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Biological investigations of the methanol extract of *Tetrastigma leucostaphylum* (Dennst.) Alston ex Mabb. (Vitaceae): *In vivo* and *in vitro* approach

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ABSTRACT: *Tetrastigma leucostaphylum* (Family: Vitaceae) is popular for its medicinal value in Bangladeshi tribal communities. This study aims to investigate several pharmacological values of methanol extract of *T. leucostaphylum* (METL). *In vivo* analgesic and anti-inflammatory researches have been implemented by using acetic acid-induced writhing and formalin-induced paw licking test protocols in mice. Furthermore, *in vitro* thrombolytic and anthelmintic studies have been performed by following the blood clot lysis method and nematode mortality measurement method. In the *in vivo* study, METL did not minimize the acetic acid-induced writhes prominently but significantly attenuate both the peripheral and inflammatory pain in mice in a dose-dependent manner. In early and late phase, METL 400 (mg/kg, b.w; p.o) showed 39.63 % and 48.73 % paw licking inhibition. Again, METL (100 µL) reflected 56.62 % clot lysis in thrombolytic research. Besides, METL causes the death of nematodes in a dose-dependent manner. The bioassay of the methanol extract of *T. leucostaphylum* justified the analgesic, anti-inflammatory, thrombolytic and anthelmintic activities of the crude extract and finally suggests the test extract as a wellspring of anti-inflammatory, thrombolytic and anthelmintic agents as a crude drug source.

KEYWORDS: *Tetrastigma leucostaphylum*, Writhing, Formalin, Clot lysis, *Tubifex tubifex*.

INTRODUCTION

Pain is a troubling perception often resulting from severe or harmful stimuli [1] where inflammation is the part of a complex physiological reaction to harmful stimuli including pathogenes, injured cells, or irritants by body tissue. It is a defensive response involving immune cells and blood vessels [2]. In spite of conducting several types of researches to develop pain therapy, still, the place of operative and promising analgesic candidates especially for the management of chronic pain is not entirely up for grabs [3]. Phytoconstituents derived from herbal plants are considered as a noteworthy source of novel bioactive molecules exhibiting promising therapeutic activities [4]. Phytochemical studies based on folkloric usage of the medicinal plant to alleviate pain have become rational and promising to discover newer analgesic agents [5]. Alkaloid (morphine), one of the vastly used pain relievers is widespread in treating intense and remorseless pain including injury or cancer-induced pain though it has few severe side effects and thus the

attention of pharmaceutical companies is grabbed to evaluate different medicinal plant extractives to bring newer pain killers with less side effect and more specificity to light [6]. Studies, stratified into various types of pain tests reflect remarkable anti-nociception activity achieved by systemic administration of several alkaloids, terpenoids, and flavonoids such as rutin, quercetin, pectolinarin, and gossypin [3]. Intense breakdown of homeostasis like injury, infection and/or exposure to contaminants can provoke innate immune receptors by pointing out the pathogens with a target of eliminating those from bodies is known as inflammation categorized by redness, pain, heat, swelling, and loss of function in the affected zone [7, 8]. In recent practice, both NSAIDs (Nonsteroidal Anti-Inflammatory Drugs) and steroidal drugs are taken under consideration to treat pain and inflammation through the use of NSAIDs for a prolonged period. But it can impose several side effects and organ damage including liver and gastrointestinal tract along with initiation of cardiovascular complications and renal failure [9, 10]. Thus, to alleviate pain bioactive constituents isolated from plants have been inspected for centuries to establish phytochemicals as anti-inflammatory agents [11]. Besides, phytomedicines acquired from plant extracts may satisfy the desire of nontoxic, more potent, fruitful, and safe drugs to cure pain and inflammation more efficiently [8].

Thrombosis is a fatal disease categorized by thrombus formation in the circulatory system [12]. Alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (tPA) are abundantly used anti-thrombus agents that are administered to dissolve blood clot characterized as thrombolysis process [13, 14]. Though aspirin and heparin are safe to use, they are not profoundly effective to lysis clots and to prevent occlusion. Again, Thrombin inhibitors and antiplatelet agents that are more selective and are supposed to be more potent are having dubious safety [15]. Streptokinase and urokinase are frequently used first-generation drugs to treat thrombosis which possess serious side effects including anaphylactic reaction, systemic fibrinolysis, and hemorrhage due to their weak substrate specificity [16]. Giving streptokinase can also trigger immunogenicity which can confine numerous other treatments for a patient [17]. Besides, selective third-generation thrombolytic agents including monoplane, tenecteplase, reteplase, etc. are contributing with notable angiographic potency in patients with acute myocardial infarction in spite of showing parallel mortality rates with those few agents that have been evaluated in large-scale trials [14, 18]. Thus, persistent research is ongoing to offer advanced thrombolytic agents with utmost coronary arterial thrombolysis with nominal bleeding [19] as currently almost all available synthetically derived thrombolytic agents are possessing notorious drawbacks [20]. In accordance with a research, almost 30 % of all manufactured pharmaceuticals are plant-derived which are less toxic and safe from adverse effects than synthetically derived ones [21]. Herbs and natural food sources along with their supplements having anticoagulant and antiplatelet activity can provide protection from detrimental coronary events and strokes [17]. This incidence accredits extensive researches to ascertain new natural plant-derived sources of thrombolytic agents, as well as antimitotic agents [22].

