Comparative Study on Acute Toxicity, Antioxidant and Cholesterol Lowering Activities of Po-Sa (Leaves And Stem)

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Abstract

Po-sa plant possesses many useful biological activities which have been the reason for the selection of this plant for the present paper. The present paper is concerned with detection of acute toxicity, antioxidant and cholesterol lowering activities of Po-sa (leaves and stem).In acute toxicity tests, watery and 80% EtOH extracts of both leaves and stem were free from acute toxic effects up to the dose of 12g/ kg body weight. Antioxidant activity of Po-sa (leaves and stem) extracts was performed by using spectroscopic DPPH assay method. The IC_{50} values of EtOAc, EtOH and watery extracts of leaves were 2.39µg/mL, 2.11µg/mL and 2.15µg/mL respectively. . The IC_{50} values of EtOAc, EtOH and watery extracts of stem were 2.94µg/mL, 1.21µg/mL and 1.20µg/mL respectively. The EtOH and watery extracts of stem showed more potent radical scavenging activity than those of leaves. Cholesterol lowering activity of crude extracts of leaves and stem were also studied by Zlatkis, Zak and Boyle method on rats model. The cholesterol levels were determined after orally administrated rats with 600 mg/kg/day of plant extracts for 10 days. It indicated that watery extract of stem has the ability to reduce bad cholesterol (TC, TG and VLDL) and it also could effectively increase the good blood cholesterol (HDL) level. Therefore, Po-sa stem has good antioxidant and cholesterol lowering activities. It can be used as a natural source of antioxidants to prevent the progression of many diseases.

Keywords: *Acute toxicity, Antioxidant activity, cholesterol lowering activity*

Introduction

Medicinal plants still play an important role in emerging and developing countries. Medicinal plants are the major components of all indigenous or alternative systems of medicine. *Morus alba* linn, a popular medicinal plant belongs to family Moraceae, has long been used commonly in Ayurvedic and many of traditional systems of medicine (Banskota, 2001). *Morus alba* L. commonly known as Tut in india. *Morus alba* is a moderately sized tree, three to six meter high. *Morus alba* is commonly known as white mulberry. White mulberry is cultivated throughout the world, wherever silkworms are raised. The leaves of white mulberry are the main food source for the silkworms (Kalia,2009). In the present work, *Morus alba* Linn. (Po-sa) was chosen to investigate some biological activities such as antioxidant and hypocholesterolemic activities (Kim *etal.* ,1999).

It belongs to one of ten species in the genus *Morus*, known as the common mulberries, which more or less juicy fruits, native to temperate Asia and North America(Wealth of India,1962). The plant is a very good source of ascorbic acid, which over 90% is present, and also contains carotene, vitamin B_1, folic acid, isoquercetin, tannins, flavonoids saponins, coumarins, volatile oil, alkaloids, amino acid and other organic acid (Doi,2001).Stem possesses ntirheumatic, antispasmodic, diuretic, hypotensive and pectoral activities. They are used in the treatment of rheumatic pains and spasms, especially of the upper half of the body, high blood pressure (Sulochana, 2012). A tincture of the bark is used to relieve toothache. The

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branches are harvested in late spring or early summer and are dried for later use. A fiber is obtained from one-year old stem, it is used in weaving clothes etc. The stem bark is fibrous and is used in China and Europe for paper making (Chen *etal.*, 2005). *These* reports are very encouraging and indicate that herb should be studied more extensively for its therapeutic benefits.

Materials and Methods

Plant Material

Morus alba Linn. was produced from Pyin Oo Lwin Township, Mandalay Division. Samples were ground to get a fine powder. They drug powder was then stored in an air-tight container.

Preparation of Crude extracts

Morus alba Linn (leaves and stem)

The powdered sample 500 g of *Morus alba* L. (Leaves and stem) was extracted with 80% EtOH. When the filtrates were evaporated; leaves extract 30 g and stem extract 20 g were obtained. And then, there were partitioned with PE and EtOAc. There were obtained 2 g of PE and 9 g of EtOAc fractions from leaves extract and 1 g of PE and 7 g of EtOAc fractions from stem extract.

Biological Activities

(a) Acute Toxicity

The acute toxicity test was done by the method of Litchfield and Wilcoxon (1949).A total of 130 adult mice weighing (30-35 g) were used. Mice were treated with 80% EtOH and watery extracts of Po-sa (leaves & stem). The doses were (3, 6, 12g/kg) respectively.

