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Research Article

ANTI-ALLERGIC, ANTI-INFLAMMATORY AND ANALGESIC ACTIONS OF STEM BARK EXTRACT OF *LANNEA WELWITSCHII (ANARCADIACEAE)* IN MICE

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ABSTRACT

Objective: Various parts of *Lanneawelwitschii* find use in traditional medicine in the treatment of pain and oedema. This study evaluated the anti-allergic, anti-inflammatory and analgesic effects of a 70 % (v/v) aqueous ethanol extract of the stem bark of *Lanneawelwitschii,* LWE in mice.

Methods: IgE-mediated anaphylaxis in a local allergic reaction was studied in the pinnal inflammation model while endotoxic shock was induced by the injection of lipopolysaccharide, LPS and survival rates of mice monitored. The indirect anti-histamine effect of LWE was evaluated on clonidine-induced catalepsy. The effect of LWE assessed on the maximal and total oedema responses in the carrageenan–induced paw oedema was used to evaluate the anti-inflammatory action of the extract while the tail immersion test was employed to study the analgesic effects of LWE.

Results: LWE dose-dependently inhibited the antigen-induced pinnal inflammation and also offered protection to mice in the LPS-induced endotoxic shock. LWE showed significant inhibition of the clonidine-induced catalepsy in mice and suppressed the mean maximal swelling as well as the total paw swellings induced over 6 h in the carrageen an paw oedema. LWE caused a significant and dose-dependent increase in the mean reaction time of treated mice in the analgesic test.

Conclusion: The aqueous ethanol extract of *Lanneawelwitschii* has anti-allergic and anti-inflammatory actions mediated through the inhibition of histamine release from mast cells. Additionally, *L. welwitschii* exhibits analgesic actions in mice. Our results contribute towards validation of the traditional use of *Lanneawelwitschii* in the treatment of pain and oedema.

Keywords: Allergy, Anti-histaminic activity, Anti-inflammatory, Analgesic, *Lanneawelwitschii*

INTRODUCTION

Inflammatory events are initiated, enhanced, or co-ordinated by the action of various immune-competent cells such as mast cells, platelets, and leukocytes. Mast cells play a central role in the development of inflammation through the release of inflammatory mediators. These chemicals include preformed mediators such as histamine and serotonin[1, 2], low molecular weight lipids derived from arachidonic acid, and cytokines and chemokines, which promote inflammation and further function to amplify the inflammatory response [3]. The inflammatory response, though primarily a defensive mechanism, may lead to tissue damage which may be serious enough to call for pharmacological intervention to ameliorate the process. Orthodox drugs in current use for the treatment of inflammation and allergic conditions include the non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and the disease modifying anti-rheumatic drugs (DMARDs)[4]. Reportedly, there are serious adverse effects and even life-threatening side effects associated with the use of these drugs.

Medicines from plant sources have shown great promise with actions most likely mediated through interference with the predominant pathophysiological processes underlying inflammation. One of such plants is *Lanneawelwitschii* Synonym: *Ricinodendronstaudtii, Calesiamwelwitschii* of the family Anarcadiaceae*. Lanneawelwitschii* is widely distributed in West and Central Africa (Côte d'Ivoire to Uganda, as well as Congo and Angola) and found in abundance in Cameroonian forests. The tree reaches 30 m. tall with a straight cylindrical trunk to 2.70 m girth. Its fruit is small and is an ellipsoid to nearly globose, slightly compressed drupe 6–8 mm long, smooth, blackish purple when ripe and usually 1-seeded [5].Various parts of the plant find use in traditional medicine. The aqueous bark extract is commonly used in decoctions administered to treat diarrhoea [6], swellings, oedema, gout, haemorrhoids, emesis [7] and back ache [8]. The presence of oils, alkaloids, saponins, flavonoids, tannins, anthraquinones, cyanogenic glycosides and reducing sugars have been reported in a phytochemical analysis [6].Literature reviews indicated that no studies combining the analgesic and anti-inflammatory actions of the bark of *L. welwitschii*have so far been undertaken.

Taking this in view and as a part of our on-going research on anti-inflammatory actions of medicinal plants, the present study aim to pioneer studies into the aqueous ethanol extract of *Lanneawelwitschii* on allergy, acute inflammation and analgesic actions in mice.

