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EVALUATION OF THE METHANOLIC EXTRACT OF LEAVES OF *PERSICARIA CAPITATA* FOR PHARMACOLOGICAL ACTIVITY

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Abstract

The aim of the study was to evaluate the in vivo cytotoxic and analgesic activities of methanol extract of leaves of *Persicaria capitata*. The cytotoxic bioassay was evaluated using brine shrimp (Artemiasalina Leach) lethality test where the LC50 value for the extract was found to be 12.31 µg/ml where the LC50 value for the standard potassium dichromate was 31.26 µg/ml. The extract showed significant writhing inhibition in acetic acid induced Swiss Albino mice at doses of 250 and 500 mg/kg body weight and percentage (%) of inhibition was 58.66% and 62.36% respectively compared to the standard diclofenac at a dose of 10 mg/kg body weight. The overall result obtained from the study suggests the cytotoxic and analgesic properties of leaves of the plant. The effect of both cytotoxic and analgesic of the methanol extract was significant.

Keywords: *Persicaria capitata1, Polygonum capitatum2,* methanol extract3, leaves extract4, cytotoxic5, brine shrimp lethality6, analgesic7, writhing8.

Introduction

Medicinal plants are spread all over the world and various plants have different uses in remedies of diseases in different culture and region. These plants and their ethnopharmacological uses help in the discovery and development of new drug molecules. More than 50% of all the drugs in modern therapeutics are derived from natural products and their derivatives (Pan et al., 2013). According to World Health Organization (WHO, 1993), 74% modern medicine derived from about 119 plants are used in ways directly correlated with their traditional uses as medicine (Nyarkoet al. 2012). Bangladesh is also a land of various medicinal plants.Most rural people here depend on thousands of different plants for traditional medicine among the 5000 phanerogam and pteridophyte species growing in forests, wastelands and roadsides. (Ghani, 2003).Different randomised surveys have been conducted at different times in various areas of Bangladesh to find out the medicinal plants used by local kaviraj or traditional healthcare practitioners. They are reliable sources of natural remedies because of being safe and economical as well as possessing lesser side effects. A number of plants used for traditional medicine have been found to contain various efficacious compounds against different diseases. Extracts from different Bangladeshi medicinal plants were studied in vitro to see the effect on the proliferation of breast cancer cell lines (Lambertiniet al., 2004). There are traditional uses of medicinal plants for the treatment of urinary tract infection and sexually transmitted disease (Hossanet al., 2010).Some traditionally used plants have been found to possess anti-hypotensive and antidiabetic activities. There are also some plants used in respiratory problems (Rahmatullah et al., 2009).A number of functional foods have been identified having pharmacological properties (Rahmatullah et al., 2009).

Persicaria capitata is one of the medicinal plants found in Bangladesh. *P. capitata*, also known as *Cephalophilon capitatum*, *Polygonum capitatum*,is an Asian species of perennial herb having ascending or creeping stem. It is commonly known as pink-head smartweed, pink-headed persicaria etc. The plant is reported to contain different antioxidant models and compounds which have been screened and identified e.g., gallic acid, progallin, kaempferol, quercetin, tricosanol, lignoceric acid, oleanolic acid etc (Liu et al., 2008) (Yang et al., 2009).

The plant is traditionally used in China in the treatment of urinary tract infection and the plant is reported to contain anti-bacterial and anti-inflammatory agents (Shang-Gao Liao et al. 2011). In vivo pharmacological experiment showed that aqueous extract of *Polygonum capitatum* (*Persicaria capitata*) decreased the WBC and RBC level in urine of pyelonephritis mode in rats, death rate of E. coli infected mice and the temperature of feverish rabbits. After oral administration of *Polygonumcapitatum*, the animal urine inhibited the growth of E. coli (Ren G et al.1995). No other biological activity has been studied yet. The present study was conducted in vivo to evaluate the cytotoxic and analgesic actions of the methanol extract of leaves of *P. capitata*.

Methods

Collection of Plant Material

The plant of investigation was collected from wasteland of Mymensingh, Bangladesh at the end of November, 2017 and was identified by experts of National Herbarium, Bangladesh.

Extraction

The collected plants were cleaned and the leaves were separated from the stem. The leaves were washed using portable water and kept under shade for drying. The leaves were shade-dried for around 7 days and then crushed into coarse powder using an electric grinder. Approximately 300 g of the powdered leaves of *P. capitata* were taken in a 1 L beaker and soaked in about 600 ml of methanol at room temperature. The opening of the beaker was sealed using aluminium foil paper and this was kept for 3 days with occasional stirring. After 3 days, the powder was filtered using cotton followed by filter paper. The filtrate was then concentrated at 50° C using a rotary evaporator. The yield was a blackish green crude methanol extract of the leaves of *P. capitata*.

Pharmacological Studies

Brine Shrimp Lethality Bioassay

Cytotoxic effect of plant extract is evaluated by Brine Shrimp lethality bioassay (Meyer et al. 1982) performed on a simple zoological organism (Brine nauplii) which is very comprehensive in monitoring the presence of any bioactive natural product. Since sea water is a friendly media for hatching of Brine Shrimp eggs, simulated sea water was prepared by dissolving 76g of iodine-free sodium chloride in 2 litres of distilled water taken in a rectangular glass chamber to make a concentration of 3.8%. Shrimp eggs were placed in the water of the chamber and one side of the chamber was covered. The hatching of the eggs was accomplished after 24 hours by maintaining constant oxygen supply and controlled temperature. The hatched shrimps were attracted to the light using an electric bulb and nauplii larvae were collected for the test from the illuminated part of the tank using a pipette.40 mg of crude plant extract was measured and dissolved in 10 ml of pure Dimethylsulfoxide (DMSO) making a concentration of 4000 µg/ml. This stock solution was used to prepare 10 different sample solutions by serial dilution with concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.57 and 0.78 µg/ml. Similar concentrations of solutions for positive control was prepared using Potassium dichromate and 50 µml of DMSO was added to three test tubes containing 4.95 ml sea-water to prepare negative control. 100 µl of every prepared solutions were taken in different test tubes and the final volume was adjusted to 5 ml using sea water. 10 nauplii were taken in every test tubes and kept for incubation at 30±2° C. After 24 hours of incubation, the test tubes were inspected and nauplii were counted using a magnifying glass. The obtained data was transferred in Microsoft Excel to determine the LC50value.