(b) Antioxidant Activity

For the examination of *in vitro* antioxidant activity of Po-sa (leaves & stem) by DPPH staining method and spectrophotometric method were used. In DPPH staining method, the silica plate was based on the procedure of the Soler-Rivas *et al.* (2000). In spectrophotometric method, the sample solutions were measured by using spectrophotometer.

(c)Screening of hypocholesterolemic activity

The examination of *in vivo* hypocholesterolemic activity of crude extracts, were determined by Zlatkis, Zak and Boyle method in the rat model. There were fifteen rats were divided into three groups, each containing three animals. After ten days of duration the blood was collected from the tail of rats to determine the serum TC, serum LDL cholesterol levels.

Results and Discussion

Acute toxicity

The acute toxicity test of *M. alba* Linn. (leaves and stem) extracts on mice were observed that even with the maximal permissible dose (12g/kg b.w) of 80% EtOH and aqueous extracts of the plant, the mice were found to be alive and healthy during the observation period of two weeks. All the animals remained alive and did not show any visible symptoms of toxicity like respiratory disorders, convulsions, and death etc.

Therefore, it was observed that both 80% EtOH and aqueous extracts of *M. alba* Linn were free from acute toxic harmful effects. The medium lethal dose (LD_{50}) of plant extracts were more than 12g/kg body weight. The results of acute toxicity study of plant extracts on albino mice were shown in Table 1.

Table 1. Acute Toxicity Test of EtOH and H_2O Extracts from Po-Sa (Leaves and Stem) on Mice

Group	Diet	Dosage (g/ kg bw)	Ratio of death and tested mice	% of death
1, 2, 3	PSL-EtOH extract	3, 6, 12	0/ 10	0
7, 8, 9	PSS-EtOH extract	3, 6, 12	0/10	0
4, 5, 6	PSL – H_2O extract	3, 6, 12	0/10	0
10, 11, 12	PSS-H_2O extract	3, 6, 12	0/10	0
13	distilled water	10 ml/kg bw	0/10	0

PSL – (Po – Sa) leaves
PSS – (Po – Sa) stem
Number of mice in each group = 10
Duration of test = two weeks

Antioxidant activity

(i) Rapid screening of Antioxidant Activity by DPPH staining method

It was observed that *Morus alba* Linn (leaves and stem) showed antioxidant activity on the TLC plates. After staining, white spots with strong intensity the amount of 12.5µg of dry matter for crude extracts (Figure1). The intensity of white colour depends upon the amount and nature of radical scavenger present in the sample.

EtOH extract of leaves

EtOH extract of stem

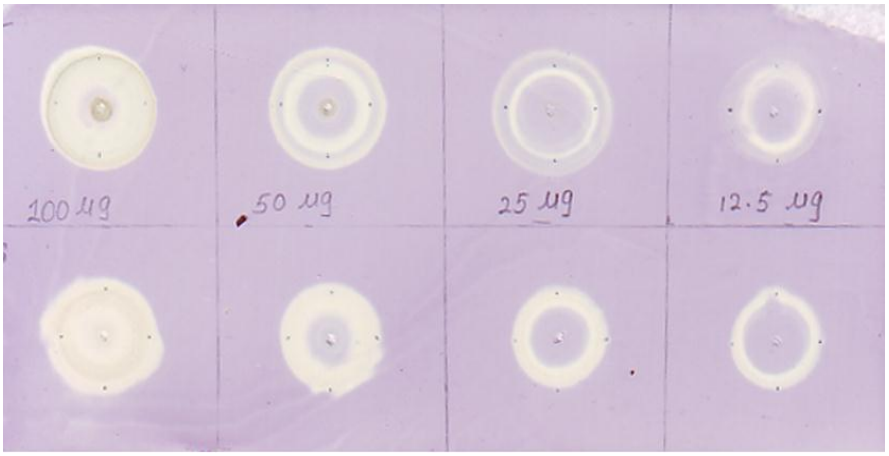

Figure 1. Screening of antioxidant activity of EtOH extracts from Po-sa (leaves and stem) by DPPH Dot-Blot assay

(ii) DPPH radical scavenging activity by spectrophotometric method

EtOAc, 80%EtOH and H_2O extracts were tested by spectrophotometric method by determining the H_2O extract have more potent than the other extracts compared with standard BHT (Table2). From these results, increase in concentration, showed increase in percent inhibition, i.e. increase free radical scavenging activity. The lower IC_{50} value indicates the greater antioxidant activity.