MATERIALS AND METHODS

Preparation of Plant Extract

The stem bark of *Lanneawelwitschii* was collected from Nkawkaw in the Eastern Region of Ghana, in November 2012 by Mr. Clifford Osafo Asare, a herbalist. The identity was confirmed as the stem bark of *Lanneawelwitschii* (Hiern) Engl. by anatomical observation and direct comparison with the authentic specimens, stored in the Herbarium in the Department of Pharmacognosy, KNUST, Kumasi. A voucher specimen(No. FP/AN/L-11/2012/) has been deposited in the same Department. The stem bark of the plant was sun dried for 7 days, chopped into pieces and air dried for another 7 days after which it was pulverized using a heavy duty blender (37BL85 (240CB6), Waring Commercial, USA). 5 kg of the powdered bark was extracted by cold maceration using 10 L of 70 % (v/v) aqueous ethanol. After 7 days the crude aqueous ethanol extract was concentrated under reduced pressure at 45°C by a vacuum rotary evaporator (R-210, BUCHI, Switzerland). This was then dried in an oven (Gallenkamp OMT, Sanyo, Japan) and stored in a desiccator. The final yield 18.5 % (w/w) obtained was freshly reconstituted in normal saline and referred to as LWE.

Animals

Both sexes of C57BL/6 and ICR mice (20-30 g) were supplied by the Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana. The animals were kept in the Animal House of the Department of Pharmacology, College of Health Sciences, KNUST, Kumasi, Ghanaunder laboratory conditions (temperature 23 ± 2°C with a 12 hour light-dark cycle). Animals had free access to commercial pellet diet (GAFCO, Ghana) and water supplied *ad libitum*. The animals were humanely handled throughout the experiment in accordance with internationally accepted principles for laboratory animal use and care (EEC Directive of 1986: 86/609 EEC). Additionally all animal

experiments were approved by the Department of Pharmacology, KNUST Ethics Committee. Each animal was used only once and at the end of each experiment animals were euthanized.

Chemicals and Reagents

λ-Carrageenan, Evans Blue, Aspirin and Dexamethasone were purchased from Sigma-Aldrich (St Louis, USA). Clonidine was purchased from BoehringerIngelheimInc, (USA). Haloperidol was obtained from Janssen-Cilag Pty Ltd (U.K.). Chlorpheniramine was bought from DWD Pharmaceuticals Ltd (India). Bovine Serum Albumin, BSA and Phosphate Buffered Saline, BPS was purchased from the PAA Laboratories (Germany) and Gibco (Karlsruhe, Germany)respectively.

Microorganism

Escherichia coli (strain: ATCC 25922) was a kind donation from the Department of Pharmaceutical Microbiology, KNUST, Kumasi.

Passive Cutaneous Anaphylaxis, PCA

The pinnal inflammation model as previously described Churchet al.,(1974)was used [9]. Briefly,8-10 week oldICR mice (20-25g) were immunized with 100µlof a solution of bovine serum albumin, BSA (0.05 mg ml^{-1}) subcutaneously. Immunization was repeated on day 14 with100µl of a solution of BSA (0.02mgml^{-1}). Seven days later mice were anaesthetized with ether and 200µlof a 1 % solution of Evans Blue dye injected into the tail vein. Promptly after this, and while still under anaesthesia, the mice were laid supine and each pinna was spread out and inoculated with BSA (0.1 mgml^{-1}) using a 21 gauge hypodermic needle. After 30 min the mice were sacrificed and their ears cut off, spread out and the area of the reaction measured by circumscribing the area of extravasation of the blue dye and matching it with the best fit of standard circles. The area of the reaction was taken as the square of the diameter (mm) of the circle of best fit. For treatments, LWE 50, 200 and400 mg kg^{-1}, dexamethasone 10 mg $kg^{-1}$and aspirin 100 mg kg^{-1}, were given orally 1 h before challenge with the antigen (BSA). Unlike LWE or reference drugs, vehicle (saline, 100µl) was injected s.c. just before the challenge.

Percentage inhibition of the inflammatory reaction was expressed as:

$$\%\ inhibition\ of\ PCA = 100\left(1-\frac{P_t}{p_0}\right)$$

Where P_o and P_t are the area of extravasation of the blue dye in the pinna of the saline control and drug or extract treated mice respectively.

