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Comparative pharmacological properties between ethanolic extracts of *Phyllanthus acidus* P. and *Sonneratia apetala* L. on Swiss albino mice

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Abstract

The present study was designed to evaluate and compare antidiabetic, analgesic activities of ethanolic extract of *Phyllanthus acidus* and *Sonneratia apetala*. We carried out this work to explore the medicinal uses of very common and cheap fruity plant among the people of all classes. The antidiabetic activity was evaluated and compared by studying the effect of ethanolic extract of *Phyllanthus acidus* (EEPA) and *Sonneratia apetala* (EESA) against blood glucose level of alloxan-induced diabetic mice in every 4 h for 16 h. To evaluate and compare analgesic, activities different tests such as acetic acid-induced writhing test. The mice were treated with both plants extract at a dose of 250 & 500 mg/kg body weight in case of antidiabetic activity test. Blood glucose level was examined and found that there was a significant reduction of blood glucose level with EEPA ($p<0.05$) and EESA ($p<0.05$) extracts were given at a dose of 250 mg/kg body weight showed significant ($p<0.05$) reduction of blood glucose level with a mean value from 19.08±2.598* mmol/L to 9.4±2.112 mmol/L and 14.6±1.025* mmol/L to 8.66±0.60465 mmol/L. EEPA and EESA was given at a dose 500 mg/kg body weight showed significant ($p<0.05$) decreased blood glucose from 23.8±6.756 mmol/L to 8.86±1.806*** mmol/L (±SEM) and 16.16±2.441 to 9.825±0.70282*** mmol/L (±SEM) in comparison with their respective diabetic control group. Although both plant extracts reduced the blood glucose level, the glucose reducing effect was higher in EEPA. Both the plants EEPA at a dose of 500 mg/kg and EESA and at a dose of 250 mg/kg body weight showed significant ($p<0.05$) analgesic effect with percentage of inhibition 32.55% and 21.70% respectively. EEPA at a dose of 250 mg/kg and EESA at a dose of 500 mg/kg body weight showed significant ($p<0.05$) analgesic effect with percentage of inhibition 67.44% and 52.71% respectively. The analgesic activity in treated mice but less significant central analgesic activity. EEPA showed higher peripheral analgesic activity than EESA. In this study, showed highly sufficient antidiabetic property in plant of *S. apetala and P. acidus* showed highly significant analgesic activity, thus administration of noil and keora may be useful for diabetic people. Keora may be more helpful for diabetic people than noil.

KEYWORDS

Phyllanthus acidus ; *Sonneratia apetala* ; Antidiabetic ; Analgesic.

1.0 INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by a deficiency in insulin production and its action or both. That leads to prolonged hyperglycemia with disturbances in most metabolic processes inside the human body [1]. Untreated cases show severe tissue and vascular damage leading to serious complications such as retinopathy [2],

neuropathy [3], nephropathy [4], cardiovascular complications [5] and ulceration [6]. Moreover, diabetes has an indirect relation with a many other diseases being the most common endocrine disorder. It was estimated that about 200 million people worldwide suffered from DM in 2010, and it is expected to reach 300 million by 2025 [7]. Both insulin and glucagon, pancreatic endocrine hormones, are responsible for controlling blood-glucose level within the body in an adequate level based on the body needs [8].

Phyllanthus acidus Skeels belonging to the family Phyllanthaceae is distributed commonly throughout India, and often used by ethnic peoples of north-east India in folk medicine. *P. acidus* have been used traditionally in the treatment of several pain, inflammatory and oxidative stress related disorders such as rheumatism, bronchitis, asthma, respiratory disorder, hepatic disease, diabetes and gonorrhoea. The plant is also important to improve eyesight, memory and to cure cough, psoriasis, skin disorders, sudorific [9-11]. Fruits of the plant used as astringent, root and seed are useful as cathartic, leaf and root use as antidote to viper venom [12]. The leaf of the plant found effective in hypertension [13]. Leaf, bark and roots are used to treat fever traditionally [14]. The latex of the plant credited with purgative and emetic activity [15]. Methanolic extract of fruit and leaves was reported to possess antimicrobial activity [16]. *In- vitro* screening of petroleum ether extract of fruits showed cytotoxic, antibacterial and antioxidant activity [17]. Adenosine, kaempferol, and hypo gallic acid were found in leaves which showed airway chloride secretion, a potential treatment in cystic fibrosis [18]. Phyllanthus genius is reach in different secondary metabolite like alkaloids, tannins, flavonoids, lignans, phenolics and terpenes [19]. Several species of Phyllanthus have shown ant nociceptive activity in mice [20]. In this study, *P. acidus* leaves extracts were assessed for analgesic, antidiabetic activity.

