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ISOLATION OF A COMPOUND (AP-I) FROM THE LEAVES OF *ABRUS PRECATORIUS* LINNAEUS RESPONSIBLE FOR BODY WEIGHT LOSS IN ALBINO RATS AND EFFECT OF SEASON ON YIELD OF THE COMPOUND

Tanaya Ghosh¹, Prasenjit Mitra², Dilip Kumar Jha³ and Prasanta Kumar Mitra^{1*}

¹Department of Medical Biotechnology, ²Department of Biochemistry, ³Department of Physiology, Sikkim Manipal University, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India.

ABSTRACT

A compound (AP-I) was isolated from the leaves of *Abrus precatorius* Linnaeus by solvent extraction, acid hydrolysis, chromatography followed by crystallization. The compound could exert body weight loss in rats. Reduction of body weight of the rats by this compound started from 10th day but significant reduction was observed from 20th day onwards. Effect of seasons on the amount of isolated compound (AP-I) from *A. precatorius* L. leaves was also studied. Results showed that leaves of *A. precatorius* L. for the months of July and August yielded maximum amount of the compound.

KEY WORDS: *Abrus precatorius* Linnaeus, Acid hydrolysis, Chromatography, AP-I.

INTRODUCTION

Since Vaidic period *Abrus precatorius* Linnaeus has been used for therapeutic purpose [1]. The plant is popularly known as Gunja, Rosary pea, Jequirity bean etc. It belongs to the family leguminosae (Fabaceae). The plant is found throughout India in hedges and bushes in exposed areas. Roots, seeds and leaves of *A. precatorius* L. are used in traditional Medicine. The plant is mainly used in the treatment of ulcer and skin infection [2].

Seeds of the plant are very much attractive but are deadly poisonous. They are used in ornaments. Pharmacological studies revealed that seeds have anti diabetic property and can induce abortion [3,4]. Seeds also have anti oxidative property as well as anti-inflammatory analgesic activity [5, 6]. Much work has been done on antimicrobial activity of the aqueous extract of *A. precatorius* L. It was found out that the plant could exert antimicrobial effect against *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *Escherichia coli*, and *Streptococcus pneumonia* [7- 9]. The plant is also found efficacious in cancer [10] and in malaria [11]. A wide range of active components including

glycoside abralin, an albuminous substance 'abrin', abrasine, abrusgenic acid-methylester, abruslectone, abrusic acid, anthocyanins etc. have been isolated from the plant [12,13].

Recently we have seen that leaves of *A. precatorius* L. could exert body weight loss in albino rats. We have also noted that leaves of *A. precatorius* L. for the months of July and August had maximum activity. Results are under communication. Tempted on this observation we have undertaken study to isolate the active constituent present in *A. precatorius* L. leaves responsible for body weight loss in rats. We also studied effect of different seasons on the amount of isolated compound to justify the maximum body weight loss during the months of July and August. Results of the experiments are being reported

MATERIALS AND METHODS

Plant material

Leaves of *Abrus precatorius* Linnaeus were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, west Bengal, India randomly and during the

periods of January – February, March – April, May – June, July – August, September – October and November – December of the year 2012. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references.

Isolation of the active constituent

Isolation of the active constituent from the leaves of *A. precatorius* L. collected randomly and during the months of January – February, March – April, May – June, July – August, September– October and November – December were separately processed by the following steps.

First step:

Leaves of *A. precatorius* L. were properly washed, shade dried and powdered. 100g of this powder were extracted with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture for ½ h on a rotary shaker . It was then centrifuged. Supernatant was collected and evaporated to dryness. Dry brown mass was obtained.

Second step:

Dry brown mass was refluxed with 100 ml of 1(N) HCL for 30 minutes on a water bath at 100 degree centigrade. It was cooled and centrifuged. Supernatant was evaporated to dryness .

Third step:

Dry brown mass thus obtained from the supernatant was extracted with 50 ml of a mixture of chloroform – ethanol mixture (1 : 1 v/v) on a rotary shaker for 15 minutes. centrifuged. The solution was centrifuged and the supernatant was evaporated to dryness . Dry brown mass was obtained.