Table 2. Average Oxidative Inhibition % in Various Concentrations and IC_{50} Values of Extracts of Po-sa (Leaves and Stem)

Extracts	**% Inhibition in various concentration (µg/mL)**					**IC_{50} (µg/mL)**
	0.625	**1.25**	**2.5**	**5**	**10**	
PS-EtOH (stem)	40.61	50.64	60.85	69.27	84.00	1.21
PS-H_2O (stem)	28.01	51.97	62.48	70.06	78.61	1.20
PS-EtOAc (stem)	28.18	37.52	48.06	59.00	77.33	2.94
PS-EtOAc (leaves)	21.70	40.42	50.97	57.15	69.03	2.39
PS-EtOH (leaves)	18.97	32.24	58.16	63.61	68.91	2.11
PS- H_2O (leaves)	37.94	44.79	52.06	64.85	76.12	2.15
BHT	14.04	54.82	74.22	77.13	87.40	1.17

BHT = Butylated Hydroxy Toluene

Hypocholesterolemic activity

In cholesterol lowering activity, 80 % EtOH and watery extract of Po-sa (leaves & stem) could reduce 5.64%, 8.84 %, 4.86% and 9.27 % of TC after ten days duration when treated with 600 mg/kg/body weight/day dose (Figure 2). All of the 80 % EtOH and watery extracts of Po-sa leaves and stem did not only decrease the serum TC, TG, VLDL-C and LDL-C levels, but also they could raise the good cholesterol, HDL levels were shown in (Table 3). Watery extract of Po-sa stem was the most effective in lowering TG as well as VLDL-C and the highest ability to increase HDL cholesterol. The 80 % EtOH extract of Po-sa stem was the highest potency to reduce LDL cholesterol.

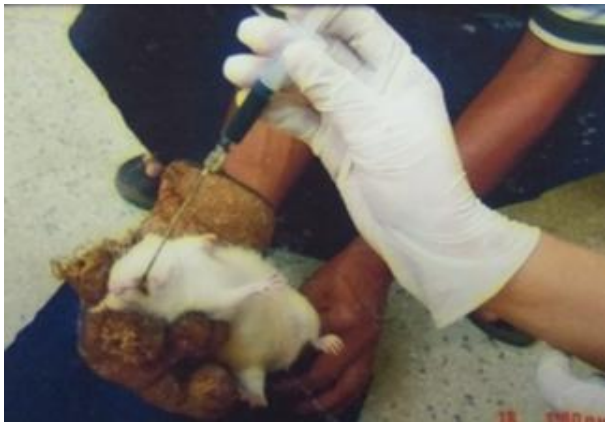

Figure 2. Photograph of administration of samples to male winster strain rat by oral route

Figure 3. Photograph of male winster strain rat for cholesterol lowering test

Table 3. Effect of Po-sa (Leaves and Stem) Crude Extracts on Rat Models (600mg/kg body weight per day dose)

Samples	TC (Decreasing %)	TG (Decreasing %)	VLDL-C (Decreasing %)	LDL-C (Decreasing %)	HDL-C (Increasing %)
80% EtOH Extract Psl	5.64	2.94	6.25	7.19	2.63
H_2O extract PSL	4.86	6.45	7.14	11.19	2.78
80% EtOH extract PSS	8.84	5.1	5.56	19.62	2.56
H_2O extract PSS	9.27	15.63	14.29	12.93	9.09
(distilled water)	-	-	-	-	-

Conclusion

Health treatments using medicinal plants are the less expensive and more accessible to the population, mainly in developing countries. Thus, studied that Po-sa (leaves & stem) possess many pharmacological activities such as acute toxicity, antioxidant and hypocholesterolemic activity. From the experimental result, EtOH and H_2O extracts of Po-sa (leaves and stem)showed low toxicity to mice since no animal death was detected at a dose of (3,6,12g/kg).The antioxidant activity of EtOH extract of Po-sa (leaves & stem) was indicated as (12.5µg (or) 0.0625 mg/ml)by using Dot-blot and DPPH staining method. Among these extracts, EtOH extract of stem was observed significant antioxidant activity than leaves extract. According to spectrophotometric method, EtOH, watery and EtOAc extracts showed antioxidant activity with IC_{50} values (1.20-2.94 µg/ml). The radical scavenging activity of EtOH and watery extracts of stem is more potent than that of leaves. In hypocholesterolemic activity, the watery extract of Po-sa (stem) has the ability to reduce bad cholesterol (TC, TG and VLDL) and it also could effectively increase the good blood cholesterol (HDL) level. Therefore, EtOH extract of stem may be utilized for diseases caused by oxidation. Po-sa plant may be used as medicinal plant due to its antioxidant and the hypocholesterolmic activities.

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