With the ability to make humans and cattle vulnerable to fungal and bacterial infections, helminths have become the most familiar infectious agents in developing countries and less developed countries which mainly impose a great threat to public health especially to millions of school-going children [23]. Synthetic anthelmintics with several drawbacks make the desired competence apocryphal including resistance [24]. Previous research conducted on the efficacy of anthelmintics reflects that albendazole, levamisole, tetramisole, and ivermectin all can be compromised with resistance issues [25]. One significant approach to embellish cheaper and efficient anthelmintics is to focus on herbal remedies. Thus, evaluation of anthelmintics derived from natural sources is becoming popular day by day in the treatment of parasite infections [4]. Treatments with synthetic regional formulations have several adverse effects and are not available to low income people due to higher drug costs. Demand of medicinal plants is increasing gradually in the search for new medicinal drug for the treatment of several disorders around the world [26].

Tetrastigma leucostaphylum is a woody climber belonging to the family of Vitaceae. Leaves of *T. leucostaphylum* (in wild) can coexist as simple, defoliate, and trifoliate patterns in the same plant [27]. The genus *Tetrastigma* is comprised of 100 species [28]

which are seemingly found in the tropical or subtropical areas of Asian, and Australian undistributed rainforest [29]. Parasitic plants of the Raffesiaceae family, producing the largest flower in the world utilize *Tetrastigma* as sole host [30]. In Malaysia, Indonesia, and Vietnam leaf poultice or extracts of *Tetrastigma* plants are applied externally or internally to combat headaches and fever [31]. Few species of *Tetrastigma* are well-known to treat sore throat, asthma, pneumonia, rheumatism, diarrhea, hepatitis, febrile convulsion, menstrual disorders, scrofula, immune system disorders, and cancer [28]. The local name of *T. leucostaphylum* is Horina lata and has folkloric importance in tribal vicinity of Bangladesh (Chakma, Marma, and Tripura). Previously conducted research suggests that extract of *T. leucostaphylum* is rich in alkaloids, cardiac glycosides, carbohydrates, diterpenes, reducing sugars, phytosterols, saponin, fixed oils and fats and can be used as anesthetics and CNS stimulants due to high alkaloid content [32]. Traditional practice of this plant also includes preventing fecundity, blood feeding, hatching of eggs along with diarrhea and dysentery management [33, 34]. Pharmacological investigations of different plant extracts reveals the medicinal properties of many botanicals, but yet a lot of medicinal plants remain out of investigations. Hence isolation and characterization of healing compounds from these medicinal plants are needed [35]. The research on the medicinal plants should be extended with the identification of the active principles in the plants. Scientific exploration of the remedies is composed of standardization and quality control of the products which is an evaluation procedure to get approval to be used in primary health care by ensuring their safety. Such research activities can also lead to the development of new drugs as in the future [36]. As the best knowledge there is no investigations have been performed regarding the pain, thrombus, and anthelmintic management of this plant extract, this study has been intended to perform the analgesic, anti-inflammatory, thrombolysis and anthelmintic investigation of methanol extract of *T. leucostaphylum.*

MATERIALS AND METHODS

Collection and identification of the plant

Leaves of *T. leucostaphylum* were collected from Hathazari hill, Chittagong hill region, Chittagong, Bangladesh in September 2018. Superior steps were taken to avoid contamination and healthy, fresh products have been reserved. The plant specimen was identified by Sajib Rudra, taxonomist, Bangladesh, and listed for ease of future reference (accession no: CTGUH SR7912) and stored in the Herbarium in the Chittagong University.

Extraction process

Around 500 g of the dried powdered plant materials were taken in separate clean glass bottles and soaked in 2.5 liters of methanol. The container with its materials was fixed by aluminum foil with a container lid and kept for a period of 14 days at 23 ± 2^0 C going with frequent shaking and mixing. The entire mixture was then filtered by cotton followed by the number 1 Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and the filtrates were condensed on a water bath at 40^0 C, in this manner solvent can be evaporated [37]. Finally, 12 g of the crude methanol extracts were yeilded from the pulverized leaves. The crude extract was preserved at the refrigerator under 4^0 C for further research.

