ORIGINAL ARTICLE

Investigation of the Anti-Inflammatory and Analgesic Effects of the Aqueous Extract Obtained from the Bark of Guiacum Officinsale (Lignum Vitae)

K Williams, AS Jacob, SA Levy

ABSTRACT

Background: *The bark of* Guaiacum officinale *(Lignum vitae) is used in folklore practice to relieve pain and swelling associated with arthritis and gout and on this basis an aqueous extract was tested on histamine-induced oedema and pain in rats in the present study.*

Subjects and method: *Intraperitoneal injections of aqueous lignum vitae extract (16 mg/kg and 32 mg/kg) were tested on oedema induced by sub plantar injection (0.2 mL) of 1 mg of /kg histamine in rat paws (n = 6). Control animals received distilled water (0.2 mL) and ibuprofen (10 mg/kg) was used as positive control. The analgesic efficacy of the extract was also evaluated using an analgesiometer for recording latency period.*

Results: *Lignum vitae extract (32 mg/kg) and ibuprofen treated groups displayed increased latency period and which confirmed analgesic efficacy (*$p < 0.05$*) when compared to control animals and animals treated with 16 mg/kg extract (*$p > 0.05$*). Significant anti-inflammatory effects were also observed with administration of 32 mg/kg lignum vitae extract. In the acute toxicity test, no signs of toxicity were observed when extract was given in doses of 300 mg/kg and 128 mg/kg.*

Conclusion: *The aqueous extract from lignum vitae bark showed significant anti-inflammatory and analgesic activity with no observable toxic effects at a dose ten times that of the other extract producing these pharmacological effects. These results are consistent with anecdotal claims of swelling and pain relief with the tea from lignum vitae bark and can provide guidelines for quantification and safety in folkloric use of this extract.*

Keywords: Analgesia, extract, *Guaiacum officinale*, inflammation, lignum vitae

Investigación de los efectos antiinflamatorios y analgésicos del extracto acuoso obtenido de la corteza del Guaiacum officinale (*Lignum vitae*)

K Williams, AS Jacob, SA Levy

RESUMEN

Antecedentes: *La corteza del* Guaiacum officinale *(*Lignum vitae*), conocido comúnmente como guayacán azul, se utiliza en la práctica tradicional para aliviar el dolor y la inflamación asociados con la artritis y la gota. Sobre esta base, en el presente estudio un extracto acuoso fue probado en ratas con dolor y edema inducidos mediante histamina.*

From: The University of the West Indies, Pharmacology Section, Department of Basic Medical Sciences, Kingston 7, Jamaica, West Indies.

Correspondence: Dr A Jacob, The University of the West Indies, Pharmacology Section, Department of Basic Medical Sciences, Kingston 7, Jamaica, West Indies. Email: Audrey.jacob@uwimona.edu.jm

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Sujetos y método: *Inyecciones intraperitoneales de extracto acuoso de* Lignum vitae *(16 mg/kg y 32 mg/kg) fueron probadas en el edema inducido por la inyección subplantar (0.2 mL) de 1 mg/kg de histamina en las patas de las ratas (n = 6). Los animales de control recibieron agua destilada (0.2 mL) y se utilizó ibuprofeno (10 mg/kg) como control positivo. La eficacia analgésica del extracto también se evaluó utilizando un analgesiómetro para registrar el período de latencia.*

Resultados: *El extracto de* Lignum vitae *(32 mg/kg) y los grupos tratados con ibuprofeno mostraron un mayor período de latencia, lo que confirmó la eficacia analgésica (*$p < 0.05$*) al comparase los animales de control y los animales tratados con extracto de 16 mg/kg (*$p > 0.05$*). También se observaron efectos antiinflamatorios significativos con la administración de 32 mg/kg de extracto de Lignum vitae. En la prueba de toxicidad aguda, no se observaron signos de toxicidad cuando el extracto se dio en dosis de 300 mg/kg y 128 mg/kg.*