Lipopolysaccharide (LPS)-induced allergy

LPS-induced systemic anaphylaxis was examined as previously described [10]. 12-14 week old male C57BL/6 mice (25-30 g) received i.p. injection of lipopolysaccharide, LPS (*Escherichia coli*, 5 mg kg^{-1}dissolved in PBS). Vehicle, dexamethasone 10 mg kg^{-1} or LWE 50, 200 and 400 mg kg^{-1} was given orally for two consecutive days before initiation of the allergic reaction. Survival rate of the animals was monitored for 7 days after challenge.

Clonidine- and haloperidol-induced catalepsy

As earlier described by Ferreet al.,(1990), clonidine (1 mg·kg^{-1}, s.c.) or haloperidol (1 mg kg^{-1}, s.c.)was administered to8-10 week old ICRmice (20-25 g) and their forepaws placed on a horizontal bar (1 cm in diameter, 3 cm above the table)[11].Either vehicle (5 mlkg^{-1}), LWE (50, 200 and400 mg kg^{-1}), or chlorpheniramine (10 mgkg^{-1}) was given orally for 2 consecutive daysending30 min before clonidine or haloperidol injection. The time required to remove the paws from the bar was noted for each animal. The duration of catalepsy was measured at 30 min intervals up to 3 h after administration of clonidine or haloperidol.

Carrageenan-induced paw oedema

Pedal oedema was induced by a method earlier described [12]. Briefly ,a 1 % carrageenan suspension in normal saline was injected (50µl, s.c.) into the subplantar tissue of the right hind paw of 8-10 week old ICR mice(20-25g). Oedema was monitored with an electronic calliper (Z22855, Milomex Ltd, Bedfordshire, UK) at 1 h intervals over 6 h as percentage increase in paw thickness. Total oedema induced during the 6 h was measured as area under the time course curves (AUC). Drug effects were evaluated by comparing the maximal and total oedema responses attained during 6 h in drug-treated groups with the corresponding values attained in drug vehicle-treated inflamed control groups. In the preventive (prophylactic) protocol, drug vehicle, LWE 50, 200 and 400 mg kg^{-1}, or aspirin 100 mg kg^{-1}, was given orally 1 h before the induction of the oedema while in the curative (therapeutic) protocol, treatment was done 1 h post oedema induction.

Tail immersion test for analgesia

The tail immersion test was carried out according to the method described by Janssen et al., (1963)[13] and modified by Savegnago et al., (2007)[14]. Reaction time, defined by the time (in seconds) to withdraw the tail from hot water maintained at 50.0±1.0°C, was measured. A cut-off time of 10 s was set to avoid tissue damage and used for the selection of mice for the experiment. Non-reactive mice within this cut–off time were excluded. Increase in mean reaction time was indicative of anti-nociception. The % increase in reaction time was calculated as:

$$\%\ Increase\ in\ reaction\ time = 100\left(\frac{\text{Reaction time (treatment)} - \text{Reaction time (control)}}{\text{Cut-off time} - \text{Reaction time (control)}}\right)$$

Animals were tested before and at 30 min intervals for 180 min after administration of LWE (50, 200, 400 mg kg^{-1}), and aspirin 100 mg kg^{-1} orally.Total analgesic effect was expressed as the area under the time-course curves (AUC).

Statistical analysis

All data are presented as the Mean ± s.e.m. (n=5). LPS-induced septic shock data was presented in a Kaplan-Meier survival plot and analyzed using Log-rank (Mantel Cox) test. In the clonidine-haloperidol-induced catalepsy, the data analysis was carried out using Two-way analysis of variance (ANOVA) followed by Bonferonni's *post hoc* tests. Pinnal inflammation, carrageenan-induced paw oedema and analgesic data were analyzed using One-way ANOVA followed by Newman-Keuls' *post hoc* test. All graphs were plotted using GraphPad Prism for Windows Version 5.00 (GraphPad, San Diego, CA).