The mangrove fruit *Sonneratia apetala* (Buch.-Ham.), locally known as keora, is abundantly grown in the world largest mangrove forest, the Sundarbans and also along the coastal areas in Bangladesh, India, Myanmar, Malaysia, China, Papua New Guinea, and some parts of Africa. People in coastal Bangladesh, India, and Myanmar extensively consume the fruit by cooking and through other preparations. Fruits and barks of the plants belonging to genus Sonneratia have remedial activities against asthma, febrifuge, ulcers, swellings, sprains, bleeding, hemorrhages, and piles [21]. Patra et al. [22] reported antibacterial, antioxidant, antidiabetic, and anticancer activities of leaf and bark extracts of *S. apetala*. Nevertheless, a little scientific attention on the fruit has been paid until today. We reported antioxidant, antidiabetic, and antibacterial activities of methanol extract of this fruit [23]

Moreover, analgesic, antidiarrhoeal, anthelmintic, and cytotoxic activities of ethanol extract of the fruit were also reported [24]. However, food insecurity is one of the major causes of hunger and malnutrition in tropical coastal population. To fulfill food security and primary health care, it is essential to explore wild food plants and for the tropical coastal countries, edible mangrove fruits could be an attractive source in this regard since they could easily be planted in vast saline water intruded areas outside the coastal embankments. Fruit of *S. apetala* is such an important fruit as has untapped potential and, therefore, the current study was undertaken.

2.0 MATERIALS AND METHODS

Drugs and chemicals

Diclofenac sodium and metformin were procured from Square Pharmaceuticals Ltd, Bangladesh. Alloxan were supplied by the Department of Pharmacy, Dhaka International University (DIU), laboratory. All solvents and reagents used were of analytical grade and solvents obtained from Merck, Germany.

Plant material

The Noil (*Phyllanthus acidus*) and Keora (*Sonneratia apetala*) were selected for the investigation. These were collected and identified from various areas of Satkhira (22.3155° N, 89.1115°E) Bangladesh during the month of December 2021.

Preparation of plant extracts

The leaves plant of *P. acidus* and *S. apetala* were

thoroughly washed with fresh water to remove all contaminants and dried under shade at room temperature for a period of 9 days. Coarse powder of the materials was produced by grinding the materials with the help of a grinder. The coarsely powdered materials were weighted and about 200 mg of both powdered materials were macerated with ethanol (1:3) at room temperature for a period of 10 days with occasional shaking and stirring. The whole mixture was filtered through cotton and then Whatman No.1 filter papers. The solvent of the filtrate thus obtained was evaporated by rotary evaporator (Witeg, Germany, Model No: 2600000) to get the final extract (viscous mass). Then, the extract was stored in refrigerator (4°C) for further use.

Experimental animals

A 4-5-week-old aged female Swiss albino mice of body weight 20-30 g (procured from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh) were used to run the antidiabetic and analgesic experiments. Before initiating the experiments, the animals were kept under standard environmental conditions, maintained 54-64% relative humidity, and exposed to alternative 12:12 h light and dark cycle at an ambient temperature of 26±2°C. Proper arrangement of foods and water ad libitum was ensured. Before the experiment, mice were habituated for 7 days in our laboratory environment and ensured the constant environmental and adequate nutritional conditions throughout the period of the experiment. Plant accession number *P. acidus* (67083) *S. apetala* (67082).