Conclusión: *El extracto acuoso de la corteza de* Lignum vitae *mostró una actividad antiinflamatoria y analgésica significativa sin efectos tóxicos observables en una dosis diez veces mayor que la del otro extracto con estos efectos farmacológicos. Estos resultados se corresponden con las aseveraciones anecdóticas según las cuales el té de la corteza de* Lignum vitae *alivia el dolor y la hinchazón, y pueden proporcionar pautas en cuanto a la cuantificación y seguridad en el uso tradicional de este extracto.*

Palabras clave: Analgesia, extracto, *Guaiacum officinale*, inflamación, *Lignum vitae*

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INTRODUCTION

Approximately 90% of the world's population is utilizing the medicinal benefits of unrefined extracts from plant leaves, roots and seeds 1). Additionally, 25% of today's therapeutic medicine contains bioactive agents from plants.

The *Guaiacum officinale* (Lignum vitae) plant has also been exploited due to its medicinal properties. It belongs to the Zygophyllaceae (2) family and is a native to Central America and also Jamaica (where it is the national flower). Common names associated with this plant are tree of life, bois de vie and iron wood (3). The leaves of the Lignum vitae are leathery, shiny and also dark green for the entire year. Mature fruits of the plant are orange, flattish and basically heart shaped. The flowers display deep blue to purple petals that gradually become white (4). The resin from the bark interacts with alcohol in blood stains to form a blue color and this characteristic feature is utilized by police and other investigators to detect blood stains at crime scenes (5).

According to anecdotal testimonies, the tea from the Lignum vitae bark is effective in relieving pain and swelling in the knee joint (6). In Britain, Lignum vitae leaf extract was used in arthritic and rheumatic conditions to relieve the swelling and the pain in the joint (7) In addition, the wood chip preparation of the plant functioned as a local anaesthetic and was used to relieve swelling in the rheumatic joints (8).

Currently, non-steroidal-anti-inflammatory drugs such as ibuprofen and celecoxib are the mainstay therapy for treatment of inflammatory arthritic conditions such as rheumatoid arthritis. The adverse effects of the non-selective NSAIDs such as ibuprofen and indomethacin can include prolonged bleeding and gastrointestinal ulcerations (9), while those related to the selective NSAIDs such as celecoxib include impaired platelet aggregation and renal function (10). These side effects continue to limit the use of NSAIDs and foster the constant search for new agents with less deleterious effects.

The present study therefore evaluated the potential of the lignum vitae bark extract to inhibit oedema and suppress pain as cardinal signs of the inflammatory process involved in arthritic conditions. Histamine was used to induce oedema and pain through its ability to evoke the release of neuropeptides, prostaglandins and monohydroxy-eicosatetranoic acid from endothelial cells (11). The subsequent release of these mediators leads to stimulation of nociceptive fibres resulting in hyperalgesia as well as other pro-inflammatory effects including vasodilation, fluid exudation and oedema formation (12). This model of histamine induced inflammation in the rat paw is well established

in screening for anti-inflammatory activity of novel extracts and compounds (13).

The significance of this study pivots on anecdotal reports of anti-inflammatory properties of lignum vitae in folk medicine. It is hopeful that findings will validate the usefulness of this extract in folklore practice and provide data to substantiate further studies and chemical characterization. Additionally, results of the acute toxicity test will shed some light on possible deleterious effects after use.

SUBJECTS AND METHODS

Ethics approval was granted by the Ethics Committee of the Faculty of Medical Sciences. The University of the West Indies, Mona and animal care and use conformed to the institutional guidelines and The Declaration of Helsinki contribution on use of animals in experiments. Male and female Wistar rats (250–300 g) were obtained from The University of the West Indies animal house and placed in polyacrylic cages (38 cm x 23 cm x 10 cm) with six rats per cage. Animals were housed in standard environmental conditions (ambient temperature, 28.0 ± 2.0ºC, and humidity 46%, with a 12-hour light/dark cycle). Animals in both test and the control groups were allowed free access to food (Purina Chow, USA) and water *ad libitum* for the duration of the investigations. Cages were cleaned daily to maintain hygienic conditions.