RESULTS

Effect of *Lanneawelwitschii extract,* LWE on Passive cutaneous anaphylaxis (PCA)

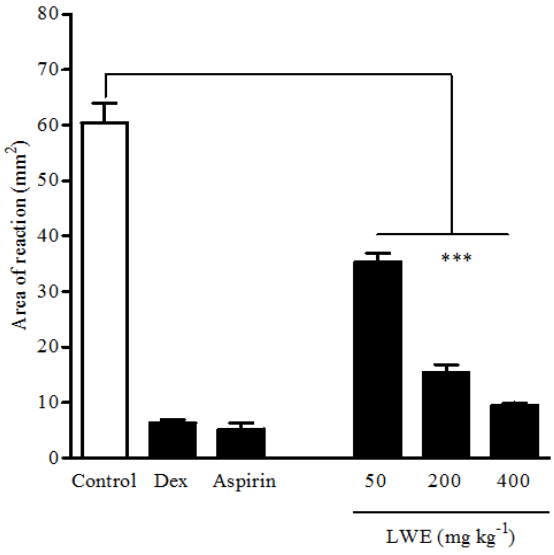

Fig. 1: It shows the effect of *Lanneawelwitschii extract* (LWE) on Passive cutaneous anaphylaxis (n=5).

Antigen-induced pinnal inflammation in bovine serum albumin-sensitized mice was significantly ($P \leq 0.005$)and dose-dependently inhibited by the*Lanneawelwitschii extract*, LWE (50, 200 and 400 mg kg^{-1}) by41.56 % , 74.94 % and 84.22 % while dexamethasone (10 mg kg^{-1}) and aspirin (100 mg kg^{-1}) suppressed same by 89.53 % and 91.38 % respectively relative to the control (Fig.1).

Effect of *Lanneawelwitschii* extract, LWE on LPS-induced allergy

100 % mortality was observed with the i.p. injection of LPS (*Escherichia coli*) in all the vehicle-treated mice in 48 h. LWE at 50mg kg^{-1}, significantly ($P \leq 0.001$) protected mice against endotoxic shock by 45 % while the 200 and 400 mg kg^{-1}both showed increased protection against endotoxic shock by 75 % respectively (Fig.2).The dexamethasone (10 mg kg^{-1})-treated group exhibited maximum protection against endotoxic shock induced with the LPS presenting a survival proportion of 100 %.

Effect of *Lanneawelwitschii extract*, LWEonclonidine-and haloperidol-induced catalepsy

Catalepsy was observed in all the group speaking at 120 min in the vehicle control group after the administration of clonidine (1 mg kg^{-1},s.c).LWE (50, 200 and 400 mg kg^{-1})and chlorpheniramine (10 mg kg^{-1}) showed significant inhibition of clonidine-induced catalepsy at all time points in the preventive protocol (Fig. 3A). In the curative protocol, LWE 50, 200 and 400 mg kg^{-1} significantly suppressed the clonidine-induced catalepsy from 90 min through 180 min(Fig. 3B). Haloperidol-induced catalepsy was neither inhibited by LWE nor chlorpheniramine (results not shown).

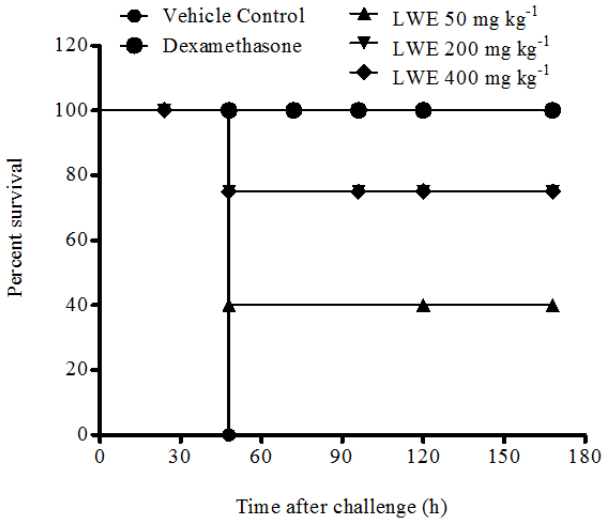

Fig. 2: It shows the effect of *Lanneawelwitschii extract*(LWE) on lipopolysaccharide (LPS)-induced allergy (n=10).