Drugs and chemicals

Analytical grade chemicals and drugs have been used in this research and were purchased from BDH (UK) Laboratory Supplies and Sigma (St. Louis, MO, USA), Sanofi Bangladesh Ltd (Tongi, Bangladesh), Square Pharmaceuticals Ltd (Dhaka, Bangladesh) and Beximco Pharmaceuticals Ltd (Dhaka, Bangladesh).

Experimental animals

Standard guidelines for the use and treatment of laboratory animals are extended to the handling and care of animals for the study [38]. Swiss albino mice (weighing about 25–30 g), had been purchased from Jahangir Nagar University, Savar, Bangladesh. The animals received standard laboratory diet and water (*ad libitum*) and followed sufficient ventilation within the room following the normal day-night cycle. All the experiments were carried out in a separate and quiet state. For 10 days before the test, the animals have been adapted to the research laboratory environs. The designed protocol was approved by the P & D (Pharm

P&D - 09/18-193) committee of Department of Pharmacy, International Islamic University Chittagong, Chittagong - 4318, Bangladesh.

In vivo investigations

Acute toxicity test

Previously described method approved by Organization for Economic Cooperation and Development (OECD) [33, 39] with slight modifications has been used to investigate oral acute toxicity of the plant extract. Group of 10 mice received oral doses of 1000 mg/kg, 2000 mg/kg and 3000 mg/kg of METL while the control group received only the vehicle (water). For 48 hours, the groups were monitored. Every day the animals were weighed, and changes were noted in their typical behavior and lack of any signs of harmfulness.

Analgesic and anti-inflammatory investigations

Acetic acid-induced writhing test

The peripheral nociceptive effect of the mice sample was determined by following the acetic acid-induced writhing test. This research has been performed by following the previously established method mentioned by Du, Junrong, et al. [40]. Mice were divided into 4 groups and each group contained 5 mice. The first group was treated as a control group. They were administered orally with vehicle (1% Tween 80 in distilled water). In the second group, mice were treated with diclofenac-sodium (10 mg/kg, b.w; p.o). Third and fourth groups were administered orally with METL 200 and 400 (mg/kg, b.w; p.o) respectively 30 minutes before intraperitoneal injection of 0.6 % acetic acid solution at a dose of 10 mL/kg body weight. After 5 minutes, each group of mice observed for 30 minutes to count the number of writhing responses.

$$\text{Inhibition (\%)} = \frac{(Number\ of\ writhings\ by\ control - Number\ of\ writhings\ by\ test\ sample)}{Number\ of\ writhings\ by\ control} \times 100$$

Formalin induced paw licking test

To evaluate the anti-inflammatory response of METL formalin mediated inflammatory studies were followed [41]. At first, control (1% Tween 80), standard (ibuprofen 10 mg/kg), and test samples (METL 200 mg/kg, and 400 mg/kg) were administered orally. One hour later, 0.2 µL of 2.5% formalin was injected into the sub-plantar of the right-hand paw. Following formalin injection, the mice were immediately kept in a 40 cm^3 diameter jar, and licking time was observed. Licking of the paw was reported and observations expressed as a total licking time in the early stage (0-5 minutes) and the late stage (15-30 minutes) which was representing as neurogenic and inflammatory pain responses respectively.

$$\text{Inhibition (\%)} = \frac{(Number\ of\ lickings\ by\ control - Number\ of\ lickings\ by\ test\ sample)}{Number\ of\ lickings\ by\ control} \times 100$$

In vitro investigations

Thrombolytic assay

Streptokinase (SK) solution preparation

5 mL of sterile distill waters have been properly mixed into the commercially produced lyophilic SK vial (15,00,000 I.U) (PolaminWerk GmbH, Herdecque). This suspension was used as a stock solution for the *in vitro* thrombolysis investigation and finally, 30,000 I.U solutions were used from the stock solution.

Specimen for thrombolytic test

The procedure referred to by Emon, et al. [42] was followed in order to perform this analysis. Volunteers' venous blood (500 µL / tube) was held in the preselected sterile Eppendorf tube, and incubating at a temperature of 37 ° C for 45 minutes. The serum was completely separated after the development of the coagulation. Each tube reweighed to verify the weight of the clot. After removing the clot, 100 µL of METL has been correctly applied to each Eppendorf. Finally, each tube was incubated again for the 90 minutes at 37 °C coagulation. After incubation, the fluid found was detached and tubes were again weighed to detect the change in weight after clot disruption. The weight difference before and after clot lysis was considered to the final percentage of clot lysis. Water and streptokinase have been used as the positive and the negative and positive controls respectively. The following formula has been followed to determine the percent of clot lysis.

$$\text{\% clot lysis} = \frac{Weight\ of\ the\ lysis\ clot}{Weight\ of\ clot\ before\ lysis} \times 100.$$

Anthelmintic assay

Previously described method [43] with slight modification, the anthelmintic research has been implemented. Nearly 6-8 worms (*Tubifex tubifex*) were taken in each of six Petri dishes, and METL at different concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL) were added in 3 Petri dishes and standard levamisole at different concentrations (1 mg/mL, 0.8 mg/mL, 0.5 mg/mL) were added in rest 3 Petri dishes. Then the beginning of the paralysis and the time of the worms' death were observed carefully. In two distinct parameters, 'time for paralysis' and 'time for death' of the worms were determined to distinguish the anthelmintic effect of the sample. Paralysis time was recorded if no movement could be detected. Upon confirmation, time taken to kill worms was noted if the worms did not react either when shaken vigorously or when plunged into the slightly warm water.