Preparation of extract

Lignum vitae bark was collected from the St Andrew area of Jamaica and dried using a solar drier. The plant was validated by Mr Patrick Lewis in the Botany Section of the Faculty of Science and Technology. A sample was deposited in the UWI herbarium and was assigned accession number 35959. The extract was prepared by boiling 34.13 g of powdered lignum vitae bark in 500 mL of distilled water for half an hour. The solution was cooled, filtered, refrigerated and the concentrated dark red residue was obtained by rotary evaporation at 100ºC. The yield was 7.9%.

Anti-inflammatory investigation

Histamine-induced oedema

In assessing the anti-inflammatory property of the plant extract, the rats were divided into four groups (n = 6 per group).The control group was given sub-plantar injection 1 mg/mL of histamine (14). Other test groups were also given right hind paw sub-plantar histamine injections and then treated with 10 mg/kg ibuprofen (as positive control) or lignum vitae extract at 16 and 32 mg/kg intraperitoneally ten minutes later. The change in paw volume of rats from each group were measured, using a Plethysmometer, every 30 minutes for two and a half hours. Readings were done in duplicate.

Analgesic investigation

Histamine-induced pain: Animals were treated orally with aqueous lignum vitae bark extract (16 and 32 mg/kg) and the tail flick latency was assessed with analgesiometer (Inco India) over a period of three hours. Ibuprofen (10 mg/kg) was administered to one group as a positive control, while the control group was given 0.5 mL of water. Chemical injury was induced by histamine (0.2 mL of img/mL) which also induced hyperalgesia in the hind paw.

The analgesiometer was adjusted to generate a constant current of six amperes during testing. The distance between the heat source and tail skin was 1.5 cm. The site of application of radiant heat in the tail was measured from root to tail. The cut off reaction time was fixed at 10 seconds to avoid tissue damage.

Acute toxicity testing

This procedure was done according to the Organization for Economic Co-operation and Development (OECD) Guidelines for Testing Chemicals; Acute Oral Toxicity-Fixed dose procedure, 2001. Twelve mice (22 g to 25 g) were fasted for three hours prior to oral administration of the extract. The mice were then divided into four groups (three mice per group) where one group served as the control group, a second group was administered 1000 mg/kg of the extract, a third group was administered 300 mg/kg of the extract and the fourth group was administered 125 mg/kg of the extract orally. The mice were observed for a period of 72 hours. One mouse died within 24 hours at 1000 mg/kg; the highest dose tested. At doses of 300 and 125 mg/kg of extract, no death occurred within 24 hours.

Statistical analysis

Analysis with the one-way analysis of variance (ANOVA) was used to note the difference observed between the treatment groups and pairwise comparison was executed with the Tukey post *hoc* test to confirm all significance. A value of $p < 0.05$ denoted significance between test and control groups. All data are presented as means ± SEM.

RESULTS

Anti-inflammatory activity

A significant decrease in paw oedema of animals treated with the lignum vitae extract at 32 mg/kg was observed in the histamine-induced paw oedema model [$p < 0.05$] (Fig. 1). Maximum inhibition was observed at 90 minutes (89% reduction in oedema).

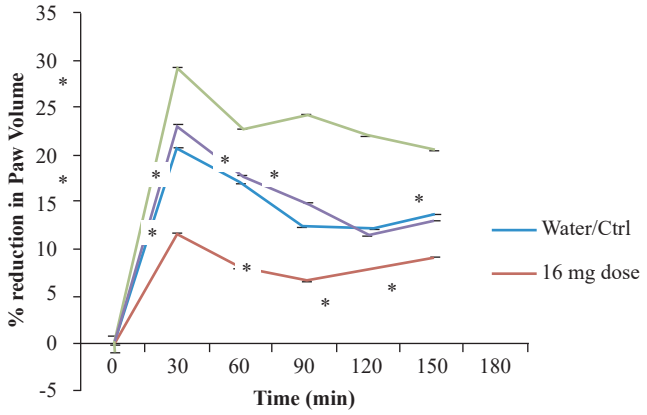

Fig. 1: Graph showing effect of aqueous *Lignum vitae* extract and ibuprofen on histamine-induced rat paw inflammation.

Pairwise comparison with the Tukey post *hoc* test, indicated that the 32 mg/kg dose of the aqueous extract as well as the ibuprofen (10 mg/kg) caused statistically significant reduction in paw volume ($p < 0.001$) when compared to the controls (water). The 16 mg/kg dose of the extract showed no statistically significant reduction in paw volume ($p > 0.05$).