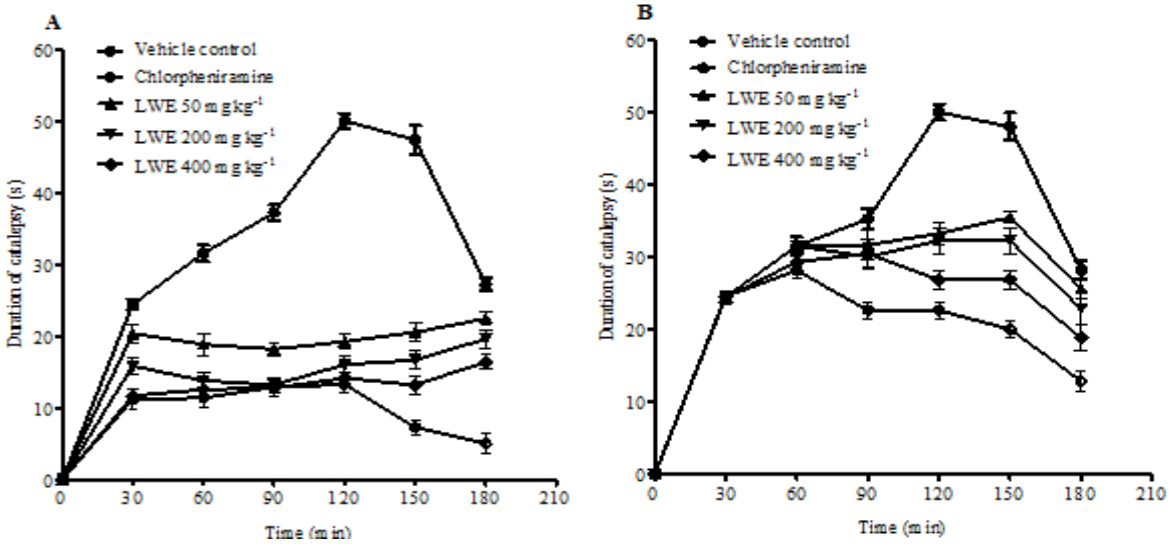

Fig. 3: It shows the effect of *Lanneawelwitschii extract* (LWE) on clonidine-induced catalepsy (n=5).

Effect of *Lanneawelwitschii extract*, LWE on carrageenan-induced paw oedema

L. welwitschii (50, 200, 400 mg kg^{-1}) when administered before (preventive) the induction of the carrageenan paw oedema caused the mean maximal swelling to be significantly suppressed to 29.08±5.73 %,17.26±3.70 %, and 39.23 ±5.64 % of the inflamed control response respectively (Fig. 4A) while the total paw swellings induced over the 6 h (measured as the area under the time course curve, AUC) were also significantly suppressed to 62.99±7.44 %,44.09±4.91 %, and 70.98±6.49 % of the inflamed control response respectively (Fig. 4B). Administered in the same doses after the induction of the carrageenan paw oedema(curative), *L. Welwitschii*ca used a suppression of the mean maximal swelling to 29.04±3.78 %,14.49 ±2.43 %, and 34.26 ±4.09 % of the inflamed control response respectively (Fig. 4C). The total paw swellings induced over the 6 h were also significantly

suppressed to 61.13±5.28 %, 37.19 ± 4.38 % and 68.11 ± 4.51 % of the inflamed control response respectively (Fig. 4D). In both protocols the maximum inhibitory effects were attained at a submaximal dose of 200 mg kg^{-1} rather than at the maximal dose of 400 mg kg^{-1}on the parameters assessed. This possibly could be due to an increase in concentration of some pro-inflammatory constituents of the crude extract with increasing dose albeit both doses causing significant suppression.

