

AN IMPRESSIVE THROMBOLYTIC AND ANALGESIC EFFECT HAS BEEN EXHIBITED IN THE METHANOLIC EXTRACT OF *HYGRORYZA ARISTATA* LEAVES

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Abstract

Hygroriza aristata is the significantly most important traditional medicinal plant practitioners all over the country since long years. Seeds are cooling and astringent to urinary tract; useful in biliousness. By the use of this plant only a few investigations was done like: anti-inflammatory and antioxidant. The purpose of the study is to determine the thrombolytic and analgesic activity of *Hygroriza aristata* extract. Phytochemical screening of the methanolic extract of whole plant of *Hygroriza Aristata* indicates the presence of alkaloids, carbohydrates, flavonoid, glycoside, and steroid. In thrombolytic assay, methanolic extract of the *Hygroriza aristata* leaves exhibited a promising effect compared standard drug Streptokinase. Here, the extract exhibited 63% (for 100 mg) clot lysis activity whereas; Streptokinase exhibited 82% (for 30,000 I.U). The analgesic activity of the methanolic leaf extract of *Hygroriza aristata* was also observed by acetic acid induced writhing in mice. This writhing was markedly reduced with increase of the amount of extract at 250 mg/kg (63.1%) and 500 mg/kg (70.24%) respectively. Here, Diclifenac-Na 10 mg/kg (54.76%) was used as a standard drug. Thus, we can assume that, *Hygroriza aristata* plant have a promising effect of thrombolytic and analgesic activity that showed a ways of inventing new lead compound having such type of pharmacological effects respectively.

Keywords: *Hygroriza aristata*, thrombolytic, analgesic, methanolic extract

Introduction

In order to avoid the negative side effects of allopathic drugs, people, especially in developing countries like Bangladesh, choose the alternative ways such as: Traditional medicine, Herbals medicine, Unani and Ayurvedic medicine. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs [1]. Different forms of traditional medicines have been used in this country as an essential means for the treatment of diseases and the management of various health problems since a long. In this country, this practice has flourished enormously in recent years along with that of modern medicine. As a result, even at this age of highly advanced allopathic medicine, a large majority of the population of this country, particularly in rural and semi-urban areas, still prefers to use traditional medicine in the treatment of the majority of their diseases despite the fact that modern medical facilities may be available in the neighborhood.

Recently, blood clot formation has been a severe problem of blood circulation as thrombus or embolus that hinders the blood flow by blocking the blood vessel therefore depriving tissues of normal blood flow and oxygen. Streptokinase is a generally utilized fibrinolytic medication as usual. All thrombolytic operators work by initiating the compound plasminogen that clears the cross-connected fibrin work [2].

Pain results in dropped muscular activities. The majority of the anti-inflammatory drugs now accessible are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, strong vasodialators and furthermore add to erythema, edema and pain. Henceforth, for treating inflammatory diseases analgesic and anti-inflammatory agents are required [3].

Hygroriza aristata (Retz.) has a remarkable place among the drug producing plants in terms of medicinal properties. It is a stoloniferous perennial plant widely distributed in tropical Asia. This plant grows in paddy fields and ponds at 400–800 meters above sea level, often forms floating clusters in

lakes and slow flowing rivers. Traditionally it is used as a diuretic, emollient, galactagogue, stranguria, diarrhea, otopathy, fatigue, general weakness [5]. *Hygroriza aristata* can also be used in aquariums with water temperatures of 20 to 30°C. The water should be soft to hard, the pH-value should be between 6 to 8 [6,7]. Seeds are used as cooling and astringent to urinary tract and soothing of biliousness [8]. The aim of this study is to evaluate the thrombolytic and analgesic activities of methanolic extract of *Hygroriza aristata* leaves.

Methods

Plant material

The leaves of the plant *Hygroriza aristata* were collected from the Babukhan, Rangpur, Bangladesh and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka Bangladesh for its authentication.