Statistical analysis

Statistical analysis was interpreted as mean ± standard error (SEM) and the data were analyzed using GraphPad Prism 5.2 (GraphPad Software, Inc., La Jolla, CA, United States). Statistical significance was determined by a one-way variance analysis (ANOVA) followed by Dunnett's test, where *P < 0.5, **P < 0.01, and ***P < 0.001 was considered statistically significant.

RESULTS

Effect of extracts on oral acute toxicity test

During the observations, there was no lethality, no behavioral change (sedation, excitability) or no allergies reaction was appeared after the oral administration of METL.

Effect of the extracts on acetic acid-induced writhing test

Peripheral analgesic activity determination by the acetic acid-induced writhing study was measured on the basis of the average number of abdominal constrictions shown during the writhing test by extending a hind paw to animals (mice). Inhibition was observed for 20 min in the writhing of the test extract. In contrast to the control group, a dose of 200 mg/kg inhibited 8.28% of writhes. Dose of 400 mg/kg inhibited 31.31 % (P < 0.05) of writhes while the standard drug diclofenac-sodium exhibited 66.58 % (P < 0.001) of inhibition. The overall results of the control and test samples have been narrated in Table 1. Comparing with standard drug diclofenac-sodium, the therapeutic activity of METL was less than the standard drug diclofenac sodium.

Table 1. Effect of test samples on the analgesic study of the METL on the acetic acid-induced writhing test in mice.

Treatments	Number of writhing
TWN 80 - 10 mg/mL	39.50 2.54$^{\#}$
DFN – 10 mg/kg	13.20 1.98***
METL - 200 mg/kg	36.25 2.38
METL - 400 mg/kg	27.13 1.32*

The values were presented as mean ± SEM; One-way analysis of variance (ANOVA) was followed by Dunnett's test. *P < 0.05, **P < 0.01 and ***P < 0.001, where # was designated as control. METL = methanol extract of *Tetrastigma leucostaphylum* leaves, TWN 80 = 1% Tween 80, and DFN = diclofenac sodium.

Effect of extracts on formalin-induced paw licking test

The formalin test exhibited moderate anti-inflammatory activity. When METL was administered orally at 200 and 400 mg/kg according to body weight, the licking time in both the early and late phases was decreased in a dose-dependent manner. METL at 200 mg/kg caused significant inhibition of licking in both early phase (28.34 %) (P < 0.01) and late phase (33.72 %) (P < 0.01). METL at 400 mg/kg also caused significant inhibition of licking in early phase (39.63%) (P < 0.01) and late phase (48.73%) (P < 0.001). Standard drug diclofenac-sodium inhibited paw licking 53.39 % (P < 0.001) in early phase and 54.67% (P < 0.001) in late phase when compared with control (1% tween 80) group animals. The summary of the findings has been enumerated in Figure 1.

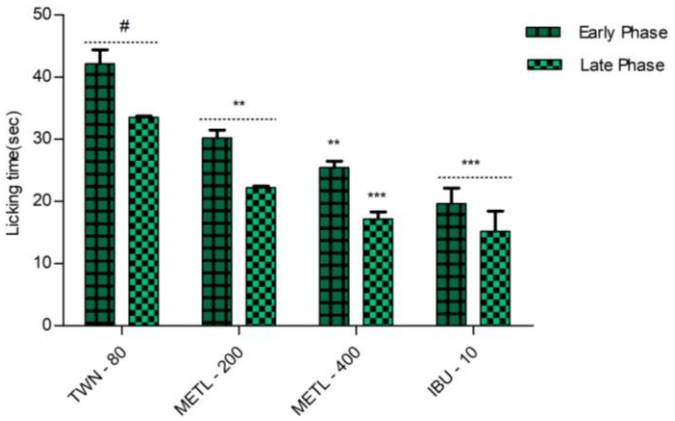

Figure 1. Effect of test samples on the anti-inflammatory investigation of the METL on the formalin-induced licking test. The values were presented as mean ± SEM; One-way analysis of variance (ANOVA) was followed by Dunnett's test. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001, where # was designated as control. METL = methanol extract of *Tetrastigma leucostaphylum* leaves, TWN 80 = 1% Tween 80, and IBU = Ibuprofen.

