Pharmacognosy Communications
Volume 2 | Issue 3 | Jul-Sep 2012
www.phcogcommn.org

Research Article

Effect of *Basella rubra* L. leaf extract on haematological parameters and amylase activity

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ABSTRACT: **Introduction:** *Basella rubra* is a plant species commonly known as Poi (family Basellacea) found in almost every part of India, except hills. Poi possesses several folkloric uses, having beneficial effects in haemorrhagic diseases and as a tonic. In the present study, the effects of ethanolic and aqueous extract of *Basella rubra* (Poi) leaves were investigated for haematological parameters on normal Swiss mice and amylase activity on Wistar rats. **Methods:** Ethanolic and aqueous extracts of the plant (dosed at 100 and 200 mg/kg body weight) were administered to mice in different test groups for a period of 14 days for determination of haematological parameters. Aqueous, ethanolic and hexane extracts were given (200 mg/kg body weight) to the test group animals (Wistar rats) for the determination of amylase activity. The control group received distilled water for a period of 7 days. **Result:** There was an increase in the haematological parameters (RBC, WBC, Hb and PCV. The plant extracts caused a significant increase in the total bilirubin content. The aqueous and ethanolic extracts of 200 mg/kg showed significant increase ($P < 0.05$) in total bilirubin content but no significant increase was observed in aqueous and ethanolic extract (100 mg/kg) treated mice. There was also an increase in the amylase content in the hexane and ethanolic group animals and a decrease of amylase content in aqueous extract containing animals as compared to control group**. Conclusion:** It can be concluded that the commom plant *B. rubra* leaf has beneficial haematological properties in Swiss mice. In addition, the extract can prevent various complications of diabetes.

KEY WORDS: *Basella rubra*, Poi, haematological parameters, amylase activity

INTRODUCTION

Basella rubra is grown as a pot herb in almost every part of India, except in hill regions. In English, *Basella rubra* is known as Indian spinach; in Ayurveda as upodika, potaki, malvaa, amritvallari; and in Siddha/Tamil as vaslakkirai.[1] *Basella rubra* belonging to family Basellaceae[1] is a succulent, branched, smooth, twining and herbaceous vine reaching a length of several meters. The stems are green or purplish. The leaves are somewhat fleshy, ovate or heart-shaped, 5 to 12 cm in length, stalked, tapering to a pointed tip and cordate at the base.[3] Leaves are used in catarrhal affections and to hasten suppuration and decoction of roots reliefs bilious vomiting.[2] *Basella rubra* is a good source of calcium, iron, and vitamins A and C. Red-stemmed types are especially nutritious.[4] Basella herb is sour, tonic, antipyretic, improves the voice, applied to burns according to "Unani" system.[1]

Basella rubra contains amino acids, vitamins, organic acids, polysaccharides and biflavonoids. A glycoprotein with strong antiviral activity (against potato virus) has been isolated from leaves. The fatty acid composition of the seed oil has also been reported.[5] Two novel antifungal peptides, designated α- and β-basrubrins have been isolated from the seeds of *Basella rubra,* that exerted potent antifungal activity toward *Botrytis cinerea*, *Mycosphaerella arachidicola* and *Fusarium oxysporum*.[6] The plant was found to be rich in calcium constituents. The fatty oils from the seeds were found to contain palmatic, oleic and linolenic acid.[7] Carotenoids have been detected in leaves of *Basella rubra* and the major carotenoids detected in all the species were beta-carotene, small amounts of alpha carotene and traces of other carotenoids.[8] The plant has previously been investigated for hypoglycaemic, antimicrobial, antifungal and for antiulcer activities although there is no information

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DOI: 10.5530/pc.2012.3.3

on the haematological parameters and amylase activity in the literature. The present study was undertaken to investigate the effect of different solvent extractions of *B. rubra* leaves on haematological parameters and on amylase activity. Because relevant experimental work on the haematological and amylase activity of the plant has not yet been explored

MATERIALS AND METHODS

Plant material

Fresh leaves of *B. rubra* (Indian spinach) were procured from the local market of Lucknow. The plant materials were identified and authenticated (Ref. No. NBRI/CIF/177/2010) by the Division of Taxonomy, National Botanical Research Institute (NBRI, CSIR), Lucknow. The leaves were initially washed in tap water, then with distilled water and shade-dried. They were subsequently reduced to a fine powder by grinding and passed through #40 mesh sieve. The dried powdered leaves were extracted with hexane, ethanol or water by Soxhlet extraction.

