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In-vivo antiemetic and antinociceptive, cytotoxic and *in-vitro* thrombolytic potential of methanolic extract of stem and leaves of *jatropha gossypiifolia*

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ABSTRACT

This study describes the biological investigations of methanolic extract of leaves and stem of *Jatropha gossypiifolia*, a plant belonging to the family Euphorbiaceae, to reveal their possible contribution as an antiemetic, antinociceptives, cytotoxic and thrombolytic factor. The leaves and stems of *Jatropha gossypiifolia* were extracted with methanol. The leaves extracts were used for the observation of thrombolytic potential. Result reveals that the leaves at a concentration of 2.5mg/mL, showed maximum activity (27.255 ± 0.90%), whereas 20 mg/mL sample solution showed the lowest activity (7.722 ± 0.26%), when comparing positive control streptokinase. The extracts were used for the investigation of cytotoxic activity considering vincristine sulfate as a positive control. The leaves and the stems portion have considerable cytotoxic property (LC50 are 0.00532 µg/ml for stems and 0.000109 µg/ml for leaves, respectively) when comparing with positive control. The methanolic extract showed potent antiemetic activity at the highest concentration both at leaves and stem extracts as compared to positive control metoclopramide. Where metoclopramide shows 73% of inhibition, there leaves and the stem portion of the plant shows 90% and 91% of emesis inhibition respectively. By using a standard protocol, the crude methanolic stem and leaves extract of *J. gossypiifolia* was conducted to attempt for antinociceptives activities, where Diclofenac Na is used as a standard. The crude methanolic extract of leaves and stem exhibit 31.07% and 30.79% writhing inhibition in test animals respectively, which are statistically significant. The results of the study showed that the plant extract has potential thrombolytic, cytotoxic, antiemetic and antinociceptive activities.

Keywords: *Jatropha gossypiifolia*, thrombolytic, antiemetic, cytotoxic, antinociceptive

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INTRODUCTION

The knowledge of herbal medicine and its assorted practices have been channelized through the senesces. For centuries, respective diseases have plagued the humanity and medicinal plant was merely the alternative for remedy. Even, most of the today's drugs have been educed from potential plants sources.[1] Concording to the World Health Organization's questionnaire, it is annunciated that in the primary health protection 70-80% of the population in the world still depends on ethnic medicines, chiefly in plant sources.[2] Even so, respective medicinal plants used as ethnic medicine have not received any scientific contribution yet. Many of the natural plant products have potent biological and pharmacological properties due to their distinct active secondary metabolites.[3] And all these properties make them a potential source in the development of modern medicine. By extending an origin of drugs in their pure form, medicinal plants can be practicable either in their primitive or advanced state.[4]

Jatropha gossypiifolia (Local name: kerong, keron) belonging to the family Euphorbiaceae[5] is a shrub-herb with 1.8-meter-high, clustered with palmate shaped 3-5 lobed leaves and dark red flowers. The preliminary phytochemical evaluation of methanolic extract of *J. gossypiifolia* confirmed the presence of glycosides, tannins, phytosterols, triterpenoids, diterpenes, saponins and phenols both at its leaves and stem portions.[6] Its leaf stipules, petioles, and margins are covered with glandular hairs. It is reported to be beneficial to vertigo, dysphonia, dyscrasia and anemia.[5] It is an antibiotic, insecticidal and used in a toothache and act as blood purifier.[7] The leaves are employed to itches, carbuncles, and eczema, as well as act as a purgative and tumefied. The extract of the leaves is practicable for venereal disease, stomachache and as blood purifier. Leaves are used to prepare tea for constipation. Extracts of the plant are used as a cathartic and emetic, and to treat diarrhea, headache, skin and mouth sores.[8] Literature reveals that the leaves parts are potent hepatoprotective against the CCl_4 induced rat models.[9] Moreover, the leaves are reported to be potent amoebicidal against cytotoxic human cell.[10]

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The plant, *Jatropha gossypiifolia*, possesses no scientific data on its different uses such as thrombolytic, antinociceptive, cytotoxic and antiemetic activities, although it is used widely in the tralatitious treatments. As a part of our present study of medicinal plants of Bangladesh, the methanol extract of leaves and stems of *J. gossypiifolia* were screened for thrombolytic, antiemetic, cytotoxic and antinociceptive activities for the first time and we, here in the report the results of our study.