Antidiabetic activity test

Experimental induction of diabetes:

Group animals were allowed to fast for 12 hr. and rendered diabetic by injection intraperitoneally with a freshly prepared solution of alloxan monohydrate (180 mg/kg body) in normal saline after baseline glucose estimation was done [3]. After alloxan induction, hypoglycemia may develop. To overcome alloxan-induced hypoglycemia, the alloxan-treated mice were allowed to food overnight. After 48 h, blood glucose concentration was measured using Rapid ViewTM blood glucose test meter using blood sample from the tail vein of the mice. The mice having blood glucose level above 11.1 mmol/L were considered as diabetic mice and selected for the study.

Experimental Design

Seven groups of mice, five in each received the following treatment schedule.

Group I: Normal control (saline 10 ml/kg, p.o.)

Group II: Alloxan-treated control (180 mg/kg, i.p)

Group III: Alloxan (180 mg/kg, i.p) + *P.acidus* plants extract (250mg/kg, p.o)

Group IV: Alloxan (180 mg/kg, i.p) +*S.apetala* plants extract (250mg/kg, p.o),

Group V: Alloxan (180 mg/kg, i.p) + Standard drug, metformin (150mg/kg, p.o)

Group VI: Normal *P.acidus* plants extract (500 mg/kg, p.o)

Group VII: Normal *S.apetala* plants extract (500 mg/kg, p.o).

Final observation

Both the plant extracts (250 mg/kg and 500 mg/kg) and standard drug metformin (150 mg/kg) were given orally according to experimental design and blood glucose concentration was measured using Rapid ViewTM blood glucose test meter using blood sample from the tail vein of the mice in every 4 hr. for 16 hr.

Analgesic activity

Acetic acid-induced writhing test in mice:

The test was conducted by modified method of Koster et al. [9]. Briefly, mice were treated with diclofenac sodium (100 mg/kg body wt.), a standard analgesic drug. Six groups of mice, five in each received the following treatment schedule.

Group I: Control (10 ml/kg distilled water p.o.)

Group II: *P. acidus* plants extract (250 mg/kg, p.o.)

Group III: *P. acidus* plants extract (500 mg/kg, p.o.)

Group IV: *S. apetala* plants extract (250 mg/kg,p.o)

Group V: *S. apetala* plants extract (500 mg/kg, p.o)

Group VI: Diclofenac Na (25 mg/kg, p.o).

After 45 min of respective treatment, each mouse was injected intraperitoneally with 0.7% (v/v) acetic acid at a dose of 10 mg/kg body weight. Acetic acid is induced to provoke writhing activity. The writhing activity comprised constriction of the abdominal muscles together with a stretching of the hind limbs.

The number of writhing responses of each mouse was counted for a 5-min period, which began 15 min late of acetic acid administration. To determine the percentage (%) of inhibition of writhing, the following formula was used.

Mean no of writhes (control)- % Inhibition = ((mean no of writhes (test)/(Mean no of writhes (control)) ×100%

Preparation of successive extracts from dry powder

75g of the dried leaf powder was re-extracted using, ethanol, and water. Each extract obtained following successive extraction was filtered using Whatman. No 1 filter paper. A stock concentration of 1 % (W/ V) of each successive extract obtained using ethanol and water was prepared using the respective solvent.

3.0 RESULTS AND DISCUSSION

3.1 Antidiabetic activity test

Diabetes is a metabolic disorder disease with chronic high blood glucose level. Now it is one of the major life threatening diseases in the world. High blood glucose level is related to different complication in the body including heart disease. During this study, an increase in blood glucose levels in diabetic animals was observed after the induction of diabetes mellitus by alloxan. The effects were comparable to the standard antihyperglycaemic drug metformin was given at a dose of 150 mg/kg body weight. The result obtained from antidiabetic activity test is described in graph 1. Both the plant extracts EEPA and EESA were given at a dose of 250 mg/kg and 500 mg/kg body weight-treated animals showed a significant reduction in both blood glucose levels.

