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EFFECTS OF ANTIDIARRHEAL AND THROMBOLYTIC ACTIVITIES OF METHANOL EXTRACT OF *CINNAMOMUM CECIDODAPHNE MEISSN.* BARKS

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ABSTRACT

Objective: To investigate the Effects of Antidiarrheal and Thrombolytic Activities of methanol extract of *Cinnamomum cecidodaphne Meissn*. Barks. Castor oil induced diarrhoea test were used to examine the *in-vivo* antidiarrheal activity in Swiss albino mice. **Results:** *In vitro* clot lysis model was undertaken to investigate the thrombolytic action of the extract. Data were analysed by using statistical software statistical package for social science (SPSS, version 19.0). In this study of antidiarrhel activity Loperamide was used as a standard. For thrombolytic test Streptokinase used as positive control. In case of antidiarrheal study value was found 76.36% 400mg/kg where standard was found 83.33% and the extract shown 35.08% of thrombolysis effect where streptokinase was found 77.06% of clot lysis effect. **Conclusion:** The extract showed significant activity as compare to standard drugs. Methanol extract *of C. cecidodaphne Meissn* barks might be triggering the premonition of novel drug discovery in future due to its antidiarrheal effect in animal model.

KEYWORDS: Antidiarrheal activity, Castor oil, *Cinnamomum cecidodaphne Meissn*. Thrombolytic activity, % lysis of clot.

INTRODUCTION

Diarrheal diseases are a major problem in third world countries and are responsible for the death of millions of people each year [1]. It is a clinical symptom marked by rapid and frequent passage of semisolid or liquid faecal Material through the gastrointestinal tract. Secretory diarrhoea occurs when an imbalance between absorption and secretion in the small intestine occurs. Various causes can explain this condition including virus, parasites and bacteria. Secretory diarrhoea is predominantly a result of active secretion of chloride and bicarbonate ions. Secretion of these electrolytes leads to an osmosis-driven water movement into the intestines. Supportive care with replacement of intestinal fluid losses using oral rehydration solutions or isotonic intravenous fluids is the primary treatment [2]. About 5 million persons are death which people age less than 5 years according to the WHO. The curve of diarrhoeal patient increases gradually day by day. It is create a painful situation of all kinds of local and international organizations. So it is very essential to estimate or identify natural and synthesis drugs which are used as a diarrheal condition. These drugs which are synthesis from the chemicals they are not free from adverse effects and side effects [3, 4, 5, 6]. Medicinal plants are a promising source of antidiarrheal drugs [7]. For this reason, international organizations including the World Health Organization (WHO) have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices [8, 9, 10].

Formation A blood clot (thrombus) developed in the circulatory system due to failure of haemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic. Cardiovascular disorders and stroke are associated with myocardial infarction, anoxia, hypertension and thrombus formation is a fatal event and account for considerable number of deaths in the whole world [11]. Thrombolytic agents mainly tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) etc. are used all over the world for the treatment of these diseases. In Bangladesh, though SK and UK are widely used due to lower cost, as compared to other thrombolytic drugs, their use is associated with hyper risk of haemorrhage [12, 13, 14].

Streptokinase is an antigenic thrombolytic agent used for the treatment of acute myocardial infarction. It reduces mortality as effectively as the non-antigenic altreplase in most infarct patients while having the advantages of being much less expensive. Tissue-type plasminogen activator (tPA) is generally preferred as being effective and safer than either urokinase or

streptokinase type activators. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs [15, 16, 17, 18, 19].

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals) Plants showing significant anti-diarrheal and thrombolytic activity, Thousands of years ago Plants and their products have been used by humans for treatments of numerous diseases. Traditional medicine (also known as indigenous or folk medicine) comprises knowledge that developed over generations within various societies before the era of modern medicine [20].The plants that demonstrated healing powers are referred as medicinal. Historically, these treatments would cure or relieve symptoms [21]. In developed countries, alternative or complementary medicine alludes to traditional medicine used outside its traditional culture. In recent years, the interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. Nevertheless, many plants have not been studied yet for the claimed biological activities and possible adverse effects. It is estimated that there is approximately 500,000 species of plants on earth [22].Only a relatively small percentage, 1% to 10%, is used as food by humans and other animal species together. On the other hand, probably more than 10% of plants are used for medicinal purposes [23].

