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In-vivo analgesic and anti-inflammatory activities in swiss albino mice and in-vitro thrombolytic activity of methanol extract of ten days mature whole plant of *Triticum aestivum*

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ABSTRACT

The aim of this study was to evaluate the analgesic, anti-inflammatory and thrombolytic activities of methanolic extract of *Triticum aestivum*. The analgesic and anti-inflammatory activities of the plant extract was evaluated by formalin induced licking and biting test and carrageenan induced paw edema methods respectively in Swiss albino mice. The thrombolytic activity was evaluated by clot lyses test found for human. The methanolic extract of this plant showed the dose dependent analgesic effect by formalin induced licking and biting test in mice. The extract was capable of inhibiting of 35.69% and 45.72% licking and biting at dose 100 and200 mg/kg, respectively (at early phase) and42.7% and 54.65% of inhibition at dose 100 and 200 mg/kg respectively (at later phase) compared to reference standard drug (10 mg/kg acetyl salicylic acid).During carrageenan induced paw edema test, at dose 400mg/kg, the extracts showed moderate anti-inflammatory potential in mice model, showing inhibition of edema 19.023% at final hour compared with reference standard drug Ibuprofen. In case of in vitro thrombolytic activity test, at dose 10 mg/ml, the plant extract showed significant thrombolytic activity, 57.90% lysis of clot compared to reference standard drug Streptokinase. The result demonstrated that the plant extract possess analgesic, anti-inflammatory and thrombolytic properties that justified the uses of the plant extract as tradition medicine for the treatment of various diseases.

Keywords: *Triticum aestivum,* analgesic, anti-inflammatory and thrombolytic.

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INTRODUCTION

Plants containing medicinal values contribute significantly to the medicinal or drug preparations. Thousands of medicinal plants have been used to treat various disorders.[1] Due to their potent pharmacological effects, the proper investigation of traditional plants containing different types of active metabolites can open a new dimension in the field of new drugs discovery.[2,3]

*Triticum aestivum*is a grain crop which is distributed in almost every countries in the world.[4] It belongs to Germineae family and is the most essential esculent grain crop. It is commonly known as wheat grass and is enriched in different type of nutrients. Recently it is becoming the most important source of supplemental foods around the world. It not only possess supplemental value but also anti-oxidant and anti-inflammatory effects due to the presence of different type of bioactive compounds in this plant such as- flavonoids, phenolic compounds, vitamins, minerals and others compounds.[5]

Moreover, this plant is rich in chlorophyllin which is the derivative of chlorophyll. Chlorophyllin possess bacteriostatic properties that helps in wound healing process. Scientists reported its wound healing, anti-inflammatory and odor reducing properties. Furthermore, it is also used in skin diseases, anal fissure, chronic sinusitis, typhoid fever, uterine cervical pain& different other types of infections.[6]

The main cause of morbidity and mortality in developed countries are deep vein thrombosis, pulmonary emboli, strokes and heart attack etc. Now a days, various thrombolytic agents are used as thrombolytic therapy to dissolve clot.[10,11]

In this investigation, we used methanol extract of this whole plant to evaluate analgesic, anti-inflammatory and thrombolytic activity.

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MATERIALS AND METHODS

Plant materials

The plant *Triticum aestivum* was collected from Barisal and identified by Bangladesh National Herbarium, Mirpur (Accession no: 35637). The collected plant materials were separated from undesirable substances. They were subjected to shade dry for one week and grinded into a course powder with the assistance of a grinder. The powder was properly preserved on an airtight container in a cool, dark and dry place until the analysis started.

Extraction process

About 400 g of course powered material of the plants were soaked in 1600 ml of 80% methanol and waited with shaking and stirring condition at a regular time intervals for 10 days. Then the entire mixture passed through a rough filtration process by a lump of fresh white cotton material. Finally it had been filtered through Whatman's filter paper. The filtrate then evaporated by water bath and the resultant was a gummy concentrate reddish black material designated as crude methanolic extract.

Animals

Swiss albino mice, weighing 24-26 g, were collected from International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB). The mice were kept under ambient environmental condition with 12 hours light-dark cycle and acclimatized for 7 days prior to the experiment. The investigation conveyed by following the approval of International Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Blood Samples

Five ml of venous blood sample was collected from each healthy male human volunteers (n=3) under standard condition. The collected blood was kept into different pre-weight eppendorf tubes, each containing 500 µl blood.

