when $p < 0.05$

RESULTS

The current study showed that the anti-inflammatory activity of *A. webbiana* was significantly lesser when compared to the standard (Figure 1) . ANOVA test was done to compare *A.webbiana* and the standard and results showed statistical significance ($p < 0.05$). The comparison of different concentrations revealed that the efficacy of *A.webbiana* significantly increased with increase in the concentration of the extract (Figure 2). The post hoc test was done to compare the mean absorbance rate of the extract at different concentrations and the results were found to be statistically significant ($p < 0.05$).

DISCUSSION

In this study we had evaluated the anti-inflammatory activity of *A. webbiana* at different concentrations such as 10 μ l, 20 μ l, 30 μ l, 40 μ l and 50 μ l. We had also compared the anti-inflammatory activity of *A.webbiana* with an NSAID (diclofenac) which was used as a standard. The current study showed that the anti-inflammatory activity of *A. webbiana* was comparatively lesser than the standard (diclofenac). However, as the concentration of the extract increased the anti-inflammatory activity also increased in a concentration dependent manner.

The current study has used diclofenac as the standard to compare the anti-inflammatory of *A.webbiana* with. Diclofenac sodium is one of the most common choices of medication for treating acute inflammatory conditions and pain(50) . It works by inhibiting the cyclooxygenase (COX) pathway and thus preventing the synthesis of prostaglandin and other eicosanoids(51) . Diclofenam acts as a potent anti-inflammatory agent and as an analgesic but the requirement for frequent dosing due to the rapid elimination rate was anticipated to potentially compromise the tolerability of diclofenac (52).

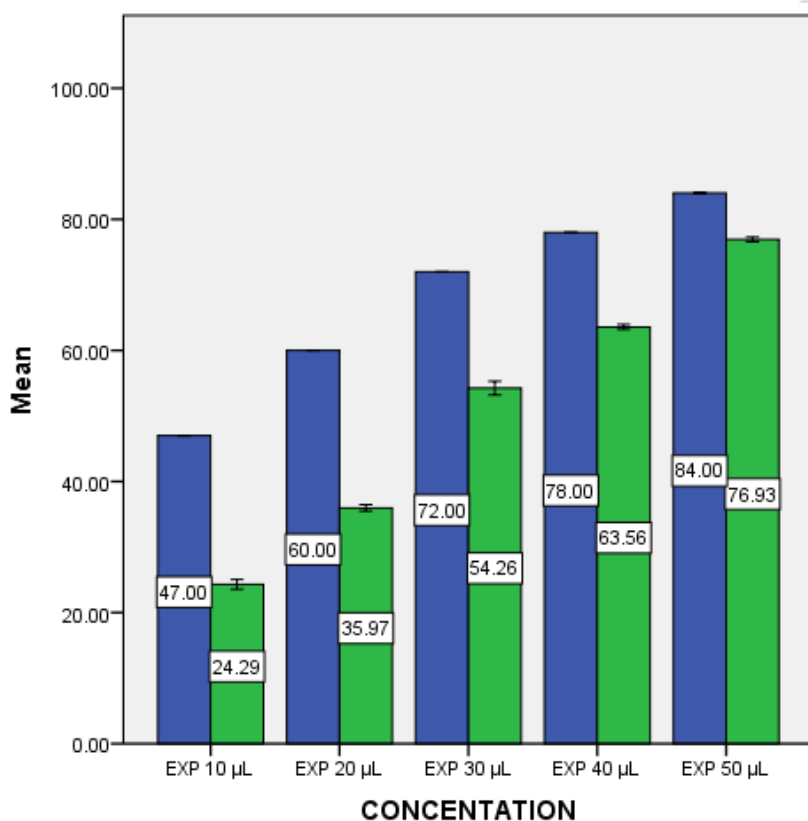
The study shows the efficacy of *A.webbiana* as an anti-inflammatory agent at different concentrations. The results showed that *A.webbiana* was less potent than diclofenac but its efficacy increases when the concentration of the extract increases. Similar studies were done on various other plants that exhibited anti-inflammatory properties of its leaves and fruit extracts. From phytochemical investigation, the presence of biologically active principles such as flavonoids, tannins, phenolic compounds and phytosterols in plants is revealed. It is suggested that one of the above constituents or a combination is responsible for producing the analgesic and (53) anti-inflammatory effects. Few studies show that the anti-inflammatory property of *A.webbiana* might be due to flavonoids or even steroids that are present in them (54,55). Further studies are in progress to isolate and characterize the active principle from the leaves and even other parts of *A.webbiana* (56,57). Researchers suggested that these plant extracts may have a reasonable safety margin with regard to acute toxicity hence justifying that it can be widely used for various purposes (58). Anti inflammatory activity of *A.webbiana* has not been reported in many studies in literature. It was only studied on rats (53). There are several other properties of *A.webbiana* such as antimicrobial and antitumor agents as well used in ayurveda (59)