Cinnamomum cecidodaphne Meissn. , Family (Lauraceae) is an important medicinal plant, which is widely distributed in the tropical and subtropical regions of Asia and the Caribbean this plant traditionally widely used for an antidiarrheal effect. C. *cecidodaphne Meissn is* an evergreen tree with straight stem, growing up to a height of 8 m and with an average girth of 1.5 m. The leaves are 12-18 cm long and 5-8 cm broad, elliptic with acuminate tip, dark green, glossy above and waxy below and with 4-5 lateral nerves. The fruits ripen during October-November in Nepal. The outer fleshy part of the fruit is peeled off immediately after collection and the seeds are washed thoroughly and sown in sand beds under shade. Germination starts in about 3-5 days after sowing and the seedlings can be transplanted into nursery bags filled with potting mixture at 3-4 leaf stage and can be maintained in the nursery[24]. Natural drugs are high efficacy, less cost. Traditionally leaves, bark and roots has antidiarrheal effects, antimicrobial effect astringent, demulcent warming, stimulating,

carminative etc. The present investigation also evaluated the antidiarrheal, membrane stabilizing, cytotoxic and thrombolytic properties of *cinnamomum Cecidodaphne Meissn*. Up to date, *C. cecidodaphne Meissn* bark extracts have not been systemically studied. Therefore, the present investigation was performed for evaluate the In vivo anti-diarrheal and thrombolytic activities of the species to find out evidence for its folk uses and to introduce it as a source of new drug candidate.

MATERIAL AND METHODS

Drug and chemicals

For Antidiarrheal activity castor oil (Taj Scientific, Anderkilla, Chittagong, Bangladesh), normal saline (NaCl 0.9%) were used. Loperamide was collected from Square pharmaceuticals Ltd., Bangladesh. For Thrombolytic activity commercially available lyophilized Streptokinase (SK) vials (Durakinase, Dongkook Pharma. Co. Ltd, South Korea) of 1500000 IU was used as positive control.All other chemicals were of analytical grade.

Animals

Swiss-albino mice (both sexes) weighing between (18-25 g) was used for the present study collected from International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh.After their purchase, the mice's were kept in standard environmental conditions (24.0 ± 0°C & 55-65% relative humidity and 12 h light/dark cycle) for one week to acclimate and fed ICDDRB formulated rodent food and water ad libitum. Guidelines of Institutional Animal Ethics Committee were followed to carry out this study [25].

Plant Material Collection

For the present investigation the barks of *Cinnamomum cecidodaphne Meissn*.Family (Lauraceae) were collected from the Cox's bazar district, Bangladesh during the month of April 2013.After that taxonomy confirmed by an expert of botany and plant Herbarium specialist Dr. Shaikh Bokhtear Uddin, Associate professor, Department of Botany, University of Chittagong. The collected plant barks were shade dried for twenty days in the low temperature and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

Extraction Process

Approximately (620 gm.) powdered material was placed in clean flat bottomed glass container and soaked in 1.5 liter methanol. The container with its content was sealed and kept for 15 days accompanied with occasional shaking and stirring for maximum wetting and extraction. The entire mixture was coarse filtration by piece of clean white cotton materials. Then the extract was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and was concentrated to obtain methanol crude extract and evaporated to dry using water bath. Then the extract was stored at 4°C until used.

Experiment procedure

Acute toxicity test

The acute toxicity test for methanolic bark extract of *Cinnamomum cecidodaphne Meis*sn was carried out to evaluate any possible toxicity using the method of Lorke [28]. Different doses of extract were injected intraperitonealy into groups of 15 mice. The given maximum dose was 600 mg/kg, while the control group only received distilled water. The number of deaths was counted at 48 h after treatment.