Preparation of the extract:

The harvested leaves of the plant were separated from undesirable materials. They were air dried for two weeks after cutting into small pieces. The parts of the plant were ground into a coarse powder with the help of a suitable grinder. The powder was put away in a airtight sealed compartment and kept in a cool, dim and dry spot. Then, the plant powder will dissolve in methanol solution in a flat bottom sealed container for several days. Then metabolic extract were evaporates by the rotary evaporator and plant extract were collect.

Animals: The examinations were done on Swiss Albino mice. Mice of 16 weeks old, weighing 30-35 gm were used. The rats were kept in gatherings of 5 in each polyvinyl confine. The animals were given standard mice feed and water kept in the laboratory environment for several days. They were fasted overnight and weighed before the experiment.

Phytochemical screening: Various phytochemical tests which were performed under the heading of phytochemical screening are Benedict's Test and Fehling's Test for carbohydrates, general test for glycosides, tests for alkaloids by Mayer's reagent and Dragendroff's reagent, test for Saponins, test for Flavonoids, test for Steroids and test for gums[9].

Detection of carbohydrates:

Extracts were dissolved independently in 5 ml refined water and separated. The filtrates were utilized to test for the presence of carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and warmed tenderly. Orange red encourage shows the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and warmed with Fehling's A and B solutions. Development of red precipitate shows the presence of reducing sugars.

Tests for Glycosides: 2 ml solution of the extract was taken into a test tube. 1ml mixture of Fehling solution A and B then added into the test tube. The tube was set in a water-bath at 60° C. On the off chance that a brick red ppt. structure that demonstrates the presence of glycosides.

Test for alkaloids:

Mayer's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. At that point 1 ml of Mayers reagent was included. Yellow color ppt. was shaped and that was shown as the presence of alkaloids.

Dragendroff's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. At that point 1 ml of Dragendroff's reagent was included. Orange brown colored precipitate was framed and that was demonstrated as the presence of alkaloids.

Test for Steroids:

Sulphuric acid test: 1 ml solution of chloroform extract was taken and then added 1ml Sulphuric acid Red color indicates the presence of steroid.

Test for gums: 5 ml solution of the extract was taken and after that molish reagent and sulphuric acid were included. Red violet ring created at the intersection of two fluids demonstrated as the presence of gums and carbohydrate.

Test for Flavonoids: Included a couple of drops of concentrated hydrochloric acid to a little measure of a alcoholic extract of the plant material. Prompt improvement of a red color demonstrates the presence of Flavonoids.

Test for Saponins: 1 ml solution of the extract was diluted with refined water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of saponins.

Thrombolytic Activity:

100 mg extract was suspended with 10 ml of distilled water and vigorously shaken on a vortex mixer. On the commercially available lyophilized SK vial of just one, 500,000 I. U., 5 ml clean refined water was included along with blended properly. This suspension was used as being a stock from which 100 µl (30,000 my spouse and i.U) was used for throughout in vitro thrombolysis[10].

Venous blood drawn from healthy volunteers and incubated at 37°C with regards to 45 minutes. After clot enhancement, serum was completely removed (without disturbing the clot formed). Each tube having clot again weighed to estimate the clot weight. (Clot weight = bodyweight of clot containing tube - weight of tube alone). 100 µl of plant extract and 100 µl standard drug was taken in the clot containing tubes and incubated at 37°C for 90 minutes and observed the clot lysis. After incubation, fluid obtained was removed as well as tubes were again weighed to observe the difference in weight right after clot disruption. Distinction acquired all through weight taken previously and directly after clot lysis was communicated despite the fact that rate. Thrombolytic activity of methanolic extract of *Hygroryza aristata* block up lysis. Streptokinase and water were used being a positive and negative (non-thrombolytic) demand respectively. The examination was repeated a couple of times with the blood samples in regards to various volunteers.