Haematological Parameters

Experimental design

The animals were divided into five groups of 6 animals (Swiss mice) in each group. The first group served as a control. The second and third group were given 100 and 200 mg/kg body weight (bw) of aqueous extract, respectively. The fourth and fifth group were given 100 and 200 mg/kg bw of ethanolic extract, respectively. Administration of the extract was performed orally once a day for a period of 14 days. Blood samples were collected from all animal through retroorbital sinus at the 14th day of the experiment. The blood samples were divided into two portions. The first portion was used to determine haematological parameters, whilst the second portion was used to determine biochemical parameters.[9] First portion of blood was put into anticoagulant and second portion of blood was allowed to clot and then centrifuged at 3500 rpm for 15 min. Serum obtained was used for the assay of total bilirubin.[10]

Blood Analysis

The blood samples were analyzed to determine the haematological parameters such as: packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, and haemoglobin concentration (Hb conc.).[11] RBC and WBC count was determined using Neubauer's chamber, haemoglobin concentration was measured by haemocytometer and Packed cell volume (haematocrit) was determined by using Wintrobe's tube. The automated haematology analyzer (STAT FAX model no. 3300) was used for determination of biochemical parameters of the serum for total bilirubin content.

Amylase Activity

Experimental design

The animals were divided into four groups of 6 animals (Wistar rats) per group. The first group served as control whilst the second, third and fourth groups were given 200 mg/kg body weight (bw) of aqueous, ethanolic and hexane extract, respectively. The control group received distilled water. Administration of the extract was done orally once a day for a period of 7 days. Urine samples were collected from the animals before 24 hours for analysis.[12]

Urine analysis

Urine analysis was performed by using Street and Close method.[12] It is a rapid method for determination of amylase activity in urine, by colorimetric absorption. This method uses amylose as substrate; it is very simple to perform and can be completed in approximately 25 minutes. Preparation of amylose and a criterion of purity of the product are given. The diagnostic reagent kit is intended for *in vitro* quantitative determination of the activity of α-amylase in serum or urine. Urine sample is preferred to be diluted 1:100 with normal saline. And the absorbance of control and test measured against distilled water at 620 nm. [Calculated in: Street-Close units/24 hours for urine sample]

$$\text{Amylase Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times \frac{}{10 \times 24 \text{ hours urine volume in ml}}$$

Statistical Analysis

All results were expressed as mean ± standard error of mean (SEM). Data was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered as statistically significant.

RESULTS

The results of the study are presented in Tables 1 to 3. Table 1 shows the effects of aqueous and ethanolic extracts of *B. rubra* on the haematological parameters. Aqueous and ethanolic extracts of 100 mg/kg bw and 200 mg/kg bw caused increases in RBC count ($P < 0.05$). The effect of the ethanolic extract (100 mg/kg) proved a more effective dose. A similar pattern of result was obtained for WBC, PCV and haemoglobin i.e. 200 mg/kg bw of aqueous extract and 100 mg/kg bw of ethanolic extract showed maximum effect. Interestingly, the ethanolic extract of 200 mg/kg did not significantly affect the Hb value compared to that of the control group. Table 2 shows the effect of plant extracts on total bilirubin content. It is evident that all plant extracts caused significant increases in the total bilirubin content. The aqueous and ethanolic extracts of 200 mg/kg showed significant increase ($P < 0.05$) but no significant increase in total bilirubin content was observed in aqueous

Table 1: Effect of aqueous and ethanolic extract of *B. rubra* leaves on some haematological parameters

