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IJPBS |Volume 2| Issue 1 |JAN-MARCH |2012|25-31

Antioxidant, antipyretic and choleretic activities of crude extract and active compound of *Polygonum Bistorta* (Linn.) in albino rats

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PHARMACEUTICAL SCIENCES ***Research Article***

RECEIVED ON 06-12-2011 **ACCEPTED ON 06-01-2012**

ABSTRACT

Objective: *To evaluate the antioxidant, antipyretic and choleretic activities of crude extract of Polygonum Bistorta and active compound (tannic acid) in experimental animal models.*

Crude extract and active compound of Polygonum Bistorta was evaluated for antioxidant activity DPPH free radical scavenging activity, antipyretic yeast induced activity and choleretic activity of bile flow and bile solids. The results indicate that active principle had the better scavenging activity and showed a promising effect, choleretic activity of extract and active compound did not show any adverse effect on liver. Aqueous extract of Polygonum Bistorta (100 mg/kg) and tannic acid (25 mg/kg) showed reduced pyrexia activities. The present study shows that aqueous extract of Polygonum Bistorta (100 mg/kg) and tannic acid (25 mg/kg has a significant antioxidant, antipyretic and choleretic properties. Thus it may be concluded that tannic acid (25 mg/kg) were found more effective values towards control.

KEYWORDS: *Polygonum bistorta, tannic acid, DPPH, antipyretic and choleretic activity.*

INTRODUCTION

Liver is one of the most essential organs in the body as it plays a pivotal role in regulating various physiological processes and has a remarkable ability to repair and restore its structure and function after physical or chemical-induced damage [1-2]. *Polygonum bistorta* Linn. (bistort or common bistort, PB) is a herbaceous flowering plant belonging to family *Polygonaceae* .This tall perennial herb grows in moist, shady areas on higher ground in the North of India (Punjab, Kashmir, Sikkim and Himmalyan Region), England, Southern Scotland, Europe, central Asia and west of the Rockies in North America, It flowers in May and June. The plant may be propagated by division of the root stock. The rhizome is odorless, but powerfully astringent in taste, as it contains tannin to the extent of 21 percent [3] The active principle of *Polygonum bistorta* is tannin. The roots contain up to 21% tannin. These are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins [4]. Its molecular wt ranges from 500 to over 20,000. "Tannic acid is the commercial term for a mixture of large gallotannins, trigallic, m-digallic, and gallic acid, which is extracted from plant material [5]. According to IARC "tannic acid is the astringent or tanning principle occurring in the wood, bark, fruit, leaves, and roots of a large number of plants. Cholestasis is reduced or stopped bile flow. Bile flow may be blocked inside or outside the liver. Liver enlargement is usually an indicator of liver disease, although there are usually no symptoms associated with a slightly enlarged liver (hepatomegaly). Symptoms of a grossly enlarged liver include abdominal discomfort or "feeling full." It occurs when a large portion of the liver is damaged due to any type of liver disorder [6]. The free radical scavenging (antioxidant) activity of this plant probably due to the presence of the flavonoids and tannins. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [7].Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature [8]. Noticed after administration of ethanolic extract of *Peperomia pellucida* and *Laportea crenulata* (80 mg/kg, *i.p.*) significant reduction in the elevated body temperature against boiled milk (0.5 ml/kg, *i.p.*) in albino rabbit, which leads to pyrexia.

MATERIALS AND METHODS

Plant material:

The plant material was collected by the authorized ayurvedic dealer and was identified by the Botany Department of Jiwaji University, Gwalior.

Preparation of plant extract:

Root material of plant was shade, dried, coarsely powdered and allowed to heat in distill water at high temperature of water bath. The inorganic material was precipitated and filtered off. The filtrate was again concentrated in a china dish and dried in vacuum. The yield of the extract was (10.4%) w/w of powdered aqueous extract, which was stored in refrigerator for further use. The aqueous extract was evaporated and dried in vacuum.

Chemicals:

All chemicals used in this study were of analytical grade and obtained from Sigma-Aldrich, E-Merck and Loba Chemicals Pvt. Ltd. All diagnostic kits used in the experiments were procured from E-Merck.

Experimental Animals:

Studies were carried out by using adult albino male rats of *Sprague Dawley* strain (150±10 g) were used for the present investigation. Animals were housed under standard conditions (25±2°C, 60-70% relative humidity and 14 h light and 10 h dark). The rats were fed on standard pellet diet (Pranav Agro Industries, New Delhi) and water *ad libitum*. Animals were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India, Ministry of Environment & Forests (Animals Welfare Division), Chennai.