Analgesic activity

Analgesics are agents that reduce pain. The test was performed following the method described elsewhere (B. A. Whittle, 1964). The analgesia or sensitivity to pain is characterized by the writhing of the mice and it is induced by injecting 0.7% acetic acid. The experimental animals, Swiss albino mice, weighing between 18 – 25 grams, were collected and divided into four groups. 30 minutes prior to the injection of acetic acid, Group-I was administered normal saline orally at the dose of 10 mg/kg body weight, Group-II was treated with Standard drug Diclofenac sodium intraperitoneally at the dose of 10mg/kg body weight, Group-III and Group-IV was administered extract solution orally at the doses of 250 mg and 500 mg/kg body weight. Then 0.7% acetic acid was injected by intraperitoneal route. After 5 minutes of waiting, writhes were counted for 10 minutes.

Results

Brine Shrimp Lethality Bioassay

The percentage (%) of mortality of the brine shrimp nauplii was calculated using the following equation:

% Mortality= (N'-N)/N' X 100

Here, N'=Number of nauplii taken

N= Number of nauplii live

The effectiveness or concentration-mortality relationship is determined by the median lethal concentration (LC50) of the compound which can be determined by plotting % mortality against the logarithm of corresponding concentration by linear regression method in Microsoft Excel. No mortality was found in negative control group. The toxicity of the sample was significant compared to the standard.

The result of Brine Shrimp Lethality Bioassay for methanol extract of *P. capitata* is shown in Table 1 and Figure 1.

Analgesic activity

The extract was also subjected to Swiss albino mice to study the analgesic activities against acetic acid induced writhing and analgesic actions were found with oral doses of 250 and 500 mg/kg which is shown in Table 2.

The statistical analysis of result is significant compared to standard Diclofenac ($P < 0.01$). P value is calculated using Anova: Single Factor Data Analysis in Microsoft Excel.

Discussion

From the result of the study, it can be suggested that the methanol extract of *P. capitata* has potential cytotoxic and analgesic properties and further isolation and purification of the crude

extract may lead to the discovery of lead compounds responsible for the activities.

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Table 1. Result of Brine Shrimp Lethality Bioassay for leaves extract of *P. capitata* and standard Potassium dichromate.

Concentration (C) (µg/ml)		400	200	100	50	25	12.5	6.25	3.13	1.57	0.78
Log C		2.60	2.30	2	1.70	1.40	1.1	0.80	0.50	0.20	-0.1
% Mortality	Extract	80	70	70	60	60	40	50	40	30	30
	Standard	70	70	60	60	40	40	40	30	20	20
LC_{50} (µg/ml)	Extract	12.31*									
	Standard	31.26**									

* Linear equation: $y = 18.788x + 29.515$; $R^2 = 0.9327$

** Linear equation:$y = 20.404x + 19.495$; $R^2 = 0.9511$

Figure 1. Comparison between % Mortality for sample and standard in Brine Shrimp Lethality Bioassay.

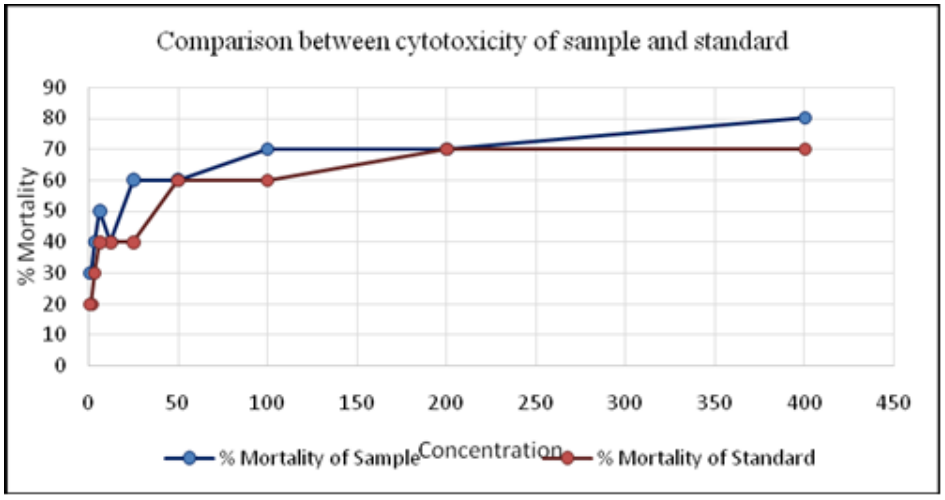

Table 2. Result of analgesic activity test forleaves extract of *P. capitata* on acetic acid induced mice.

Animal Group	Writhing Count					Mean ± SEM	% of Writhing	% of Inhibition
	Mice-1	Mice-2	Mice-3	Mice-4	Mice-5			
Control	96	83	85	86	83	86.6 ± 2.42	100	0
Standard	28	37	42	28	33	33.6 ± 2.69	38.8	61.20
TG-1	27	39	43	31	39	35.8 ± 2.94	41.34	58.66
TG-2	17	41	49	25	31	32.6 ± 5.67	37.64	62.36