Anti-diarrhoeal test

The antidiarrheal effects of plant extracts were evaluated according to the method described by Shoba and Thomas [26]. Mices fasted for 24 h and were divided into Four groups (n = 5). Group I received 10 mL/kg of Tween 80 (1% Tween 80 in water) orally and served as control animals. Animals in group II received loperamide (5 mg/kg, p.o.) and served as the standard treatment group, whereas groups III and IV received orally 200 or 400 mg/kg of methanolic extract *of* Cinnamomum cecidodaphne Meissn respectively. The extract was suspended in Tween 80 (1% v/v). One hour after oral administration of treatments, the animals received castor oil (1 mL) orally, and they were individually placed in boxes, the floor of which lined with blotting paper for observation of the number and consistency of faecal droppings. The numbers of both wet and dry droppings were counted every 60 min for 4 h, and the white paper was changed after each evaluation. The means of the stools passed by the treated groups were compared with that of the control group. The mean number of diarrheic faeces pooled by the control group was considered as 100%. The stools passed by the treated groups that's compared with the control and standard. During an observation period of 4 hours, the total number of faeces which were excreted by the animals was recorded. The level of

inhibition (%) of defecation caused by extracts was calculated relative to the control using the following relationship:

Inhibition of defecation (%) = [(NDC - NDT)/NDC] × 100;
Where, NDC = mean number of diarrheic faeces of the control group;
NDT = mean number of diarrheic faeces of the treated group.

Thrombolytic test

The thrombolytic activity of these extractives was evaluated by previously described method [27]. The dry crude extract (10 mg) was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered. Blood was collected from adult male volunteers (n = 5) who had no history of Medications for last ten days. The test was performed with the approval of Ethics Committee of Pharmacy Department, International Islamic University Chittagong (approval no. ECPDIIUC2012/09). Here, the volunteers were informed details about the research and they gave written consent on blood donation to cooperate in our study. 4 ml blood was drawn from each volunteer under the supervision of a physician and laboratory technician. The blood was then distributed to seven previously weighed sterile alpine tubes (0.5 ml/tube) and incubated at 37ºC for 45 min to form clot. The tube containing clot was weighed again to measure clot weight after completely elimination of serum. 100 µl aqueous solutions (1 mg/ml) of different fractions and crude extract was added to each tube separately. As a positive control, 100 µl of SK and as a negative non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated with the blood of the 5 volunteers. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown.

% of clot lysis = (weight of released clot / clot weight) × 100

RESULT AND DISCUSSION

Results

Acute toxicity test

Acute toxicity assay did not show any toxic effects for the animal within the due course of study.

Anti-diarrhoeal activity

Table 1 and figure 1 showed the methanolic barks extract of *Cinnamomum cecidodaphne Meissn* barks was found to be effective in a dose dependent manner against castor oil induced diarrhea on experimental mice at all tested doses. After a 30-min administration of castor oil, diarrhea was clinically apparent for the next 4 h in the control group. This condition was markedly reduced by 83.33% by loperamide at a dose of 5 mg/kg. Our extracts also demonstrated statistically significant ($P < 0.05$) inhibition of castor oil-induced diarrhea in a dose-dependent manner. At the doses of 200 and 400 mg/kg body weight, the extract produced a significant decrease in the severity of diarrhea in terms of reduction in the rate of defecation and consistency of faeces in albino mice. At the same dose, the extract showed significant antidiarrheal activity ($P < 0.05$) showing 64.84 % and 76.36 % reduction in diarrhea comparable to that of the standard drug loperamide that showed 83.33%reduction in diarrhea. The effect of the highest dose of the extract was comparatively similar to that of the standard drug, Loperamide (5 mg/kg).

Table 1: Antidiarrheal activity of methanolic extract C. *cecidodaphne Meissn* against castor oil-induced diarrhea in mice.

Group	Dose(p.o.)	No. of faeces in 4 hours	% of inhibition of diarrhea
Control	10mL/kg, p.o.	16.5±2.038688	-
Loperamide	5mg/kg	2.75±0.637377*	83.33
Methanol	200mg/kg	5.8±1.066536*	64.84
Methanol	400mg/kg	3.9±0.518411*	76.36

All value are expressed as mean ±STD (n=5). One-way analysis of variance (ANOVA) followed by Dunnett's test. Here,* $P<0.05$, significant compared with control group.