The rats were randomly allocated to six animals in each group and treated as below.

Calculation

$$\% \text{ Reduction} = \frac{\text{Elevated temp.} - \text{temp. of different intervals}}{\text{Elevated temp.} - \text{normal temp.}} \times 100$$

TOTAL ANTIOXIDANTS ACTIVITY MEASURED BY DPPH:

The free radical scavenging activity of PB extract and TA were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH·) [9]. Briefly, a 0.1 mM solution of DPPH· in ethanol was prepared and 1 ml of this solution was added to 3 ml of PB/TA solution in water at different concentrations (10-50 µg/ml). Vitamin C used as a standard. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity.

Calculation

The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH· Scavenging effect (%) = [(Ao – A_1/A_o) x 100]

Where $\mathbf{A_o}$ is the absorbance of the control reaction and $\mathbf{A_1}$ is the absorbance in the presence of the sample of PB extracts and tannic acid.

ANTIPYRETIC ACTIVITY:

Antipyretic activity in albino rats was studied with Brewer's yeast induced fever 20% [10]. Albino rats were fed uniformly till 24 h before giving drugs and then food was withdrawn. After measuring rectal temperature of the animals by introducing 1.5 cm of digital thermometer in rectum, pyrexia was induced by subcutaneous injection of 20% suspension of dried yeast in 2% gum acacia in normal saline at a dose 10 ml/kg of body weight. After 18 h of yeast injection, rats which showed a rise in temperature of at least 1ºF (0.6 °C) were taken for the study. Animals in the various groups were treated as follows.

CHOLERETIC ACTIVITY

Bile flow

Male rats are anesthetized with (25%) urethane anesthesia (6 ml/kg, *i.p.*) following an overnight fasting period, supplemental doses being administered as needed. The bile duct was surgically exposed following a midline incision (25 mm) and cannulated as close to the duodenum as possible without injuring pancreatic tissue with PE-10 tubing [11]. The rectal temperature of the anesthetized rat is maintained at 37°C with a heat lamp to prevent any hypothermic alteration in bile flow. Bile is collected for successive one hour period in glass tube located so that the opening of the cannula is at or below the level of common bile duct. At the end of the 1st (control) hour dehydrocholic acid (DHC) was used as standard (50 mg/kg) or test drug injected intraduodenally. Bile was collected hourly for 4 h following administration of the compound (test drug or DHC). The animals were sacrificed at the end of the experiment. The liver was removed and weighed. Total solids were estimated by evaporating samples to dryness and weighing the residue.

Bile volume output:

In order to estimate the activity of a choleretic agent it is necessary to measure the rate of bile flow and excretion of solids in untreated animals over an interval of time. It was observed that there was a definite decrease in bile flow from the 1st h through the 5th h. Furthermore it was found that in rats having high first hour (control) bile flows there was also high bile outputs during the 2-5 h period. The ratio for the 4 h volume to the 1 h volume averages. In treated rats, therefore, the criterion for the activity of a choleretic agent is based on the increase in bile volume (ml/rat) during 4 h collection period above the expected volume for the same period. This increase designated as the 'B' value can be calculated by using the following equation:

Calculation

$$B = Bo - (ratio)\ C$$

Where

B =calculated bile volume in ml

Bo =observed bile volume in ml obtained by adding the 2, 3, 4 and 5th h bile volume

C = observed bile volume in ml during the control h.

$$\text{Percent increase in bile volume} = \frac{B}{C} \times 100$$

Total solids output:

The excretion of total solids was measured for 5 consecutive hours. A definite decrease in the excretion of solids from the 1 h through the 5 h was observed. The ratio of the 2-5 h excretion to the 1 h excretion averaged. In treated rats, therefore, the criterion for the activity of a choleretic agent is based on the increase in excretion (mg/rat) during the 4 h collection period above the expected excretion for the same period. This increase designated as the 'S' value can be calculated by using the following equation.

Calculation

$$S = S_o - (ratio)\ C$$

Where,

S = calculated solids excretion in mg.