Fourth step:

Brown mass was dissolved in 10 ml ethanol and subjected to column chromatography using silica gel G as adsorbent. Six bands were separated. Bands were collected in separate beakers. Elution was done by 50% ethanol – chloroform mixture. Second band could exert body weight loss in albino rats.

Fifth step:

Eluent of second band was evaporated to dryness. The dry mass was extracted with 10 ml ethyl acetate for 5 minutes. It was then filtered. With filtrate polyamide column chromatography was done. Elution was made by ethyl formate : formic acid mixture (20 : 1 v/v). Four bands were separated. Third band could exert body weight loss in albino rats.

Sixth step:

Eluent of third band was evaporated to dryness. Repeated crystallization was done from ethyl acetate–cyclohexane (2:1, v/v) mixture. Crystals obtained. The compound was given a trivial name (AP-I). In each case yield of the compound was noted.

Homogeneity of the isolated compound

This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems: Acetone : ethanol - 2 : 1; n-butanol : acetic acid : acetone - 2 : 1 : 1; Chloroform: ethanol : water - 1 : 1 : 1.

Acute oral toxicity study

Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh [14]. Compound (AP-1) isolated from the leaves of *A. precatorius* L.. collected randomly was given at doses of 1, 2, 5, 10 and 30 mg/kg to different groups of mice each group containing six animals. Watery suspension of the test drug was given to the animals orally through a feeding tube.

After administering the test drug, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

Animals

Male Wister strain rats, body weight between 35 and 40g, were used for this study. Animals were housed individually in polypropylene cages, maintained under standard conditions like 12h light and 12h dark cycle, 20 - 30 degree centigrade, 35 - 60 % humidity. Rats were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and provided water *ad libitum*. The animal experiment was approved by the ethics committee of the Institute.

Experimental design

In first set of experiment, rats were divided into two groups of six each. First group of animals took normal diet while animals of the second group, in addition to normal diet, took compound (AP-I) isolated from randomly collected leaves of *A. precatorius* L. in the dose of 0.1g/kg body weight daily.

Isolated compound (AP-1) in the form of suspension in water was given to the rats orally through a feeding tube. Dose selection of the test drug was as per of our earlier studies [15-17]. Experiment was continued for 40 days.

Growth of rats

Growth of rats was measured on 10th, 20th, 30th and 40th day. Overall behavior of the animals was noted.

Statistical analysis

The values were expressed as Mean \pm SEM and was analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity studies

Acute toxicity studies revealed that the isolated compound (AP-I) from the leaves of *A. precatorius* L. did not produce any toxic symptoms when administered orally to mice in doses of 1, 2, 5, 10 and 30 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

Homogeneity of the isolated compound

This was ascertained by silica gel- G thin layer chromatography by using three solvent systems as mentioned earlier. In each case single spot was obtained. The isolated compound (AP-I) was thus pure.

Table – 1 shows effect of isolated compound (AP-I) from the leaves of *A. precatorius* L. (randomly collected) on body weight of rats. It appears from the table that (AP-I) could decrease body weight of rats. For first ten days the decrease was not statistically significant but after that up to 40 days there was significant decrease ($p < 0.001$) in body weight in those rats who took AP-I in addition with normal

diet. The animals also developed anorexia. Effect of isolated compound on body weight of rats was also shown in figure – 2. Table – 2 showed seasonal variations in the yield of the isolated compound (AP-I) from the leaves of *A. precatorius* L. Maximum yield of the compound in the leaves of *A. precatorius* L. was found during the months of July and August and it was 6.9 mg/100g of *A. precatorius* L. leave powder. The result was statistically significant at the level of $p < 0.001$ when compared to the yield of other season. Seasonal variations in the yield of the isolated compound was also shown in figure – 3.