$\% \text{ clot lysis} = (\text{Weight of the lysis clot} / \text{Weight of clot before lysis}) \times 100$ [Islam MA et al., 2013]

Analgesic Activity (Acetic acid induced writhing method):

1% Acetic acid was ingested in the mice at a dose of 10ml/kg body weight to induce pain.

Dose: Standard: Diclofenac Na 10mg/kg body weight (inject in the intra-peritoneal route)

Control: 0.5% methyl cellulose or water (oral route).

Extract: 250mg or 500mg/kg body weight (oral route)

Standard: Injected Di-clofenac Na10mg/kg body weight in the mice on the intra-peritoneal route. Wait for 15 minutes. Then 1% acetic acid was injected at 10ml/kg body weight in the mice intraperitoneally. After 5 minutes, writhing of the mice was observed up to the next 10 minutes.

Control: 0.5% methyl cellulose or water 10ml/kg body weight, were given through oral route. Wait for 15 minutes. Then 1% acetic acid was injected at 10ml/kg body weight in the mice intraperitoneally. After 5 minutes, writhing of the mice was observed up to the next 10 minutes.

Plant Extract: 250mg or 500mg/kg body weight extract are put on oral route. Wait for 30 minutes. Then 1% acetic acid was injected at 10ml/kg body weight in the mice intraperitoneally. After 5 minutes, writhing of the mice was observed up to the next 10 minutes [11].

Statistical analysis:

The results were expressed as mean \pm SEM using Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA). We used a one-way analysis of variance (ANOVA), followed by Scheffe's post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 ($p < 0.05$).

Results

Phytochemical screening

The phytochemical screening tests indicated that the different constituents such as alkaloids, carbohydrates, flavonoids, glycosides and steroids were present in the plant *Hygroriza aristata* that have pharmacological properties. The results of various qualitative chemical tests for the detection of chemical constituents of *Hygroriza aristata* is shown in the Table 1.

Thrombolytic activity

Streptokinase (SK) is a positive control (30000IU) to the blood clots and subsequent incubation for 90 minutes at 37 degree showed 82% lysis of clot. Besides, distilled water was treated as negative control which exhibited a negligible percentage (5%) of clot lysis. In this study, the methanolic extract of *Hygroriza aristata* leaves exhibited 63% of clot lysis. The mean difference in clot lysis percentages between positive (standard drug and plant extract) and normal control (water) was found significant.

Analgesic effect

Writhing was markedly reduced by the standard drug Diclofenac Na (54.76%) as well comparing to

the normal control group. The methanolic extract *Hygroriza aristata* leaves also exhibited a significant reduction of writhing at 250 mg/kg (63.1%) and 500 mg/kg (70.24%) comparing to the normal control group as shown in Table 2. In addition, the extract significantly reduced the frequency of defecation along with the number of writhing when compared with control. Percentage of writhing output was also reduced by the increased dosage of the extract.

Discussion

Differing group of phenolic compound (flavonols, flavonoids, tannic acid, anthocyanins, phenolic acid etc.) have a perfect basic science with the expectation of complimentary radical searching action [12] and exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, thrombolytic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects [13,14,15]. The phytochemical screening tests indicated that the different constituents such as alkaloids, carbohydrates, flavonoids, glycosides and steroids were present in the plant *Hygroriza aristata* that have pharmacological properties (table 1).