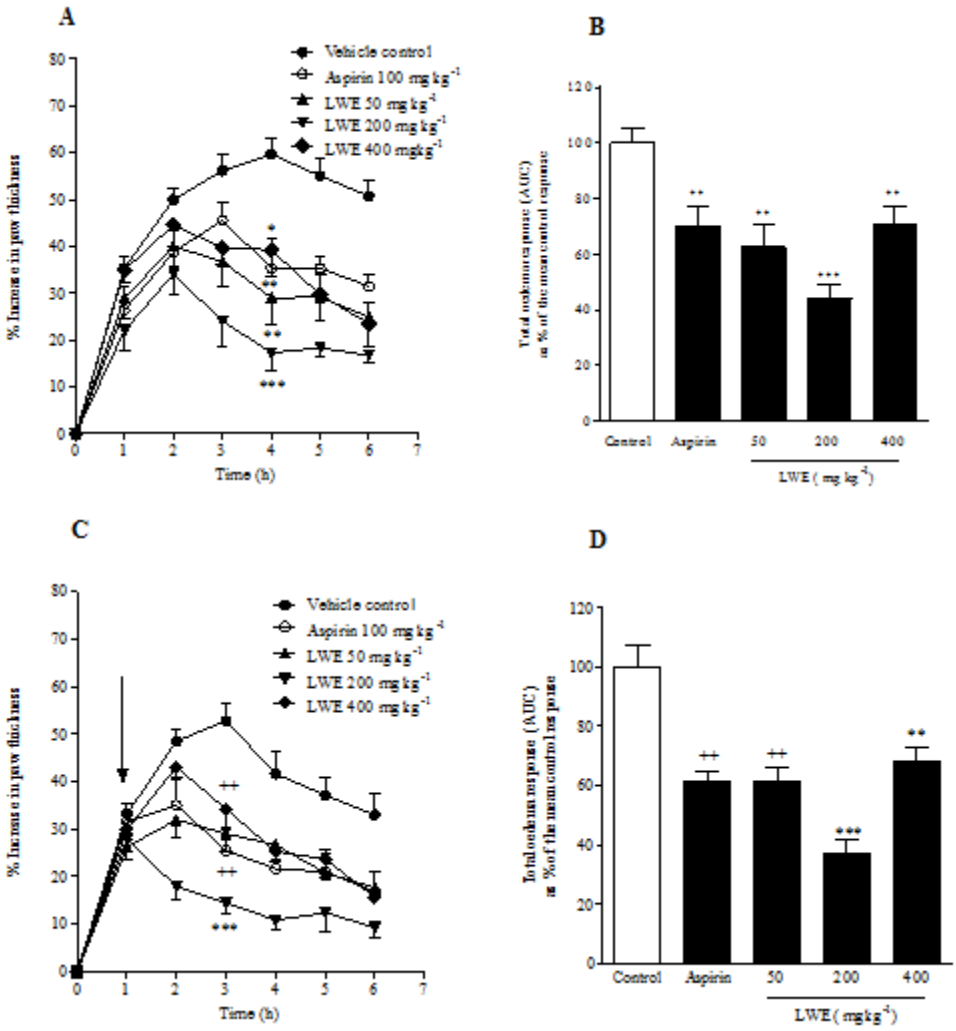

Fig.4:It shows the effect of *Lanneawelwitschii extract* (LWE) on carrageenan-induced oedema (n=5).