Figure 1. Antidiarrheal activities by Normal saline, Loperamde, Methanol extract of *C. cecidodaphne* *Meissn different* doses.

Thrombolytic activity

Addition of 100 µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed ($p < 0.01$) 77.06%.The thrombolytic result shown on table 2 and figure 2. Here used a standard control streptokinase which is used same way of procedure here shown near about 80% of clot lysis. In a negative control treated normal water which exhibited a negligible percentage of lysis of clot 2.64%.That means the difference between the positive and negative control was very significantly. The methanolic C. *cecidodaphne Meissn* extract has a moderate thrombolytic effect where percentage of clot lysis capability was ($p < 0.01$) 35.08% and p value not more than 0.5. So a thrombolytic activity plays a moderate role of this plant extract.

Table 2. Effect in vitro clot lysis activity of methanolic extract C. cecidodaphne Meissn

Extracts/ Drugs	Mean ± S.D (% of clot lysis)
Water (negative control)	2.64 ± 0.84%
Streptokinase (positive control)	77.06 ± 2.03%
Methanolic extract of C. *cecidodaphne Meissn.*	35.08 ± 8.09%*

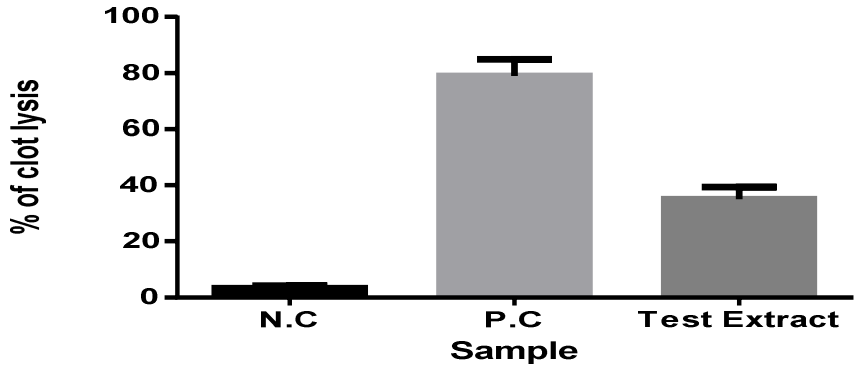

Figure 2. Clot lysis by water, Streptokinase, Methanol extract of *C. cecidodaphne Meissn.*

DISCUSSIONS

Traditional medicine or Plant-derived medicines have a positive site long history of use for the prevention and cure of human diseases. Today, many pharmaceuticals currently approved by the Food and Drug Administration (FDA) have origins to plant sources. Now a day's plant-derived medicine has led to the rigorous scientific examination to determine efficacy and safety based on the reported immune modulatory effects has emerged in recent times. [29]. Many populations who live in developing countries are deprived of the advantages of modern