RESEARCH DESIGN AND METHOD

List of chemicals

Methanol (PubChem CID: 887), calcium chloride (PubChem CID: 5284359), streptokinase (PubChem CID: 9815560), DMSO (Dimethyl Sulfoxide) (PubChem CID: 679), vincristine sulphate (PubChem CID: 249332), Tween 80 (PubChem CID: 5281955), Copper sulphate (PubChem CID: 24462), Metoclopramide (PubChem CID: 4168), diclofenac sodium (PubChem CID: 5018304), acetic acid (PubChem CID: 176).

Collection of plant materials

The whole plant of *J. gossypiifolia* were collected from Comilla, Bangladesh (The Geo position of the district is between 23˚01' to 24˚11' North latitudes and between 90˚34' to 91˚22' east longitude) and voucher specimens for each of the collections (DACB Accession no. 38200) have been deposited in Bangladesh National Herbarium (BNH), Dhaka for future references. At first, leaves were separated from the plants, washes carefully along with stem, then left for air drying. It takes about 2 weeks to make a dried one. After that leaves and stem were gone through grinding process followed by continuous sieving and finally the fine crashed powders of leaves and stems were obtained.

Preparation of extract

The preparation of extract was maintained by the standard extraction procedures.[11] The air-dried and powdered leaves and stem of the plants (500 gm) were separately soaked in methanol (1.5 L each) for 15 days at room temperature with occasional shaking and invoking. Then filtered via a newly cotton plug and eventually with a Whatman No.1 filter paper (Mesh size 42). The intensity of the filtrate then was concentrated under reduced pressure using a rotary evaporator (HEYDOLPH, Germany) maintained at 45˚C. The semi-dried methanolic extracts were further dried in a freeze drier (HETOSICC, Heto Lab Equipment, Denmark) at -55˚C temperature and stored in a reagent bottle at -8˚C in a freezer. The subsequent amount of leaves and stem extracts of *Jatropha gossypiifolia* were about 7.8 gm and 9.7 gm respectively.

Test animals

For the screening of *in vivo* antinociceptic potential of methanolic extracts of *J. gossypiifolia* leaves and stems, young Swiss-albino mice (aged 20–25 days) of either sex, average weight 20–25 g were used. From the Animal Resources Branch of ICDDR, B (International Centre for Diarrheal Disease and Research, Bangladesh), the animals were collected. After collection, they were kept for one week in a standard condition for adaptation. During this time, they were fed rodent food and water ad libitum formulated by ICDDR, B. Across the experiments, all animals' encountered human care consorting to the criteria adumbrated in the 'Acute oral toxicity studies were performed according to OECD-423 guidelines.[12]

Thrombolytic assay

In vitro thrombolysis activity of the methanolic extract of leaves of *J. gossypiifolia* was carried out with minor moderation according to the method of Prasad et al.[13] The protocol was in accordance with the Helsinki Declaration of 1975 (revised in 2008) and be approved by the institutional ethics committee. The participants were given a written informed consent and all the participants right to privacy were considered strictly. Under ethical considerations with aseptic forethoughts, venous blood drawn from healthy volunteers (n = 10) was immediately transferred to different pre-weighed sterile micro-centrifuge tube (500 µL/tube). 200 µL of 2% calcium chloride was then added to each of these tubes, mixed well and incubated at 37°C for 45 minutes for clotting to occur. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). Each micro-centrifuge tube containing clot was properly labeled and 500 µL of different concentrations of the plant leaves extract, 2.5 mg/mL (n = 10), 5 mg/mL (n = 10), 10 mg/mL (n = 10) and 20 mg/mL (n = 10) or saline (negative control) (n = 10) or 30,000 I.U. and 15,000 I.U. of streptokinase [(Popular Pharmaceuticals Ltd, Bangladesh), reference drug (n = 10)] was added to tubes with clots. All the tubes were incubated at 37°C for 90 min. The fluid left was then carefully removed and the tubes were weighed again. The difference in weight before and after clot lysis was expressed as % clot lysis.

$$\% \text{ of clot lysis} = (\text{wt. of released clot})/(\text{clot wt}) \times 100$$