In Vivo Analgesic Activity Test

Formalin induced licking and biting test

The analgesic activity of methanolic extract of the plant was observed on ***Swiss albino*** mice by a liking and biting test. During this study, 12 mice were randomly divided into four groups where each group contained 3 mice. Control group (receiving normal distilled water), standard group(receiving acetyl salicylic acid) and two study groups with 200 and 400 mg/kg dose of plant extracts were injected through intra peritoneal route prior to injecting any kind of pain inducer. In this test, 1% formalin act as pain inducer agent. Here right hind paw of mice was subcutaneously injected with 20 µl 1% formalin.[7]

The licking and biting time is mainly counted for the measurement of pain response. At the time of 5 minutes (early phase) and 20 minutes (late phase) after formalin injection, the response of the mice was observed sincerely.

In Vivo Anti-inflammatory Test

Carrageenan-induced paw edema method

The investigative ***Swiss albino*** mice were divided into four groups and each group containing five mice. Here we actually observed acute inflammation test on Swiss albino mice. 1% carrageenan was applied as inflammation inducer agent. Prior to injecting inducer agent, the plant extract of different doses (200 and 400 mg/kg), normal saline (1ml/kg) and Ibuprofen (10 mg/kg) were injected via intra peritoneal route. 1hr later, 0.1 ml 1% carrageenan was injected into the plantar area of the mice hind paw. The paw volume of the mice were measured at 0, 1, 2, hours by using a vernier caliper.[8] This vernier caliper mainly determine the edema diameter. The reading differences at different time interval were the indicator of the diameter of edema.

Thrombolytic activity

The plant extract was used in *in vitro* thrombolytic test according to method of clot lyses test.[9] 5 ml of venous blood was drawn out from 3 healthy volunteers. Then the 500 µl blood samples were transferred to different pre weighed eppendorf tubes (1ml). These eppendorf tubes were incubated in incubator at temperature 37°C for about 45 minutes. After the clot formation, the serum of the blood samples was completely removed from the tube and the clot weight determined by the following formula:

Clot weight = (Weight of clot containing tube – Weight of tube alone).

After that, 100 µl of plant extract of each dose(2, 4, 6, 8, 10 mg/ml) were added to each eppendorf tube containing only clot. In this investigation, 100 µl of streptokinase and 100 µl of distilled water were used as positive control and negative control respectively. Then these eppendorf tubes were again incubated about 90 minutes at 37°C for observing lysis of clot. After the incubation, the fluids obtained from the lysis of clot were discard and weighed the clot containing tubes for determining the percent of lysis of clot. The percent of lysis of clot can be calculated by the following equation:

% lysis of clot = (Wt. of released clot / Wt. of clot before treatment) × 100%

RESULTS

Investigation of formalin Induced liking and bitingTest

The following data represents the analgesic effect of the plant extracts by formalin induced liking and biting test. At the later phase, the highest inhibition of 54.65% was shown at a dose of 200 mg/kg when compared to the reference drug acetyl salicylic acid. Besides, the inhibition rate of 42.7% at dose of 100 mg/kg was also observed.

Carrageenan induced paw edema test

The plant extract showed moderate anti-inflammatory effect [when compared to reference standard drug Ibuprofen (10 mg/kg)] by reducing paw edema during experiment. At dose of 400 mg/kg, the percent inhibition of edema by the plant extracts at the 0, 1 and 2 hours were 16.88%,18.22%, and 19.023% accordingly.

Investigation of Thrombolytic Activity

The following data showed the clot lysis capability of the plant extracts. Enzyme (100µl) as positive control provided 62.18% clot lysis and water (as negative control) was providedonly 3.87% clot lysis. In this study groups, crude methyl alcohol extract (ME) at 10 mg/ml dose showed 57.90% clot lysis where the negative control group showed very negligible amount of clot lysis.

DISCUSSION

Medicinal plants have a long history of serving people in many regions worldwide. 80% of the world population still uses herbal and medicinal plants in treating various ailments because such plants possess different types of phytoconstituents which exert a variety of pharmacological effects in human body.[16,17] According to the previous studies, phytochemical screening on most

Table 1 **Comparative analgesic activity of methanolic extract of *Triticum aestivum* and standard drug acetyl salicylic acid**

Groups	Dose (mg/kg)	Early phase	% Inhibition	Late phase	% Inhibition
Group I	Vehicle	37 ± 2.1	-	27.20± 1.71	-
Group II	10 (standard drug)	11.25 ±0.98	71.88	8.25 ± 0.98	67.65
Group III	100 (ME)	20.1 ± 0.26	35.69	16.91± 3.11	42.7
Group IV	200 (ME)	11.3±0.68**	45.72	9.45± 1.23	54.65

Here, ME= Methanolic extract and values were expressed as mean± SEM (standard error mean), n=3. Significance value *p<0.05 compared with vehicle control. Statistical comparison was done by using one way ANOVA followed by Dunnet t test.