Here standard drug metformin reduced blood glucose level from 11.62±0.74726 mmol/L to 10.52±0.37297 (± SEM) mmol/L within 0 h to 16 h. On the other hand blood glucose change observed by plant extracts EEPA and EESA in experimental mice significantly was determined at every 4h. During 0 h to 16h observation, EEPA and EESA extracts were given at a dose of 250 mg/kg body weight showed significant ($p<0.05$) reduction of blood glucose level with a mean value from 19.08±2.598* mmol/L to 9.4±2.112 mmol/L and 14.6±1.0251* mmol/L to 8.66±0.60465 mmol/L. EEPA and EESA was given at a dose 500 mg/kg body weight decreased blood glucose from 23.8±6.756 to 8.86±1.806*** mmol/L (±SEM) and 16.16±2.441 to 9.825±0.70282*** mmol/L (±SEM). In alloxan-induced diabetic mice compared with metformin and blood glucose change observed by Kawra (*S. apetala*), also alloxan-induced diabetic mice compared with metformin which indicate Noil (*P. acidus*) has more hypoglycemic effect than Keowra.

3.2 Analgesic activity

The results of acetic acid-induced writhing test are displayed in Graph 2. EEPA at a dose of 500 mg/kg and EESA and at a dose of 250 mg/kg body weight showed significant ($p<0.05$) analgesic effect with a mean value of 17.4±5.095* and 20.2±5.598* respectively, with percentage of inhibition 32.55% and 21.70% respectively. EEPA at a dose of 250 mg/kg and EESA at a dose of 500 mg/kg body weight showed significant ($p<0.05$) analgesic effect with a mean value of 8.4±0.86002 and 12.2±3.821 and with percentage of inhibition 67.44% and 52.71% respectively. Diclofenac sodium (100 mg/kg) used as standard drug which showed comparatively highly significant ($p<0.01$) analgesic effect with mean value of 3.6±1.023** with 86.04% of inhibition.

Diabetes is a metabolic disorder disease with chronic high blood glucose level. Now it is one of the major life threatening diseases in the world. High blood glucose level is related to different complication in the body including heart disease. During this study, an increase in blood glucose levels in diabetic animals was observed after the induction of diabetes mellitus by alloxan. The effects were comparable to the standard antihyperglycaemic drug metformin was given at a dose of 150 mg/kg body weight.

Here standard drug metformin reduced blood glucose level 11.62±0.74726 mmol/L to 10.52±0.37298 (± SEM) mmol/L within 0 hr. to 16 hr. Diabetes mellitus is a chronic, endocrine disorder characterized by inability of cell to utilize insulin or by the ineffectiveness of the insulin production by pancreas. The International Diabetes Federation SEA Region,

which composes of the countries of India, Sri Lanka, Bangladesh, Bhutan, Mauritius and Maldives, has a high prevalence of diabetes. It has been reported that there were more than 72 million adults with diabetes in 2013, a number that is expected to exceed 123 million by 2035. Nearly 95% of people of the total diabetic patients have type 2 diabetes (T2DM) [25]. Moreover, another 24.3 million people have impaired glucose tolerance [26]. Both rural and urban population has reported increases in the number of diabetic people [27]. In a cross-sectional survey of 402 people among the urban middle class in Bangladesh, it has been reported that 35% had T2DM and 45% had metabolic syndrome [28]. Diabetes can progress to other disorders such as cardiovascular, kidney, and eye disorders. In fact, a high prevalence of chronic kidney disease has been seen following a community survey of urban people of Bangladesh, which has been correlated with insulin resistance [29].

We evaluate and compare the antidiabetic effect of EEPA and EESA. In this study, we found that standard drug metformin reduced the blood glucose level from 18.74 mmol/L to 10.66 mmol/L (±SEM) within 0-16h. On the other hand, EEPA (250 mg/kg) decreased blood glucose level from 19.08 to 9.45 mmol/L (±SEM) in alloxan-induced diabetic mice within 0-16h. EEPA (500mg/kg) decreased blood glucose level from 23.8 to 8.6 mmol/L (±SEM) in alloxan-induced diabetic mice within 0-16 h and EESA (250mg/kg) decreased blood glucose level from 14.6 mmol/L to 8.63 mmol/L (±SEM) in alloxan-induced diabetic mice within 0-16 h and EESA (500 mg/kg) decreased blood glucose level from 16.16 mmol/L to 9.82 mmol/L (±SEM) in alloxan-induced diabetic mice within 0-16 h which Indicate that EESA has higher hypoglycemic effect.