So = observed excretion in mg; obtained by adding the 2, 3, 4 and 5th hour excretion

C = observed excretion in mg during the control hour.

$$\text{Percent increase in bile solid} = \frac{S}{C} \times 100$$

Statistical analysis:

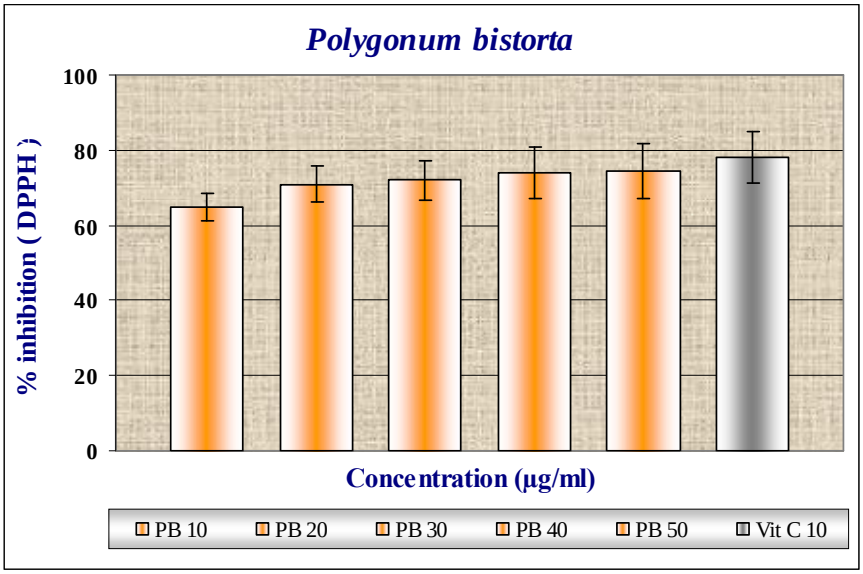
The data were expressed as mean value ± S.E. statistical significance of difference between various treatments were analyzed by Student's't' test followed by one-way analysis of variance (ANOVA)[12]. P values ≤0.05 were considered as statistically significant.

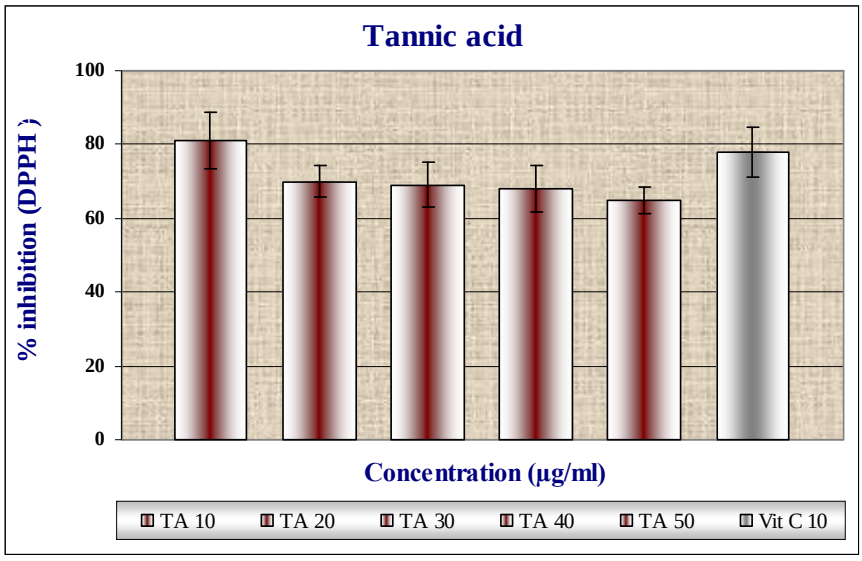
RESULTS AND OBSERVATIONS

Free radical scavenging activity (DPPH)

Graphs illustrate a significant decrease in the concentration of DPPH radical due to scavenging ability of the extracts and active principle. The results indicate that active principle had the better scavenging activity and showed a promising effect, with the dose of 10µg/ml in comparison of extract therapy of 50µg/ml. The subsequent DPPH free radical scavenging activity of tannic acid was guided by its antioxidative effects.

Effect on free radical scavanging activity





Abbreviations: PB= *Polygonum bistorta*; TA= Tannic acid; Vit C= Vitamin C

Choleretic activity (Bile flow and Bile Solids)

Studies on the effects of bile flow and bile solids (choleretic activity) revealed that tannic acid showed marked choleretic activity in anaesthetized normal rats where as *polygonum bistorta* showed only marginal activity at 1st h and 2nd to 5th hour after administration. PB extract and TA did not showed any adverse effect on the choleretic activity of liver. Solid volume in control group was 1:2.74 and 1: 2.19 thus was increased by PB extract and TA (**Table 1**).