Effect of the extracts on thrombolytic activity

In the *in vitro* thrombolytic assay, the extract exerted 26.08 % (*P* < 0.001) lysis of the blood clot while standard streptokinase was obtained 56.62 % (*P* < 0.001) blood clot lysis. The summary of the findings has been enumerated in Figure 2.

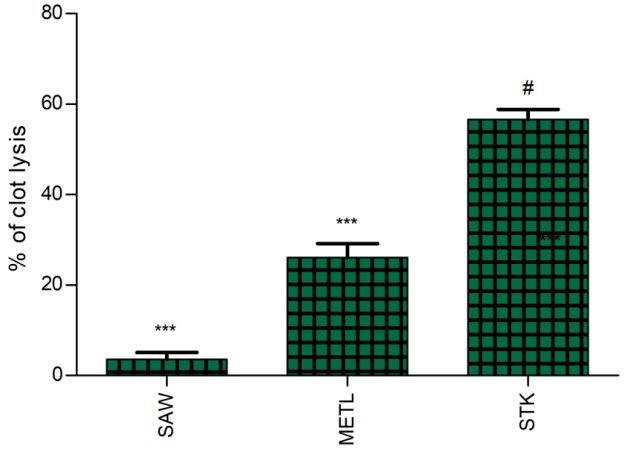

Figure 2. Clot lysis effects by Saline water, Streptokinase, and METL. The values were presented as mean ± SEM (n = 10); One-way analysis of variance (ANOVA) was followed by Dunnett's test. ****P* < 0.001 was considered as significant compared with the control, where # is designated as control. METL = methanol extract of *Tetrastigma leucostaphylum* leaves, SW = saline water, and STK = streptokinase.

Effect of extracts on anthelmintic activity

A methanol extract of *T. leucostaphylum* leaves was used to determine the anthelmintic activity and the extract showed the effect in a dose-dependent manner. METL took 9.3 ± 1.16 minutes for paralysis and 32.45 ± 2.35 minutes for death at the maximum concentration (10 mg/mL) while standard levamisole took 4.5 ± 1.38 minutes for paralysis and 8.55 ± 1.33 minutes for death at a concentration of 1 mg/mL (Figure 3).

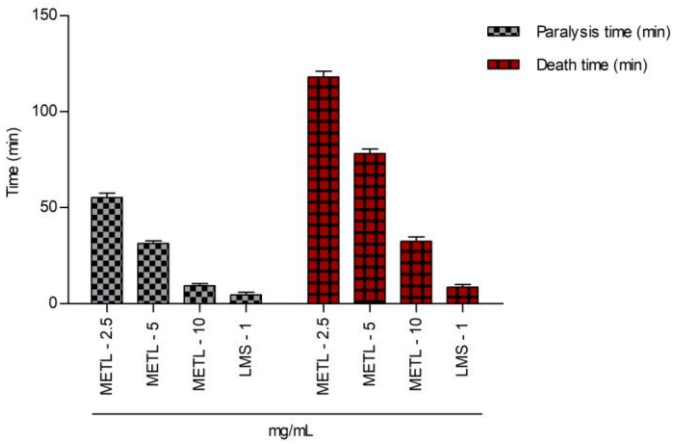

Figure 3. Effect of various test doses of the METL and LMS on nematodes (n = 6) in the anthelmintic study. Values were presented as mean ± SEM. METL = methanol extract of *Tetrastigma leucostaphylum* leaves, and LMS = levamisole.

DISCUSSION

The study was performed to investigate the analgesic, anti-inflammatory, thrombolytic and anthelmintic significance of methanol extract of *T. leucostaphylum* leaves. The experiments showed negligible anti-nociception activity after the oral administration of METL and not significantly decreased abdominal writhing in mice. Acetic acid-induced abdominal restriction response is an established procedure in peripheral analgesic agent research. Prostaglandin receptors are thought to mediate this response [44]. METL has generated less anti-nociceptive behavior and therefore suggests that the prostaglandin receptor secretion could not be attenuated strongly by the components of METL. Throughout the scientific studies, METL has shown dose-dependent activity in anti-inflammatory research. For the justification of pain and analgesia the formalin-induced pain approach is quite worthwhile [45]. The formalin-induced pain experiment includes two stages of painful exposure [46]. The first stage is the early (neurogenic pain) stage which usually results from peripheral stimulation through C-fibre [47]. This stage begins immediately after the injection of the formalin solution and takes 5-10 minutes. The 2nd (late) stage (inflammatory pain) is intensified by inflammation of local tissues and physiological alteration of the dorsal horn of the spinal

cord which begins about 20 minutes after the formalin injection. These process has been inhibited by opioid and anti-inflammatory medicines [41]. Therefore, central analgesics such as morphine often block early and late phases while medications like adrenaline (dexamethasone), or NSAIDs (aspirin), often inhibit pain in late-phase [48]. In formalin study, it was assumed that METL substantially ($P < 0.01$ and $P < 0.001$) attenuated the hyperalgesia in both phases which has been mediated throughout the formalin injection. Disruption of hemostasis can cause a blood clot (thrombus) in the circulatory system and results in vascular blockage followed by detrimental consequences in thrombolytic diseases including acute myocardial or cerebral infarction which may lead to death [1].