4.0 CONCLUSION

It can be concluded that both EEPA and EESA showed its effectiveness as an antidiabetic and peripheral analgesic agent and EESA has higher antidiabetic activity and EEPA has higher peripheral analgesic activity. We hope that further detailed investigation is underway to determine the exact bioactive phytoconstituents that are responsible for those activities of those plants. Moreover, it could be a potential source for novel "lead" discovery for antidiabetic and analgesic drug development.

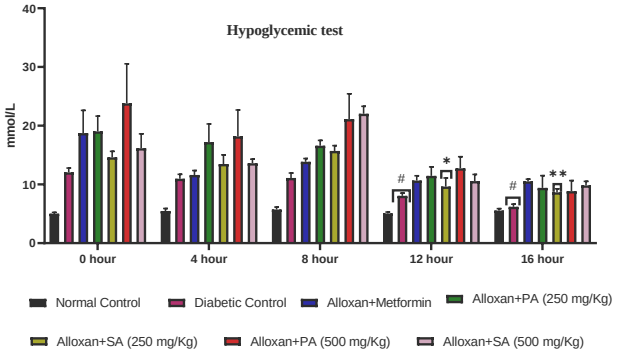


Fig. 1: Effects of *P. acidus* and *S. apetala*. extract on blood glucose level in alloxan-induced diabetic mice.

The blood glucose level was expressed as mean (±S.E.M.) values. Level of significance ***= p<0.001, **=p<0.01, *=p<0.05 compared to diabetic control. (ANOVA followed by Dunnett's t-test). EEPA: Ethanolic extract of *P.acidus*, EESA: Ethanolic extract of *S.apetala*. *P. acidus*: *Phyllanthus acidus*, *S. apetala*: *Sonneratia apetala*.

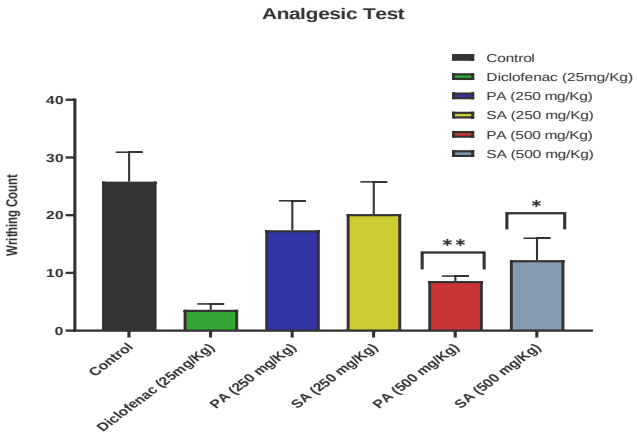


Fig. 2: Effects of *P. acidus* and *S. apetala* extract on acetic acid-induced mice.

The graph showed that the application of different plant extracts have very little analgesic effects in the animals under investigation. Acetic acid control group

gives maximum writhing 25.8 (±SEM). The plant extract reduce the count 8.4 (± SEM) in noil (*P. acidus*) and 12.2 (±SEM) in Kawra (*S. apetala*). Here standard diclofenac control group reduces the writhing count much more than the plant extract.

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AUTHORS' CONTRIBUTIONS

Tasnia Tabassum carried out the collection of plant, extraction process. Nowrin Ferdiousi, Tasnia Tabassum, Md Mizan, and Asma Akter conducted the research work, conception and design of the study, statistical analysis and interpretation of data, and wrote the manuscript. Debendra Nath Roy supervised the research project and edited the writing. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

No conflicts of interested

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