In the present study the anti inflammatory effect was evaluated using albumin denaturation assay. There are several other assays such as membrane lysis assay and lipoxigenase inhibition assay to confirm the anti-inflammatory activity of any plant extracts (60). Albumin denaturation assay is a cheap , easy yet effective method to find out the anti-inflammatory activity of plant extracts. Similar studies were done on various other plants such as *F. racemosa* and *M. scandens* and they have shown similar results (61). The efficacy of the plant extract always increases with the increase in the concentration of the extract. Studies have also used different NSAIDs such as ibuprofen as standards (62,63). The same study can be done using different methods of extract preparation, different assays as well as by using different NSAIDs

as standard to obtain more value to this study(64) (65) (66) (67) (68) (69) (70) (71) (72) (73) (74) ((74,75) (76) (77). Since many chronic inflammatory conditions demand long term usage of anti inflammatory agents the herbal formulation will be a viable alternative to long term non steroidal anti inflammatory therapy. Further research on isolating different components may be undertaken and may be incorporated into existing anti-inflammatory herbal compositions to improve their efficacy. Promising results regarding the anti inflammatory effect of Abies webbiana can be further validated with future in vivo studies to find the safe and effective concentration for clinical usage.

CONCLUSION

From the study it can be concluded that the anti-inflammatory property of A.webbiana is not as efficient as Diclofenac but it can be more efficient when its concentration is raised to safe levels.



Error Bars: +/- 1 SD

FIGURE 1: The bar graph shows the comparison of the mean absorbance of the standard and Abies webbiana at various concentrations. The X-axis represents the concentration in units of µL and the Y-axis represents the mean absorbance. Blue represents the standard and green represents A.webbiana. The graph shows that the mean absorbance of A. webbiana is significantly lesser when compared to the standard at all the concentrations even though the magnitude of difference was lesser at higher concentrations ($p < 0.05$) (unpaired t test)

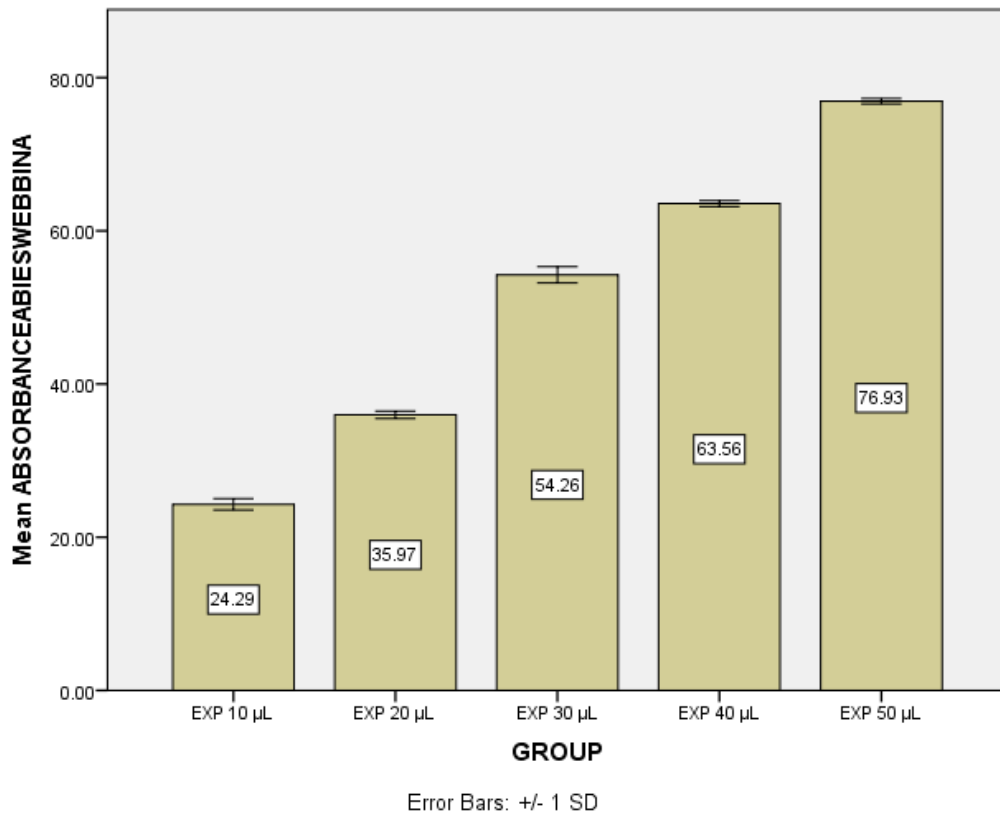


FIGURE 2 : The figure shows the mean absorbance of *A. webbiana* at different concentrations. The X-axis represents the group (different concentrations of *A. webbiana*) and the Y-axis represents the mean absorbance. There is a significant increase in the mean absorbance from lower concentration to higher concentrations. ($p < 0.05$) (One Way anova followed by Tukey's post hoc test was done to compare the mean absorbance of *A. webbiana* at different concentrations).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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