Evaluation of cytotoxicity

The cytotoxicity assay was acquitted by using brine shrimp lethality test abiding by the method of Firdaus *et al*.[14] To become brine shrimp nauplii, the eggs were placed in 1 L of seawater, oxygenated and hatched for 48 hrs at 37 °C. After 48 hrs, ten brine shrimp nauplii were placed in a test tube made full with seawater. Methanolic extract of *J. gossypiifolia* leaves and stems, serially diluted with DMSO (Dimethyl Sulfoxide), was then added to the test tube. After 24 hrs, with such treatment, the mortality of brine shrimp nauplii was taken under consideration. From the mortality rates, the LC_{50} was calculated. During the whole procedure, vincristine sulphate was used as positive control with the same condition.

In vivo antiemetic activity test

The *in vivo* antiemetic assay study was determined by estimating the mean decrease number of retching in a 3-days old chicken.[15] Young chicks of either sex, weighing from 32-52 g were obtained from a local poultry store. All chicks were kept under laboratory conditions at room temperature with 12 h light and dark cycles. Throughout the experiments, all animals received human care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals', 8th edition, prepared by the National Academy of Sciences and published by the National Institute of Health (US).

At the experiment day, the chicks were divided into seven groups of five chicks each were kept in a large beaker at room temperature for 10 mins for their adaptation. The crude methanolic extracts of *j. gossypiifolia* leaves and stems were dissolved in 0.9 % saline containing 5 % DMSO and 1 % Tween 80 and administered orally at a dose of 150 mg/kg of body weight. Whereas the control group experienced the 0.9 % saline containing 5 % DMSO and 1 % Tween 80. Copper sulphate at a dose of 50 mg/kg body weight was orally administered after 10 min. After that, the number of retching was ascertained during next 10 min. Metoclopramide with the same conditions was used as a standard drug (50 mg per kg body weight) orally. The antiemetic effect was assessed as the decrease in a number of retches in the treated group in contrast to the control. The inhibition (%) was calculated by the following equation:

$$\text{Inhibition } \% = ((A-B)/A) \times 100$$

Where A is the control frequency of retching and B is the frequency of retching of the treated group.

In vivo antinociceptive activity test

The antinociceptive activity of the crude methanolic extract of *J. gossypiifolia* was studied using acetic acid induced writhing model in mice.[16] The animals were divided into four groups including control (Group I), positive control (Group II) and four test groups (Group III–IV). The animals of test groups were administered test substance at the dose of 500 mg/kg body weight. The positive control group received diclofenac sodium (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1 % Tween 80 in distilled water at the dose of 10 ml/kg body weight. Test samples, standard drugs, and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7 % acetic acid. After 15 min of the time interval, the writhing (constriction of the abdomen, turning of trunk and extension of hind legs) was observed in mice for 5 min.

Statistical analysis

The results are expressed as mean ± SD. Statistical comparisons were made using one-way ANOVA with Dunnett t test carried out with SPSS 16.0 for Windows® software. Significance was set at $p < 0.05$. Dose dependencies were determined by the regression coefficient (r).

RESULTS

Thrombolytic activity test

The effective clot lysis percentage by the extracts of the plant, positive thrombolytic control and the negative control is tabulated in Figure 1. From the Figure 1, it is evident that the percentage of clot lysis was 47.2188 ± 0.35% and 24.732 ± 0.59% when 100 µl of streptokinase (30,000 I.U. and 15,000 I.U. respectively) was used as a positive control, while in case of negative control (saline) the percentage of clot lysis was negligible (5.35 ± 0.95%). Among different concentrated leaves extract, the crude methanolic extract at 2.5mg/mL, showed the highest activity (27.255 ± 0.90%), whereas 20 mg/mL concentrated solution showed the lowest activity (7.722 ± 0.26%), which was higher than the negative control.