Groups	Treatment	Dose (mg/kg)	RBC ($x10^6/mm^3$)	WBC ($x10^3/mm^3$)	Hb (%)	PCV (mm/hr)
Control	Dist. water	0	1.9 ± 0.03	10.05 ± 0.65	5.8 ± 0.11	20 ± 0.38
Group 1	Aqueous extract	100	2.18 ± 0.05*	11.70 ± 0.26*	6.0 ± 0.09*	21 ± 0.31*
Group 2	Aqueous extract	200	2.19 ± 0.04*	12.10 ± 0.62*	6.4 ± 0.13*	28 ± 0.51*
Group 3	Ethanolic extract	100	6.88 ± 0.07*	23.85 ± 0.74*	7.8 ± 0.11*	30 ± 0.28*
Group 4	Ethanolic extract	200	2.31 ± 0.03*	14.65 ± 0.50*	5.8 ± 0.10*	24 ± 0.25*

Values are expressed as Mean ± SEM. n = 6. * P < 0.05 when compared to control group.

Table 2: Effect of aqueous and ethanolic extract of *B. rubra* leaves on total bilirubin (serum enzyme)

Biochemical Parameter	Group 1 (control)	Group 2 (aq. extract 100 mg/kg)	Group 3 (aq. extract 200 mg/kg)	Group 4 (ethanolic extract 100 mg/kg)	Group 5 (ethanolic extract 200 mg/kg)
Total bilirubin (mg/dl)	1.70 ± 0.26	182 ± 0.58*	2.58 ± 0.43*	1.93 ± 0.25*	2.44 ± 0.47*

Values are expressed as Mean ± SEM. n = 6. * P < 0.05 when compared to control group.

Table 3: Effect of *B. rubra* extract on Amylase activity

Test	Group 1 (control)	Group 2 (hexane extract 200 mg/kg)	Group 3 (ethanolic extract 200 mg/kg)	Group 4 (aq. extract 200 mg/kg)
Determined Amylase content	222.39 ± 0.18	233.48 ± 0.72*	228.77 ± 0.77*	205.26 ± 0.62*

Values are expressed as Mean ± SEM. n = 6. * P < 0.05 when compared to control.

and ethanolic extracts (100 mg/kg) treated mice. Table 3 shows the effect of *B. rubra* leaf extract on amylase activity. As seen from the experimental analysis on rats, it was found that the aqueous extract of 200 mg/kg had greater effect than that of hexane and ethanolic extracts of 200 mg/kg bw treated rats. Observed values showed that the extracts of hexane and ethanol (200 mg/kg bw) did not decrease the amylase content while the aqueous extract decreased the amylase content in treated animals.

DISCUSSION

The present study shows that the extracts of *Basella rubra* leaves have positive effect on the haematological parameters. The result showed that the extract of *B. rubra* caused an increase in the WBC count. This finding suggests that the extract of the plant contains agents that stimulate the production of leucocytes. The presence of such agents had been reported for *Viscum album* (mistletoe) and other commonly prescribed medicinal plants.[13,14] The significant increase in RBC, Hb and PCV implies that there was a change in the oxygen carrying capacity of the blood and the transferring respiratory gases.[15] The higher values of RBC and other associated parameters are suggestive of polycythemia.[16] WBC differentials are the indicators of the ability of an organism to eliminate infection. The increase in Hb, RBC, WBC and PCV observed in this study suggested that *B. rubra* extracts may be pursued for their clinical relevance in the management of anaemia and immunity-dependent disorders. The present study revealed significant increase in the activities of serum bilirubin levels, indicating considerable hepatocellular injury.[17] Administration of aqueous and ethanolic extracts of *Basella rubra* leaves shows that 200 mg/kg bw of aqueous extract is more effective than the ethanolic extract (200 mg/kg). The value of aqueous extract of 200 mg/kg bw is greater than the 100 mg/kg bw of the aqueous extract. Through the complete literature search it is found that the plant is rich in sterols which may inhibit the absorption of cholesterol from the diet and, therefore, prevent heart and blood vessel disease.[18]

The experimental study on *Basella rubra* leaves for amylase activity shows the aqueous extract has greater values than the ethanolic and hexane extracts (or control group). The study shows the significant increase of ($P < 0.05$) when compared with the control group. Amylase activity is most often used to diagnose or monitor acute pancreatitis. It may also detect some digestive tract problems.[19]

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