Thrombolysis is the sum of several detrimental cascades related to arterial diseases connected with acute coronary disorders such as pulmonary emboli, deep vein thrombosis, strokes, heart attacks, and venous thromboembolic complications which can result in abrupt morbidity and mortality [12]. In the physiological system, vascular barricade results from thrombosis, and recuperating events can lead to fatal consequences including cerebral or myocardial infarction followed by death [20]. The hypothesis of cascade and waterfall [49, 50] shows that the coagulation process takes place in three stages: the formation of the thrombin, activation of prothrombin, and formation of fibrin. The results of this study have shown that METL has enabled lysis to the extrinsic coagulation which may act as the driving force of antithrombotic and thrombolytic action. Furthermore, the low dose intake of crude extract has no or fewer side effects and the body's coagulation system will not be hampered. For long-term thrombosis prevention, low dose METL is therefore recommended.

In the anthelminthic study, METL increased the paralysis and death of *Tubifex tubifex* within a short time in a dose-dependent manner. Helminths, worm-like organisms reside inside the living host and assemble nourishment from it which results in disruption in hosts nutrient absorption mechanism. Through skin or gastrointestinal tract juvenile forms of the parasites can invade human beings and go under maturation to form adult worms by arresting nutrition [51]. Anthelmintics, a group of antiparasitic drugs perform locally to expel worms from the gastrointestinal tract [52] or systematically to restrict helminths from doing any noteworthy damage to host [53]. METL's anthelmintic behavior may be exhibited due to the involvement of the phytoconstituents of the plant extract. Phytoconstituents can interfere with the generation of energy by de-aligning oxidative phosphorylation, or due to the interfere of phytoconstitents with the glycoproteins of the cell surface.

CONCLUSIONS

The study revealed significant anti-inflammatory, thrombolytic, and anthelmintic potentiality of methanol extract of *T. leucostaphylum* leaves and can be considered as a prominent candidate to be a lead compound for drug discovery and drug designing. Chemical dynamics of the plant extract has been presumed to trigger the biological activity of the plant extract. Finally, further investigations following chemical profiling are recommended to confirm the biological activity of the plant extract.

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AUTHOR CONTRIBUTIONS

SR, SUS, and NUE conceptualized, designed, and prepared the research protocols. SUS and NUE performed the laboratory experiments. MMRK, AMSC, ANH, and MS curated the data. SAS, SR, SA and NUE interpreted the data. SA, NUE and SUS drafted the manuscript. SMT and MAS supervised while MNI monitored the research. Finally, all the authors revise the final draft and agreed to publish the manuscript as an original research article.

CONFLICTS OF INTEREST

Authors declared that they have no conflict of interest.

REFERENCES

[1] Viana F. Nociceptors: thermal allodynia and thermal pain. Handbook of clinical neurology: Elsevier; 2018. p. 103-19.

[2] Ferrero-Miliani L, Nielsen O, Andersen P, Girardin S. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clinical & Experimental Immunology. 2007;147:227-35.

[3] Calixto JB, Beirith A, Ferreira J, Santos AR, Filho VC, Yunes RA. Naturally occurring antinociceptive substances from plants. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2000;14:401-18.

[4] Farnsworth N. Screening Plants for New Medicines. Biodiversity. Part II. Washington: National Academy Press; 1989.

[5] Elekwa I. Preliminary phytochemical screening and gas chromatographic FID evaluation of Garcinia kola seed extracts. Journal of Pharmacognosy and Phytochemistry. 2014;2.

[6] Musa Y, Haruna A, Ilyas M, Yaro A, Ahmadu A, Usman H. Phytochemical, analgesic and anti-inflammatory effects of the ethylacetate extract of the leaves of Pseudocedrella kotschyii. African Journal of Traditional, Complementary and Alternative Medicines. 2008;5:92-6.

[7] de Melo MS, Quintans JdSS, Araújo AAdS, Duarte MC, Bonjardim LR, Nogueira PCdL, et al. A systematic review for anti-inflammatory property of clusiaceae family: a preclinical approach. J Evid Based Integr Med. 2014;2014.

[8] Yasmen N, Aziz M, Tajmim A, Akter M, Hazra AK, Rahman S. Analgesic and anti-inflammatory activities of diethyl ether and n-hexane extract of polyalthia suberosa leaves. J Evid Based Integr Med. 2018;2018.

[9] Ingrasciotta Y, Sultana J, Giorgianni F, Fontana A, Santangelo A, Tari DU, et al. Association of individual non-steroidal anti-inflammatory drugs and chronic kidney disease: a population-based case control study. PLoS One. 2015;10.