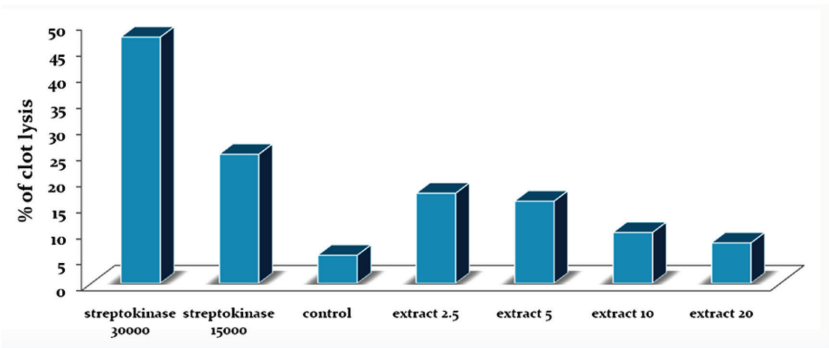

Figure 1 Thrombolytic activities of methanolic leaves extract *J. gossypiifolia* leaves

Table 1 **Cytotoxic activities of methanolic extract *J. gossypiifolia* leaves and stem**

Sample	LC_{50} (µg/ml)	Regression equation Y=mx+c	R^2
Vincristine sulphate	0.839	y = 34.02x + 52.58	0.952
Methanolic extract of stem	0.00532	y = 11.07x + 75.18	0.841
Methanolic extract of leaves	0.000109	Y=8.254x + 82.70	0.835

Table 2 **Antiemetic activities of methanolic extract *J. gossypiifolia* leaves and stem**

Drug/sample treatment	Dose	No. of retches (Mean ± SD)	% inhibition (Mean ± SD)
Negative Control	10 ml/kg	70 ± 1.07	-
Metoclopramide	50 mg/kg	17 ± 0.89	73± 0.02
MES	150 mg/kg	6 ± 0.45*	91± 0.05
MEL	150 mg/kg	7 ± 0.68*	90 ± 0.03

Values are expressed as mean ± standard deviation (SD). MES: Methanolic extract of stem, MEL: Methanolic extract of leaves. Values were found out by using ONE way ANOVA followed by Dunnet's t -test. Significance level *P = ≤0.05, **P = ≤0.01, ***P = ≤0.001

Table 3 **Effect of leaves and stem methanolic extract of *J. gossypiifolia* on acetic acid induced writhing in mice**

Groups	Treatment	Writhing count (Mean ± SD) (% writhing)	% writhing inhibition
Group I (Negative control)	1 % tween-80 solution in water orally	11.87 ± 0.58	-
Group II (Positive control)	Diclofenac sodium (25 mg/kg) orally	7.31 ± 0.7*	41.95±0.08
Group III	Methanolic extract of leaves (500 mg/kg) orally	8.05±0.83*	31.07±0.09
Group IV	Methanolic extract of stem (500 mg/kg) orally	7.98 ± 0.23*	30.79±0.02

Values are expressed as mean ± standard deviation (SD). MES: Methanolic extract of stem, MEL: Methanolic extract of leaves. Values were found out by using ONE way ANOVA followed by Dunnet's t -test. Significance level *P = ≤0.05, **P = ≤0.01, ***P = ≤0.001

Cytotoxic activity

The lethal concentration (LC50) of the test samples after 24 hours was determined by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. The lethality of the leaves and stem extracts to brine shrimps was determined and the results are given in Table 1. Vincristine sulphate (VS) was used as positive control and the LC50 value was found as 0.839µg/ml. The LC50 values of crude methanolic stem and leaves extract were found to be 0.00532 µg/ml and 0.000109 µg/ml respectfully.

Figure 2 Thrombolytic activity comparison of standard and extract. A: Blood clot, B: Standard Streptokinase induced thrombolysis, C: Sample leaves extract of J. gossypiifolia induced thrombolysis

Antiemetic activity

The result of the antiemetic activity of the extracts of *J. gossypiifolia* leaves and the stem is given in Table 2. After administration of a dose of 50 mg/kg-body weight metoclopramide and the extracts of leaves and stem, the numbers of retches were reduced. The positive control metoclopramide shows 73% of inhibition where leaves and the stem portion of the plant show 90% and 91% of inhibition respectively. On the basis of these results, it may be stated that extracts of *J. gossypiifolia* leaves and stem have antiemetic potential which is comparable with that of metoclopramide (control drug). Although the results are significant (P<0.05), the mode of action is still unknown.