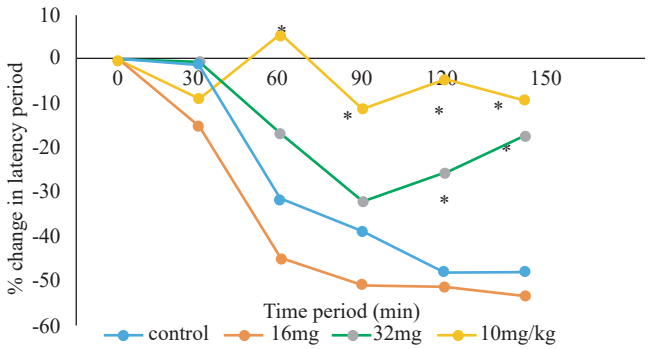

Fig. 2: Graph showing the effect of aqueous *Lignum vitae* bark extract and ibuprofen on latency period in histamine- induced pain model.

Pairwise comparison with the Tukey post *hoc* test, showed that higher dose of the plant extract (32 mg/kg) as well as the ibuprofen (10 mg/kg) groups had a significantly higher latency period than the control treated animals ($p = 0.001$ and 0.039, respectively). The difference between the control and the low dose (16 mg/kg) treated animals was not significant ($p = 0.077$).

Acute toxicity testing

Oral administration of lignum vitae extract at 125 mg/kg and 300 mg/kg produced no mortality. One animal died in the group treated with 1000 mg/kg. No significant changes in behaviour were recorded during the observation period.

DISCUSSION

As the search for efficacious anti-inflammatory agents with very little toxic effects heightens; the search continues for bioactive plant sources. Our findings confirm the anti-inflammatory and anti-nociceptive properties of Lignum vitae bark extract in a histamine induced model of oedema and pain. Treatment with the extract at 32 mg/kg showed inhibition of swelling caused by histamine in comparison with the 16 mg/kg dose and negative controls (water). This anti-inflammatory activity of the extract was comparable to that of ibuprofen which is a standard NSAID used in the treatment of inflammatory arthritis clinically. This suggests that that the anti-edematous effect of the extract is in part mediated through inhibition of histamine induced prostaglandin release (15).

The mechanism of histamine induced oedema involves stimulation of prostaglandins and monohydroxy-eicosatetraenoic acids (HETEs) from endothelial cells resulting in dilation of local blood vessels and the exudation of fluid into the interstitial spaces (16).

In the tail flick method, the tail flick latency was significantly prolonged by *Lignum vitae* extract at 32 mg/kg but not 16 mg/kg. This observation might provide some pharmacological rationale for its use in the treatment of pain in folklore medicine. In acute toxicity experiments no adverse effects were seen at a dose below 500 mg/kg. One animal died at a dose of 1000 mg/kg. Mice showed no signs of hyperactivity. Observation at 72 hours after toxicity studied showed that animals were active and well-oriented.

Previous studies have shown that anti-inflammatory and analgesic effects can be a result of the flavonoids which are known to prevent the synthesis of prostaglandins associated with arachidonic acid and the cyclo-oxygenase and 5-lipoxygenase enzyme systems (18). The *Lignum vitae* plant belongs to the Zygophillaceae family which usually contains many active compounds including, glycosides, steroidal saponins, alkaloids and flavonoids (19). It is possible that the anti-inflammatory and analgesic actions of the extract are attributed to flavonoids present in the extract (20). Biochemical investigations on the mechanism of action

of flavonoids have shown that these compounds can inhibit a wide variety of enzymes also (21).

In concluding, investigations are needed to purify and characterize the crude aqueous extract and sub-chronic and chronic toxicity studies will give vital information. These experimental results of the anti-nociceptive and anti-inflammatory actions of the *Lignum vitae*, thus, confirm that that plant was in fact relieving pain and inflammation according to folklore claims in Jamaica. Further investigation of the biochemical pathways of the pure bioactive agents from the crude extract can serve as a guide in the development of an agent with potent anti-inflammatory agent and analgesic effects.

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