Table 2 **Comparative anti-inflammatory activity of methanolic extract of *Triticum aestivum* and standard drug Ibuprofen**

		Edema diameter (Mean±SEM) mm			Percent Inhibition of edema		
Groups	Dose (mg/kg)	0 min	1 hr	2 hr	0 min	1 hr	2 hr
Group I	Vehicle	3.85±0.24	3.86±0.41	3.86±0.45			
Group II	10 (Standard drug)	2.75±0.45*	1.88±0.46*	1.63±0.46*	32.2	51.4	58.12
Group III	200 (ME)	3.03±0.62	3.01±0.52*	2.92±0.56	16.6	17.44	17.97
Group IV	400 (ME)	3.11±0.82*	2.88±0.72	2.76±2.14*	16.88	18.22	19.023

Here, ME= Methanolic extract and values were expressed as mean ± SEM (standard error mean), n=5. Significance value *p<0.05 compared with vehicle control. Statistical comparison were done by using one way ANOVA followed by Dunnet t test.

Table 3 **Comparative thrombolytic activity of methanolic extract of *Triticum aestivum***

Treatment Group	Dose	% of clot lysis
ME	2 mg/ml	31.44
ME	4 mg/ml	32.95
ME	6 mg/ml	42.66
ME	8 mg/ml	50.94
ME	10 mg/ml	57.90
Water		03.87
Streptokinase		62.18

Here, ME= Methanolic extract.

of the medicinal plants revealed the presence of several phytochemicals like alkaloids, carbohydrates, tannins, phenol, terpenes, fats and fixed oils. These phytochemicals are responsible for various biological actions including antioxidant activity.[17]

By acting in the CNS or on the peripheral pain mechanism, analgesic compounds selectively relieves pain without significant alteration of consciousness. Actually analgesics are applied when the noxious stimulus cannot be removed or as adjuvants to more etiological approach to pain.[18,19] Formalin induced analgesic test on mice model is one of the most effective methods for explaining the mechanism of pain.[13] Formalin induced pain in mice paw is bi-phasic. In early phase involve neurogenic pain and the late phase involves inflammation related pain. Here most probably early phase pain occur by the stimulation of nociceptor in the mice paw, on the other hand late phase pain occur due to the release of different types of mediators of inflammation. The centrally acting drugs show inhibition activity against the both phases of pain and peripherally acting drugs show the only early phase pain inhibition effect.[13,14] In this investigation the plant extract showed significant inhibition of pain (54.65% of inhibition at dose 200 mg/kg) by showing protection against both mechanisms of pain.

One of the maximum vital pathological sicknesses is inflammation. It is a part of non-particular immune reaction that takes place in response to any kind of bodily injury, is a complicated biological response of vascular tissues to harmful stimuli.[20] Non-steroidal anti-inflammatory drugs (NSAID) are many of the most generally prescription drugs because of their consistent effectiveness within the remedy of pain, fever, inflammation and rheumatic disorders. However, their use are associated with unfavourable effects at the extent of digestive tract, starting from dyspeptic symptoms, gastrointestinal erosions and peptic ulcers to extra serious complications, which include over bleeding or perforation.[20] Development of recent anti-inflammatory tablets remains necessary and the natural product such as medicinal flowers could lead in discovering new anti-inflammatory drugs with less undesirable effects.[20] In carrageenan induced paw edema methods, at dose 400 mg/kg, the present medicinal plant extract showed significant (*$p<0.05$) moderate anti-inflammatory effect compare to the reference standard drug Ibuprofen (10 mg/kg).Here we observed the effect of the plant extract against acute inflammation. The possible mechanism of inflammation is release of chemical mediator bradykinin which acts as an inducer of biosynthesis of autacoids and prostaglandin. These chemical mediators mainly responsible for exudation of plasma proteins and edema occur.[12]

In our thrombolytic assay, the investigative plant extract showed very potent thrombolytic activity compared to reference standard drug streptokinase. At dose of 10 mg/ml, the plant extract showed more significant thrombolytic property (57% lysis of clot) when compared to the reference standard drug Streptokinase. The synthetic drug Streptokinase act as plasminogen activator, which convert plasminogen to plasmin. And this plasmin act as thrombolytic agent. But due to the some adverse effects of this synthetic drug, researchers are conducting their research in searching different natural plant sources of thrombolytic properties.[15]

CONCLUSION

In the context of the above discussion, it can be revealed that the crude extract of *Triticum aestivum* possess significant analgesic, moderate anti-inflammatory and potent thrombolytic activities. However, further studies on this plant extract require to find out the bioactive compounds which are mainly responsible for these pharmacological activities.

CONFLICT OF INTEREST

Authors has no conflict of interest.