Antinociceptive activity

The effect of the methanolic extract of *J. gossypiifolia* leaves and stem on acetic acid induced writhing in mice is shown in Table 3. At the dose of 500 mg/kg of body weight, the crude methanolic extract of leaves and stem produced 31.07% and 30.79% writhing inhibition in test animals respectively. The results were statistically significant (P<0.05) compared to the standard drug diclofenac sodium, which showed 41.95 % at a dose of 25 mg/kg-body weight.

DISCUSSION

Thrombosis is a decisive consequence by the dethronement of platelet or tissue factor, resultant damage or blockage of blood vessels. The process of thrombus formation is opened up when the activated platelets form platelets form a platelets bonds. The resultant activated platelets in advance bind to the leucocytes and contribute to the optimum growth and formation of plaque which is a complex operation.[17] On the contrary, it is the thrombolytic agents that burst clot by interrupting the fibrinogen and fibrin comprised in a clot. As a natural anti-thrombotic agent, plasmin, activated from cell surface bound plasminogen, ultimately contribute to fibrinolysis.[18] From numerous sources, scientists have discovered several thrombolytic agents like streptokinase. Even more, to make those drugs site specific and effective in extent, reliable recombinant technology has been applied. A widely used thrombolytic agent, Streptokinase which is basically a bacteria derived plasminogen activator, works as the same mechanism. As like other synthetic drugs, this drug has bleeding and embolism like several adverse effects. These complications lead to light upon new natural sources as well as their supplements having thrombolysis effect with minimal contrary effect.[9] Regarding that in mind, this research work is done on *J. gossypiifolia* leaves extract whether it possess any clot lysis potentiality or not. During the experiment, a negligible amount of clot lysis was performed by negative control (water) when comparing with the positive control (streptokinase) (Figure 2). Earlier it was accounted that saponin, alkaloids and tannin like phyto-constituents are creditworthy for thrombolytic potentiality.[19] The phytochemical screening of *J. gossypiifolia* extract conforms the presence of saponin and tannin like phyto-constituents that make sense of having the properties of such significant thrombolytic activities of leaves extract.

Brine shrimp lethality bioassay is a wide and established method to ascertain the cytotoxic effect of plant extract. Organic extracts are ample in higher bioactive compound, and it reported that saponins, tannins and flavones like constituents are free radical scavenger and antioxidant enzymes activator.[13] So, the methanolic extract of stem and leaves of *J. gossypiifolia* having these bioactive compounds may be accountable for the possible cytotoxicity, although the exact mode of action is yet to be ascertained.

According to the findings, leaf and stem methanolic extracts of *J. gossypiifolia* have interference effect against copper sulfate stimulated emesis in young chicks. Surprisingly, both the stem and leaves extract possesses significant antiemetic activity was observed, although the mechanism of action of these extracts is not clear. Well, it is established that 5-HT4 on peripheral nervous system act as the main role in copper sulfate induces emesis.[19] Several studies claimed that, flavonoids, terpenes, alkaloids etc. may be involved in the antiemetic potency of plant extracts.[15] As the phytochemical screening confirm the presence of flavonoid and alkaloid in the methanolic extract, therefore they might play the vital role for such kind of significant properties.

The antinociceptive potency of the plant extract of *J. gossypiifolia* was evaluated acetic acid induced writhing syndrome test. Here, NSAIDs conquer the cyclooxygenase enzyme (COX) in the peripheral tissues and therefore, interpose with primary afferent nociceptors transduction mechanism.[20] Basically, this test method establishes a correlation of analgesic doses in humans with that of the ED_{50} values obtained in animals from this test.[21] Both the stem and leaves extract equally possess antinociceptic effect at a significant level, hence leaves possess slight higher potency than the stem portion. Although the precise mechanism is still not keyed out, but it may be presumed that the extract may have the similar kind of mechanism of action as like NSAIDs. Moreover, the hydrocarbon soluble fraction possesses a good amount of alkaloids, flavonoids and saponins and these phyto-constituents are leavened to be the cardinal compounds for the antinociceptive activity reported by several studies.[22] All these facts may be responsible for the antinociceptive activity of the plant extracts.

CONCLUSION

In the perspective of the findings of this study, it can be summed up that the plant extracts have notable thrombolytic, antiemetic, antinociceptive and cytotoxic activities. Therefore, it may have recommended further investigation for better understanding the underlying mechanism of such actions scientifically.

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