[10] Shih S-C, Chang C-W. Nonsteroidal anti-inflammatory drug-related gastrointestinal bleeding in the elderly. International Journal of Gerontology. 2007;1:40-5.

[11] Howes M-JR. Phytochemicals as anti-inflammatory nutraceuticals and phytopharmaceuticals. Immunity and Inflammation in Health and Disease: Elsevier; 2018. p. 363-88.

[12] Mahmud S, Akhter S, Rahman M, Aklima J, Akhter S, Merry SR, et al. Antithrombotic effects of five organic extracts of Bangladeshi plants in vitro and mechanisms in in silico models. J Evid Based Integr Med. 2015;2015.

[13] Fathima SN, Ahmad SV, Kumar BR. Evaluation of in vitro thrombolytic activity of ethanolic extract of Curcuma caesia rhizomes. International Journal of Pharma Research & Review. 2015;4:50-4.

[14] Sayeed MA, Kabir H, Rashid MMU, Bhuiyan MFA, Rashid MA. Thrombolytic activity of methanolic extracts of Desmodium paniculatum (L.) and Sarcochlamys pulcherrima (Roxb.). Bangladesh Pharmaceutical Journal. 2014;17:67-9.

[15] Dibyajyoti S, Swati P. Phytochemical Screening and Thrombolytic Activity of Chloroform Extract of Urena Sinuata (L.). Journal of Fundamental and Applied Sciences. 2014;6:208-19.

[16] Mannan A, Kawser MJ, Ahmed AA, Islam NN, Alam SM, Khan MAE, et al. Assessment of antibacterial, thrombolytic and cytotoxic potential of Cassia alata seed oil. Journal of Applied Pharmaceutical Science. 2011;1:56.

[17] Furie B, Furie BC. Mechanisms of thrombus formation. New England Journal of Medicine. 2008;359:938-49.

[18] Emon NU, Kaiser M, Islam M, Kabir MFI, Jamir M, Uddin MAJ, et al. Anxiolytic and thrombolytic investigation of methanol extract of Piper nigrum L. fruits and Sesamum indicum L. seeds.

[19] Collen D. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator? Annals of internal medicine. 2020.

[20] Nicolini FA, Nichols WW, Mehta JL, Saldeen TG, Schofield R, Ross M, et al. Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue-plasminogen activator. Journal of the American College of Cardiology. 1992;20:228-35.

[21] Annapurna A, Mahalakshmi DK, Krishna KM. Antidiabetic activity of a poly herbal preparation (tincture of panchparna) in normal and diabetic rats. 2001.

[22] Wagnor H, Hikino H, Farnsworth R, London N. The economic significance of plants and their constituents as drug. Economic and medicinal plant research. 1989;3:1-17.

[23] Silva Soares SC, de Lima GC, Carlos Laurentiz A, Féboli A, dos Anjos LA, de Paula Carlis MS, et al. In vitro anthelmintic activity of grape pomace extract against gastrointestinal nematodes of naturally infected sheep. International journal of veterinary science and medicine. 2018;6:243-7.

[24] Zenebe S, Feyera T, Assefa S. In vitro anthelmintic activity of crude extracts of aerial parts of Cissus quadrangularis L. and leaves of Schinus molle L. against Haemonchus contortus. BioMed research international. 2017;2017.

[25] Wakayo B, Dewo T. Anthelmintic resistance of gastrointestinal parasites in small ruminants: a review of the case of Ethiopia. J Veterinar Sci Technol S. 2015;10.

[26] Herrera-Ruiz M, García-Beltrán Y, Mora S, Díaz-Véliz G, Viana GS, Tortoriello J, et al. Antidepressant and anxiolytic effects of hydroalcoholic extract from Salvia elegans. J Ethnopharmaco. 2006;107:53-8.

[27] Krishna T, Krishna T, Chithra N, Deepa P, Darsana U, Sreelekha K, et al. Acaricidal activity of petroleum ether extract of leaves of Tetrastigma leucostaphylum (Dennst.) Alston against Rhipicephalus (Boophilus) annulatus. The Scientific World Journal. 2014;2014.

[28] Adarsh Krishna T, Ajeesh Krishna T, Sanyo Raj V, Juliet S, Nair S, Ravindran R, et al. Evaluation of phytochemical constituents and proximate contents of the ethanolic leaf extract of Tetrastigmaleucostaphylum (Dennst.) Alstone (Vitaceae) found in Western Ghats of Kerala, India. Research Journal of Pharmaceutical Sciences ISSN. 2013;2319:555X.

[29] Hossain MA, Shah MD, Gnanaraj C, Iqbal M. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah. Asian Pac J Trop Med. 2011;4:717-21.

[30] Barcelona JF, Pelser PB, Cajano MO. Rafflesia banahaw (Rafflesiaceae), a new species from Luzon, Philippines. Blumea-Biodiversity, Evolution and Biogeography of Plants. 2007;52:345-50.