medicine because of its high cost; hence, poor people are more vulnerable to infectious diseases. For all these reasons, there is a foremost need to identify new, safe, and cost-effective antidiarrheal agents that would help to alleviate the problems of diarrheal diseases. Plant-derived medicine represents an attractive source of antidiarrheal agents because they are natural and affordable, especially for rural people in developing countries [30]. People customarily using plant(s) or plant-derived medicine consider them to be efficacious against diarrheal disorders without any scientific basis to explain the action of such plants. The aim of this study was to experimentally evaluate the use of *C. cecidodaphne Meissn* barks which are regarded to confer protection in diarrhoea in Bangladeshi traditional medicine [31]. Diarrhoea remains a potential cause of morbidity and mortality, especially in children in developing countries. More than 80% of childhood deaths in Africa and South Asia are due to diarrhoea [32]. Traditional medicines, such as *Punica granatum*, *Psidium guajava*, *Ocimum gratissimum, Phyllanthus emblica, and Aegle marmelos*, are commonly used in Bangladesh, and their use against diarrhoeal has now been scientifically established [33, 34].Consequently medicinal plants are a promising source of antidiarrheal drugs [35, 36]. For this reason, WHO has incited studies that evaluate traditional medicinal practices to treat and prevent diarrheal diseases [37, 38]. This experimental model was therefore employed to validate antidiarrheal efficacy of *C. cecidodaphne Meissn* extract in the current study. Diarrhoea may be characterized as the abnormally frequent defecation of faeces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines. In spite of multiplicity of aetiologies, the four major mechanisms responsible for the pathophysiology in water and electrolytes transport are (i) Rises luminal osmolarity (osmotic diarrhoea), (ii) rises electrolytes secretion (secretory diarrhoea), (iii) Reduce electrolytes absorption, and (iv) deranged intestinal motility causing a decreased transit time [39]. It is widely known that after oral ingestion of castor oil, ricinoleic acid is released by lipases. This acid causes irritation and inflammation in the intestinal lumen of inflammatory mediators, such as prostaglandins, histamine, and so forth. The prostaglandins thus released promote vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines. Prostaglandins of the E series are considered to be good diarrheogenic agents in experimental animals as well as in human beings. The inhibitors of prostaglandins biosynthesis are therefore considered to delay castor oil– induced diarrhoea [40]. Methanol extract of *C. cecidodaphne Meissn* (200–400 mg/kg, p.o.) significantly ($p < 0.05$), reduced the faecal output produced by castor oil. At doses of 200–400 mg/kg (p.o.), the plant extract significantly ($p < 0.05$), and dose dependently delayed the onset of diarrhoea induced by castor oil when compared with the untreated

controls. *C. cecidodaphne Meissn* (200 mg/kg, p.o.) reduced the number of faecal episodes by 64.84% while the dose of 400 mg/kg (p.o.) significantly ($p < 0.05$), reduced the number of animals suffering from diarrhoea by reducing defecation by 76.36%. Loperamide (5 mg/kg, p.o.) profoundly ($p < 0.05$), reduced the faecal output produced by castor oil. Loperamide (5 mg/kg) reduced the number of faecal episodes by 83.33%. In terms of protection from diarrhoea at 4 h, the 400 mg/kg dose of C. *cecidodaphne Meissn* extract was comparable with the standard drug loperamide and significant inhibition of castor oil induced diarrhoea in extract dependent manner in experimental mice (Table 1).

A number of traditional plants source conspicuously several fruits and vegetables have been studied for their supplements having anticoagulant, antiplatelet and fibrinolytic activity and there is evidence that consuming such food leads to prevention of coronary events and stroke [41-44]. In our thrombolytic assay, the comparison of positive control with negative control clearly illustrate that clot dissolution does not occur when water was added to the clot. When compared with the clot lysis percentage obtained through SK and water, an extremely significant ($p < 0.01$) thrombolytic activity was observed after treating the clots with *C. cecidodaphne Meissn* extract. We found that crude have tremendous effect ($P < 0.01$). Here, the prominent effect was shown by crude (35.08 %) compare with positive control (SK) [77.06 %] (table 2). At acute toxicity level, the extracts did not cause any mortality or visible signs of toxicity or differences in food and water uptake in the animals up to 600 mg/kg According to the results of the present investigation, it can be concluded that the methanolic bark extract of *C. cecidodaphne Meissn* has significant, anti-diarrheal and Thrombolytic effects that support to the traditional use of this plant for the treatment of related diseases. This study also suggests for the further detail investigation of mechanisms of the pharmacological effects and also to isolate the active compound(s) responsible for those properties.

CONCLUSIONS

In above discussion we can come in a point that the methanolic extract of *Cinnamomum cecidodaphne Meissn* has presences strong antidiarrheal activity and moderate thrombolytic activity**.** Further studies are required to observe and isolate the main principles to establish the exact mechanism of action of the test extract.

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CONFLICT OF INTEREST STATEMENT:

The authors declare that there is no conflict of interest.

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