Fluck and Pharm [18] showed influence of climate on the active principles in medicinal plants. Thereafter, series of experiments were conducted in this direction. Now a days numerous reports are available in literature which suggest that accumulation of chemical compounds in roots, stem and leaves of plants varies with season [19-23]. In the present study we also noted that accumulation of compound (AP-I) in leaves of *A. precatorius* L. varies with season and was maximum during the period July to August.

We noted earlier that maximum growth inhibition of the rats by the leaves of *A. precatorius* L. occurred during the period July to August. Results are now under communication. It is, therefore, clear from the present result that this is due to maximal accumulation of the active compound (AP-I) in the leaves during that period.

We are now interested to characterize the compound (AP-I) isolated from *A. precatorius* L. and to see the underlying mechanism by which it can exert body weight loss in rats. Experiments are going on in this direction.

Figure 1. *Abrus precatorius* Linn



Figure 2. Effect of (AP-I) isolated from leaves of *A. precatorius* L. (randomly collected) on growth of rats (Changes of body weight in gram)

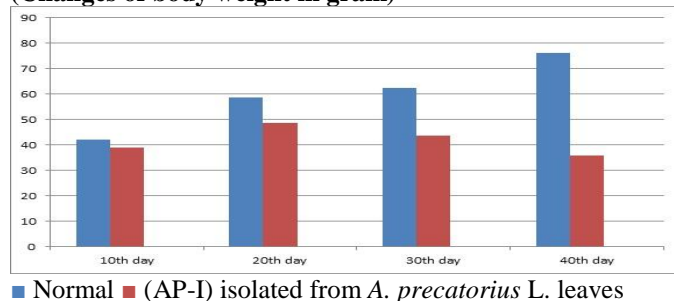


Figure 3. Seasonal variation in the amount of (AP-I) isolated from *A. precatorius* L. leaves. The amount was in terms of mg/100g of *A. precatorius* L. leave powder.

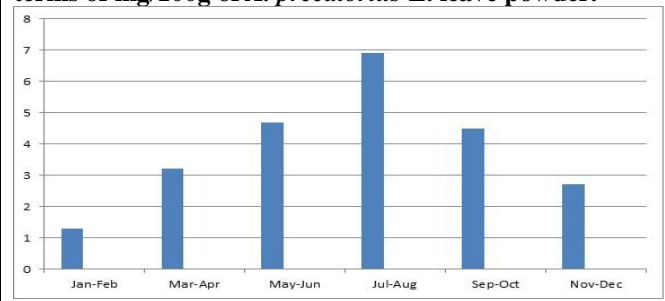


Table 1. Effect of isolated compound (AP-I) from randomly collected leaves of *A. precatorius* L. on growth of rats (Changes of body weight in gram)

Group	Treatment	10 th day	20 th day	30 th day	40 th day
1	Normal	42.1 ± 1.3	58.6 ± 2.3	62.5 ± 2.8	76.2 ± 2.1
2	Isolated compound (AP-I) from <i>A. precatorius</i> L. leaves	38.9 ± 0.8	48.7 ± 1.8*	43.8 ± 1.2**	35.7 ± 1.3**

*p<0.001, *A. precatorius* L. : 1 g / kg, . * p < 0.05, ** p < 0.001.

Table 2. Seasonal variations in the yield of the isolated compound (AP-I) from the leaves of *A. precatorius* L.

Season	Yield of the compound (AP-I) (mg/100g of <i>A. precatorius</i> L leave powder)
January – February	1.3 ± 0.03
March – April	3.2 ± 0.04
May – June	4.7 ± 0.06
July – August	6.9 ± 0.09**
September – October	4.5 ± 0.05
November - December	2.7 ± 0.04

Results are mean of six sets of experiments. ** p<0.001.

CONCLUSION

An active compound (AP-I) was isolated from the leaves of *A. precatorius* L. The compound could exert body weight loss in rats. Seasonal variation in accumulation of

the compound in *A. precatorius* L. leaves was studied. It revealed that leaves contained maximum amount of the compound (AP-I) during the months July and August.

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