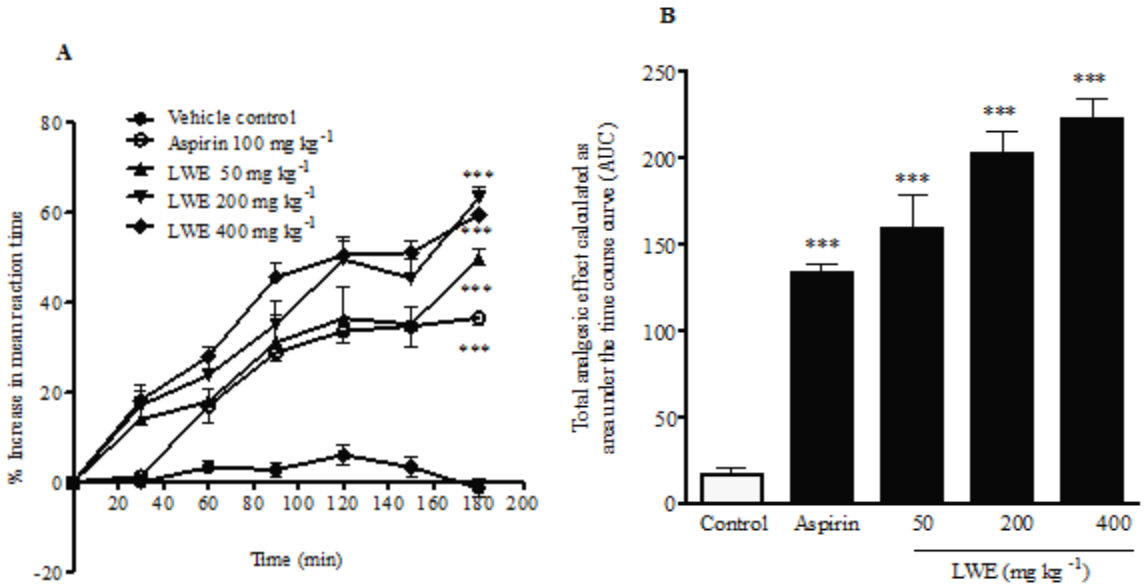

Fig. 5: It shows the effect of *Lanneawelwitschii extract*, LWE on tail immersion test for analgesia (n=5).

Effect of *Lanneawelwitschii extract,* LWE on tail immersion test for analgesia

As pain is one of the cardinal signs of inflammation and not all analgesic drugs pass as anti-inflammatory drugs we investigated the analgesic effect of the plant extract using an animal model that predicts centrally mediated pain. LWE (50, 200, 400 mg kg^{-1}) caused a significant ($P< 0.0001$) and dose-dependent increase in the mean reaction time of treated mice to 49.67 ± 2.18 %, 63.20 ± 2.54 % and 59.42 ± 0.84 % respectively compared to vehicle control group (Fig. 5A) while the total analgesic effect (AUC) was significantly ($P< 0.0001$) and dose-dependently increased to 159.20 ± 19.65, 202.30 ± 12.44 and 228.8 ± 11.29 respectively (Fig. 5B).

DISCUSSION

In this study, the aqueous ethanol extract of the stem bark of *Lanneawelwitschii* was considered as a possible candidate for anti-inflammatory and analgesic activity based on its use in traditional medicine among other things for the treatment of oedema, menstrual pain and pain after childbirth. The dose levels used were informed by acutetoxicity tests carried out by Amole et al. (2010) that presented a well tolerated effect of the drug via the oral route with a dose of 20 g kg^{-1}producing no death in the animals and an $LD_{50}$of 631 mg kg^{-1}i.p. estimated by the Log dose-probit analysis [15, 16].

Allergy a component of the inflammatory response is a consequence of the action of released chemicals such as histamine, lipid derivatives and cytokines from allergen–specific IgE-activated mast cells. Anti-allergic effects of natural products have been demonstrated through examples of *in vitro* and *in vivo* models in which mast cells degranulated by the treatment of cells with the mast cell degranulator compound 48/80 or subjection of animals to passive cutaneous anaphylaxis respectively [17, 18, 19, 20]. Wershil et al. (1987) reports of IgE-mediated pinnal inflammation, as one of the most important *in vivo* models of anaphylaxis in a local allergic reaction induced through the release of histamine [21]. LWE caused a dose-dependent inhibition of this passive cutaneous anaphylaxis. It is conceivable that LWE inhibits the initial phase of immediate type allergic reactions, probably through interference with the degranulation mechanism suggesting that LWE might be useful in the treatment of allergy. Again, in the present work, a true model of immuno-mediated anaphylactic shock in which mice collapsed to death when challenged with a specific allergen as in the LPS-induced allergy was employed. The male C57BL/6 mice are known to be very sensitive to LPS-induced shock [22, 23]. Present in the outer membrane of gram-negative bacteria such as *E. coli*is endotoxin a lipopolysaccharide (LPS) involved in the pathogenesis of gram-negative septic shock [24]. In this test macrophages release endogenous mediators among which istumour necrosis factor alpha, TNFα, a cytokine which causes cardiovascular injury and death [25].Santos et al.(1993)[26] demonstrated a reduction in the cytokines TNFα and IL-6 and lately de Kruif et al.(2007)[27]also reported the possible inhibition of IL-IRA release when glucocorticoids are administered before sepsis induction with LPS. The extract, LWE showed a dose-dependent protection against LPS-induced endotoxic shockup to 75 % survival in the challenged animals that received the highest dose of extract probably due to reduction or inhibition of release of some mediators.