In spite of the fact that there are a few thrombolytic drugs with those gotten by recombinant DNA technology, yet symptoms identified with a portion of these medications that lead to facilitate troubles have been reported [16,17,18]. Platelets play and significant role in the advancement of atherothrombosis just as harm the regions of endothelial surface (created by receptive oxygen species). The stimulated platelets form platelets to platelets bonds, binds also to leucocytes carrying them into an intricate process of plaque development and progression. Plasmin, a characteristic fibrinolytic specialist, lyses clot by breaking down the fibrinogen and fibrin contained in a coagulation. Phlorotannin, disengaged from marine brown algae, have an exceptional property in advancement of dissolution of intravascular blood clot by means of antiplasmin inhibition. A few examinations reveal that *A. bilimbi*, *C. viscosum* and *D. quercifolia* possesses tannin, alkaloid saponin [19,20] which could be participated for its clot lysis activity [21]. The present study of 100 μ l SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C,

showed 82% lysis of clot. On the other hand, distilled water was showed negligible percentage of lysis of clot 5%. In this study *Hygroriza aristata* displayed highest thrombolytic activity 63% (table 2).

The brain and spinal cord assume a noteworthy job in central pain mechanism. The dorsal horn of the spinal cord is enriched with a few neurotransmitters and receptors including: substance P, somatostatin, neuropeptide Y, inhibitory amino acid, nitric oxide, endogenous narcotics and the monoamines which are the significant focuses for pain and inflammation [22]. Narcotic analgesics restrain both peripheral and focal system of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain [23, 24]. On the other hand, acetic acid-induced writhing model represents pain by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid [25]. The response is thought to be mediated by peritoneal mast cells [26] acid sensing ion channels and the prostaglandin pathways [27]. Flavonoids may rise the amount of endogenous serotonin or may connect with 5-HT_A and 5-HT₂ receptors which might be engaged with the mechanism of central analgesic activity. Past specialists detailed the presence of a few therapeutically esteemed flavonoids from the *E. prostrate*. In this study, the plant extract inhibits pain by reducing the acetic acid induced writhing in the experimental mice (table 3), which propos that the plant extract may have strong analgesic activity.

Conclusion:

The plant *Hygroriza aristata* exhibited promising effect for thrombolytic and analgesic activity. Now it can be concluded on the basis of results obtained from investigation that the plant may be useful as thrombolytic and analgesic drug (crude drug). However, further investigation is necessary to identify the lead compound.

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Table 1. The phytochemical constituents of the experimental plant *Hygroriza aristata* obtained by phytochemical screening tests.

Phytochemical constituents	Methanolic extract of <i>H. aristata</i> leaves
Alkaloids	+
Carbohydrates	+
Saponins	-
Flavonoid	+
Glycoside	+
Gums	-
Steroids	+

(+) = Present; (-) = Absent

Table 2. Thrombolytic effect of *Hygroriza aristata* leaves extract.

Sample	Blank tube weight (gm.)	1 st clot + tube weight (gm.)	1 st clot weight (gm.)	2 nd clot + tube weight (gm.)	2 nd clot weight (gm.)	Lysis weight (gm.)	% of lysis
Standard (Streptokinase)	0.81±0.37	1.70±0.65	0.89±0.44	0.97±0.56	0.16±0.21	0.73±0.25	82%
Normal Control (Distil water)	0.80±0.34	1.82±0.72	1.02±0.65	1.77±0.75	0.97±0.19	0.05±0.12	5%
<i>Hygroriza aristata</i> leaf extract	0.79±0.33	1.69±0.42	0.90±0.56	1.12±0.53	0.33±0.22	0.57±0.21	63%*

* indicates significant difference ($p < 0.001$) from normal control group. Data are expressed as means ± SEM.

Table 3. Result of analgesic effect of *Hygroriza aristata* leaves extract on swich albino mice.

Treatment	Dose	Writhing Counting (Mean)	Inhibition %
Normal control	Water 5mg/kg.	42±0.50	-
Diclofenac-Na	10 mg/kg	19±0.45	54.76%
Methanolic leaf extract of <i>Hygroriza aristata</i>	250 mg /kg	15.5±0.60	63.1%*
Methanolic leaf extract of <i>Hygroriza aristata</i>	500 mg/kg	12.5±0.75	70.24%*

* indicates significant difference ($p < 0.001$) from normal control group. Data are expressed as means \pm SEM.