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REFERENCES

1. Qayum RA, Khan MD, Moghal MMR, Amin MN, Hossain MS, Hossain MD, et al. Analgesic and antipyretic activities of two medicinal plants – Salvinia minima and Dactyloctenium austrate in experimental animal models. Der Pharmacia Sinica. 2013; 4(3): 183-187.
2. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives. 2001; 109: 69–75.
3. Leonti M, Casu L. Traditional medicines and globalization: current and future perspectives in ethanopharmacology. Frontiers in Pharmacology. 2013; 4: 92.
4. Zhang M, Ma CY, Lv DW, Zhen SM, Li XH, Yan YM, et al. Comparative phosphoproteome analysis of the developing grains in bread wheat (*Triticuma estivum* L.) under well-watered and water-deficit conditions. J Proteome *Res*. 2014;13(10):4281-97.

5. Suriyavathana M, Roopavathi I. Phytochemical Characterization of *Triticuma estivum* (Wheat Grass). Journal of Pharmacognosy and Phytochemistry. 2016;5(1):283.
6. Jain G, Jain N, Argal A. Ameliorative Effect of Young Leaves of *Triticuma estivum* on Carrageenin Induced Inflammation. IOSR J Pharm Biol Sci. 2014; 9(2):86-90.
7. Okpo SO, Fatokun F, Adeyemi OO. Analgesic and anti-inflammatory activity of *Crinum glaucum* aqueous extract. J Ethnopharmacol. 2001;78(2-3):207-11.
8. Saha A, Masud MA, Bachar SC, Kundu JK, Datta BK, Nahar L, et al. The Analgesic and Anti-Inflammatory Activities of the Extracts of *Phyllanthus reticulatusin* in Mice Model. *Pharm Biol*. 2007;45(5):355-9.
9. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF, et al. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thrombosis Journal. 2006;4(1):14.
10. Millat MS, Hussain MS, Amin MN, Ibrahim M, Sultana F. Appraisal of In-Vitro Antimicrobial, Thrombolytic and Membrane Stabilizing Potentials of Chloroform Extract of Avicennia marina Leaves and Barks: An Approach to Develop Novel Drug. Nov Appro Drug Des Dev. 2017; 1(2): 555560.
11. Banik S, Ibrahim M, Amin MN, Moghal MMR, Majumder MS, Alam MK, et al. Determination of biological properties of *Alocasia marcrorrhizos*: A medicinal plant. World Journal of Pharmaceutical Research. 2014; 3(9): 193-210.
12. Ueno A, Naraba H, Ikeda Y, Ushikubi F, Murata T, Naramiya S, et al. Intrinsic prostacyclin contributes to exudation induced by bradikinin or carrageenan: A study on the paw edema induced in ip-receptor deficient mice. Life Sci. 2000;66:155–160.
13. 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 ethnopharmacol. 2011;137(1):543-6.
14. Deng JS, Chi CS, Huang SS, Shie PH, Lin TH, Huang GJ, et al. Antioxidant, analgesic, and anti-inflammatory activities of the ethanolic extracts of *Taxillus liquidambari* cola. J Ethnopharmacol. 2011;137(3):1161-71.
15. Bhowmick R, Sarwar MS, Dewan SMR, Das A, Das B, Uddin MMN, et al. In vivo analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and in vitro thrombolytic activity of hydro alcoholic extract from *Litsea glutinosa* leaves. Biological research. 2014;47(1):56.
16. Uddin SMN, Amin MN, Shahid-Ud-Daula AFM, Hossain H, Haque MM, Rahman MS et al. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of *Stephania japonica* Linn. J Med Plant Res. 2014; 8(37): 1127-1133.
17. Tanna MTH, Nath AK, Amin MN, Ibrahim M, Chowdhury MI, Mukul MEH. Evaluation of antioxidants, membrane stabilizing, cytotoxic and anthelmintic activity with phytochemical screening of *Chromolaena odorata*: A medicinal shrub. International Journal of Pharmacy. 2016; 6(1): 53-61.
18. Ghosh A, Banik S, Amin MN, Ahmed J. Evaluation of anti-nociceptive, antihyperglycemic, and membrane stabilizing activities of *Garcinia lancifolia R*oxb. Journal of Traditional and Complementary Medicine. 2018; 8(2): 303-307.
19. Dewan SMR, Amin MN, Adnan T, Uddin SMN, Shahid-Ud-Daula AFM, Sarwar G, et al. Investigation of analgesic potential and in vitro antioxidant activity of two plants of Asteraceae family growing in Bangladesh. Journal of pharmacy research. 2013; 6(6): 599– 603.
20. Baul S, Amin MN, Hussain MS, Mukul MEH, Millat MS, Rashed MSU et al. Phytochemical Nature and Pharmacological Evaluation of Chloroform Extract of *Pandanus fascicularis* L. (Fruits): An *in vivo* Study. J Bioanal Biomed. 2017;9: 223-228.