The dose-dependent suppression of clonidine-induced catalepsy demonstrates an indirect anti-histaminic activity of the extract. Clonidine, an α_2-adrenoceptor agonist is known to induce a dose-dependent catalepsywhich is potentiated by pre-treatment with a precursor of histamine, L-histidine[28]. There is evidence also to show that clonidine releases histamine from mast cells in a similar manner to a selective degranulator compound 48/80 [28] and cause degranulation without any damage to the cell wall [29]. Catalepsy produced by clonidine in the mouse is mediated by histamine release from mast cells acting via H_1 receptorand consequently inhibited by histamine H_1 receptor antagonist but not by H_2 receptor antagonist. Our findings that LWE inhibited clonidine-induced catalepsy in mice when administered prophylactically and therapeutically isconsistent with earlier reports that extracts having anti-histaminic or mast cell stabilizing effect inhibit clonidine-induced catalepsy [30].

In this work carrageenan-induced mouse paw oedema was employed to evaluate the acute anti-inflammatory activity of *L welwitschii* stem bark extract. Carrageenan-induced oedema is primarily a vascular event in which exudation of inflammatory cells and fluids at the site of injury initiated by a dilatation of arterioles results in an eventual increase in permeability of post capillary venules [31].The events in carrageenan-induced oedema have several features in common with the early exudative phase of the inflammatory process and the inhibition of this acute phase of inflammation will therefore ultimately attenuate the inflammatory process. Molecular mechanisms underlying carrageenan-induced oedema are a multi-mediated phenomenon that liberates a diversity of mediators. It is believed to be bi-phasic; the first phase (1 h) involves the release of serotonin and histamine while the second phase (over 1 h) is mediated by prostaglandins, the cyclo-oxygenase products, and the continuity between the two phases is provided by kinins [32, 33].Established anti-inflammatory activity for a drug administered before initiation of the inflammatory response does not necessarily imply an ability to act therapeutically. For example, paradoxically, when administered prophylactically, cyclosporin prevented the onset of collagen-induced inflammation in rats but treatment with the drug after the onset of disease exacerbated the condition [34].The suppression of the carrageenan-induced swellings by the *L.welwitschii* extract when administered either before or after the onset of the inflammatory reaction indicates the presence of compounds most likely with actions mediated through interference with the predominant pathophysiological processes underlying inflammation.On account of its effect on carrageenan-induced acute inflammation, the extract may inhibit or interfere with the production of some inflammatory mediators, like the vasoactive amines (serotonin and histamine) as well as the eicosanoids, prostaglandins and thromboxanee specially prostaglandins since the latter mediates the second phase (over 1 h) given the extract showed reduction of induced inflammation after 1 h.

D'Mello and Dickenson (2008) reported that in the transmission of pain following nociceptor stimulation, tissue injury causes among others the release of autocoids such as bradykinin, serotonin, histamine and prostaglandins that may further sensitize and/or activate nociceptors [35]. Despite the nociceptive defense system, inflammatory pain ensues and there are reports to suggest inflammatory response contributes to pain hypersensitivity [36, 37]. Interestingly, phytochemical screening of the plant revealed the presence of several therapeutically valued constituents including flavonoids and tannins [6] and therefore several mechanisms could be responsible for the actions of the extract. Moreover, LWE showed significant analgesic activity in the entire experimental model which may be due to its high flavonoid content. The role of flavonoid, a powerful antioxidant [38, 39], in analgesic activity is primarily to target prostaglandins [40, 41]through inhibition of eicosanoid biosynthesis. Eicosanoids, such as prostaglandins are involved in various immunological responses and are the end products of the cyclooxygenase and lipo-oxygenase pathways [42]. Flavonoids may increase the amount of endogenous serotonin or may interact with 5-HT_2A and 5-HT_3 receptors which may be involved in the mechanism of central analgesic activity [43] as well as suppress the intracellular Ca^{2+} ion elevation and release of pro-inflammatory mediators such as TNFα [44]. Anti-nociceptive activity is also mediated in part by tannins [45]. As pain is one of the cardinal signs of inflammation the inhibitory effect of LWE on nociception demonstrates a pass for analgesic and anti-inflammatory actions.

CONCLUSION

We conclude that the aqueous ethanol extract of *Lanneawelwitschii*has anti-allergic and anti-inflammatory actions mediated through inhibition of histamine release from mast cells. Additionally,*L. welwitschii* exhibits analgesic actions in mice. Our results contribute towards validation of the traditional use of *Lanneawelwitschii* in the treatment of pain and oedema.

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