TABLE1: CHOLERETIC ACTIVITY OF LIVER IN NORMAL RATS

Treatments	Bile flow (ml)			Bile solid (mg)		
	1 h	2-5 h	Ratio	1 h	2-5 h	Ratio
Control	0.47 ± 0.036	1.30 ± 0.092	1:2.74	0.046 ± 0.003	0.101 ± 0.006	1:2.19
DHC 50 mg/kg	0.21 ± 0.014	0.77 ± 0.041	1:3.66	0.041 ± 0.002	0.127 ± 0.010	1:3.09 (Standard)
PB 100 mg/kg	0.37 ± 0.027	1.12 ± 0.102	1:3.02	0.018 ± 0.001	0.040 ± 0.002	1:2.24
TA 25 mg/kg	0.44 ± 0.036	1.36 ± 0.074	1:3.09	0.026 ± 0.002	0.063 ± 0.004	1:2.42
S 50 mg/kg	0.25 ± 0.019	0.91 ± 0.060	1:3.64	0.048 ± 0.004	0.152 ± 0.023	1:3.16
F value	19.7@	12.6@		21.7@	17.6@	

Data are mean ± S.E., N = 6; @ =Significant at $P \leq 0.05$ for ANOVA;
Abbreviations: DHC= Dihyrocholic acid; PB= *Polygonum bistorta*; TA= Tannic acid; S= Silymarin

Yeast induced antipyretic activity

The experimental rats showed a mean increase of about 0.86°C in rectal temperature 18 h after Brewer's yeast injection. Aqueous extract of *polygonum bistorta* (100 mg/kg) and tannic acid (25 mg/kg) showed reduced pyrexia activities (**Table 2**).

Table 2: Effects of PB extract and TA in yeast induced hyperthermia in rats (Rectal temperature °C at time h)

Treatments	Before yeast	18 h After yeast	After treatment			
			1 h	2 h	3 h	4 h
Control	37.1 ± 2.95	38.4 ± 3.08	38.1 ± 2.83	37.9 ± 3.46	37.8 ± 3.38	37.5 ±3.15
APAP 33 mg/kg	37.2 ± 3.02	38.6 ± 3.24	37.6 ± 2.44	37.5 ± 3.15	37.3 ± 3.00*	37.1 ± 2.95
PB 100 mg/kg	37.2 ± 3.02	38.2 ± 2.91	37.9 ± 3.46	37.7 ± 3.30	37.6 ± 2.44	37.4 ± 3.08
TA 25 mg/kg	37.1 ± 2.95	38.5 ± 3.34	37.8 ± 2.59	37.4 ± 3.08	37.2 ± 3.02	37.1 ± 2.95
F value			0.028ns	0.042ns	0.017ns	0.043ns

Data are mean ± S.E., N = 6; ns = Non significant at $P \leq 0.05$ for ANOVA;
Abbreviations: APAP= Acetaminophen; PB= *Polygonum bistorta*; TA= Tannic acid.

DISCUSSION

One of the important functions of the liver is secretion of bile which, among other things, has a role in digestion. The most important bile acid in mammals is cholic acid, thus in this present investigation DHC was used as a standard drug. It is of interest to note that *Polygonum bistorta* and active principle tannic acid was effective at a concentration of 100 and 25 mg/kg to increase the bile flow. This herb may be of use in certain biliary disorders in view of its stimulatory effect on bile output. Thus this plant root is an attractive candidate for the development of an invaluable drug and/or hepatoprotective herbal formulations. Similar result also stimulated by *Taraxacum officinale* [13].

Fever may be due to infection or one of the sequel of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are agents, which reduce the elevated body temperature. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature [14]. Treatment with *P. bistorta* and tannic acid reduced the yeast induced body temperature significantly and thus showed antihyperthermia activity. So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of APAP [15]. Similar study showed protection with the plant extracts of *Acacia catechu* Wild [16].

Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. The DPPH test provides information on the reactivity of the test compounds with a stable free radical. In the present study treatment with plant extract of *Polygonum bistorta* and its active principle tannic acid showed free radical scavenging activity in dose dependent manner.

The presence of flavonoids and tannins in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids, tannins and plant phenols are a major group of compounds that act as primary antioxidants or free radical scavengers [17]. Flavonoids are commonly found in plants and have been shown to display a remarkable spectrum of biological activities, such as hepatoprotective, anti-inflammatory and antiviral activities. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Other plants *Cassia sophera* and *Alpinia rafflesiana* showed similar results [18].

ACKNOWLEDGEMENTS

The authors feel indebted to Prof. Sangeeta. Shukla and Prof. R. Mathur, School of Studies in Zoology, Jiwaji University, Gwalior for their invaluable suggestions. The financial assistance from Jiwaji University and Central Council of Research in Unani Medicine, New Delhi-India, is acknowledged.

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