[31] Dao PTA, Le Quan T, Mai NTT. Some compounds from stem of tetrastigma erubescens planch.(vitateae). Journal of Engineering Technology and Education. 2013;9:54-7.

[32] Madziga H, Sanni S, Sandabe U. Phytochemical and elemental analysis of Acalypha wilkesiana leaf. Journal of American Science. 2010;6:510-4.

[33] Van den Heuvel M. A New Approach to the Classification of Substances and Preparations on the Basis of their Acute Toxicity: A Report by the British Toxicology Society* Working Party on Toxicity. Human Toxicology. 1984;3:85-92.

[34] Ashraf MA, Khatun A, Sharmin T, Mobin F, Tanu AR, Morshed T, et al. MPDB 1.0: a medicinal plant database of Bangladesh. Bioinformation. 2014;10:384.

[35] Tasleem F, Azhar I, Ali SN, Perveen S, Mahmood ZA. Analgesic and anti-inflammatory activities of Piper nigrum L. Asian Pac J Trop Med. 2014;7:S461-S8.

[36] Pattanayak P, Behera P, Das D, Panda SK. Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. Pharmacognosy reviews. 2010;4:95.

[37] Alam S, Emon NU, Rashid MA, Arman M, Haque MR. Investigation of biological activities of Colocasia gigantea Hook. f. leaves and PASS prediction, in silico molecular docking with ADME/T analysis of its isolated bioactive compounds. BioRxiv. 2020.

[38] National Research Council Committee for the Update of the Guide for the C, Use of Laboratory A. The National Academies Collection: Reports funded by National Institutes of Health. In: th, editor. Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US) National Academy of Sciences.; 2011.

[39] Walum E. Acute oral toxicity. Environmental health perspectives. 1998;106:497-503.

[40] Du J, Yu Y, Ke Y, Wang C, Zhu L, Qian ZM. Ligustilide attenuates pain behavior induced by acetic acid or formalin. J Ethnopharmaco. 2007;112:211-4.

[41] Emon NU, Jahan I, Sayeed MA. Investigation of antinociceptive, anti-inflammatory and thrombolytic activity of Caesalpinia digyna (Rottl.) leaves by experimental and computational approaches. Advances in Traditional Medicine. 2020:1-9.

[42] Emon NU, Jahan I, Rudra S, Aktar M, Islam N. In Vitro Comparative Cardioprotective Activity of Methanol Extract of Caesalpinia Digyna (Rottl.) Stems and Senna Sophera (L.) Roxb. Stems.

[43] Tariq K, Chishti M, Ahmad F, Shawl A. Anthelmintic activity of extracts of Artemisia absinthium against ovine nematodes. Veterinary parasitology. 2009;160:83-8.

[44] Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH, et al. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol. 2000;387:111-8.

[45] Jimoh AO, Chika A, Umar MT, Adebisi I, Abdullahi N. Analgesic effects and anti-inflammatory properties of the crude methanolic extract of Schwenckia americana Linn (Solanaceae). J Ethnopharmaco. 2011;137:543-6.

[46] Moniruzzaman M, Imam MZ. Evaluation of antinociceptive effect of methanolic extract of leaves of Crataeva nurvala Buch.-Ham. BMC Complement Altern Med. 2014;14:354.

[47] Wooten M, Weng H-J, Hartke TV, Borzan J, Klein AH, Turnquist B, et al. Three functionally distinct classes of C-fibre nociceptors in primates. Nature communications. 2014;5:1-12.

[48] Pelayo VdRT, Villalba BS, Guadarrama AAD, Reyes AM, Pernas MÁC. Thermal nociceptive tail-flick reflex development in Wistar male rats. Revista Médica de la Universidad Veracruzana. 2007;7:8-11.

[49] Riddel Jr JP, Aouizerat BE, Miaskowski C, Lillicrap DP. Theories of blood coagulation. Journal of Pediatric Oncology Nursing. 2007;24:123-31.

[50] Toussaint EF, Short AE. Transoceanic stepping–stones between cretaceous waterfalls? The enigmatic biogeography of pantropical Oocyclus cascade beetles. Molecular phylogenetics and evolution. 2018;127:416-28.

[51] Joseph B, George J, Mohan J. Pharmacology and traditional uses of Mimosa pudica. International journal of pharmaceutical sciences and drug research. 2013;5:41-4.

[52] McSorley HJ, Chayé MA, Smits HH. Worms: Pernicious parasites or allies against allergies? Parasite immunology. 2019;41:e12574.

[53] El-Rahman MA, Abdel-Nabi IM, Omran MA, Mohamed MF. Cytotoxic effects of albendazole, an antiparasitic drug, on the liver of the rat: subchronic study. Egyptian journal of Biology. 1999;1:16-29.