



**ISOLATION OF A COMPOUND (AP-II) FROM THE LEAVES OF ABRUS
PRECATORIUS LINNAEUS AND ITS EFFECT ON BODY WEIGHT LOSS IN
ALBINO RATS**

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ABSTRACT

By solvent extraction, acid hydrolysis, chromatographic experiments followed by crystallization a compound (AP-II) was isolated from the leaves of *Abrus precatorius* Linnaeus. The compound could exert body weight loss in albino rats. Body weight loss started from 10th day but significant loss was observed from 20th day onwards.

KEYWORDS: *Abrus precatorius* Linnaeus, solvent extraction, acid hydrolysis, chromatography, AP-II.

INTRODUCTION

Abrus precatorius Linnaeus (family, Fabaceae), known as Gunja, Rosary pea, Jequirity bean etc., found throughout India in hedges and bushes in exposed areas, has been used for therapeutic purpose since ancient times.^[1]

A. precatorius L. has several good effects. It is good for treatment of ulcer and skin infection.^[2] It has anti-diabetic property^[3], anti-inflammatory analgesic activity^[4,5], as well as antimicrobial activity.^[6-8] The plant is also found efficacious in cancer^[9] and in malaria.^[10]

A. precatorius L. has several bad effects too. The plant has Teratogenic activity^[11] Spermicidal effect^[12], Luteal suppressant effect^[13], Anti-gonadotropin effect^[14], CNS depressant activity^[15] etc. Seeds of the plant is toxic and induce abortion^[16]. We have also noted that *A. precatorius* L leaves could cause body weight loss in normal healthy growing albino rats.^[17,18]

In this communication we report results of a study on isolation of a compound from *A. precatorius* L. leaves and its effect on body weight loss in rats.

MATERIALS AND METHODS **Plant material**

Leaves of *A. precatorius* L. were collected during July 2015 in morning hours (9 – 10 AM) from the medicinal plants garden of the North Bengal University, Dist.

Darjeeling, West Bengal, India. 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 the Sikkim Manipal University, Gangtok, Sikkim, India for future references.



Fig. 1: Abrus precatorius Linnaeus leaves

Isolation of the active constituent

Isolation of the active constituent from the leaves of *A. precatorius* L. was done by the following steps. Principles of standard isolation procedures were followed.^[19-23]

First step

Leaves of *A. precatorius* L. were properly washed, shade dried and powdered. 50g of this powder were extracted with 500ml of 1: 1 (v/v) acetone –chloroform mixture for 15 minutes 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 100ml of 1(N) HCL for 15 minutes on a water bath at 100°C. It was cooled and centrifuged. Supernatant was evaporated to dryness.

Third step

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

Fourth step

Brown mass was dissolved in 10ml acetone and subjected to column chromatography using silica gel G as adsorbent. Seven bands were separated. Bands were collected in separate beakers. Elution was done by 50% acetone–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 10ml acetone for 5minutes. It was then filtered. With filtrate polyamide column chromatography was done. Elution was made by acetone: formic acid mixture (50: 1v/v). Six 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 (1:1, v/v) mixture. Crystals obtained. The compound was given a trivial name (AP-II).

Homogeneity of the isolated compound

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

Acute oral toxicity study

Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh.[24] Isolated compound (AP-II) from the leaves of *A. precatorius* L. 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 observed for any abnormality in behavior as well as 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

Rats were divided into two groups of eight each. Normal diet was given to first group of animals while animals of the second group, in addition to normal diet, took the compound (AP-II) isolated from *A. precatorius* L. leaves in the dose of 0.1g/kg body weight daily. The compound, as suspension in water, was given to rats orally through a feeding tube. Dose selection of the test drug was on the basis of our earlier studies.^[17,18] Experiment was continued for 40 days.

Growth of rats

Growth of rats was measured on 10th, 20th, 30th and 40th day. Animals was observed for overall behavior.

Statistical analysis

The values were expressed as mean \pm SEM and were 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**

Results showed that the isolated compound (AP-II) 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 30mg/kg. Animals were healthy, behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

Homogeneity of the isolated compound

As mentioned earlier this was ascertained by silica gel- G thin layer chromatography using three solvent systems In each case single spot was obtained. The isolated compound (AP-II) was, therefore, pure.

Effect of isolated compound on growth of rats

This was shown in Table – 1.

It appears from the table that the isolated compound (AP-II) could induce body weight loss in rats. For first ten days the loss was not statistically significant as compared to control group of animals (Body weight of treated group - 39.3 ± 0.9 g, Control group - 40.2 ± 1.1 g).

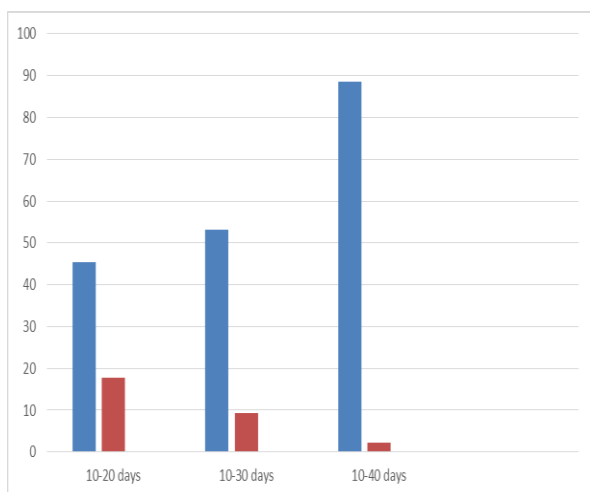
Thereafter loss in body weight in rats induced by the isolated compound AP-II was found statistically significant ($p < 0.001$) when compared to that of controlled rats. On 20th, 30th and 40th days body weight of control rats were 58.5 ± 1.2 g, 61.6 ± 2.1 g and 75.8 ± 2.0 g respectively while those for rats took AP-II were 46.3 ± 1.1 g, 43.0 ± 1.1 g and 40.2 ± 1.2 g respectively.

From the table it also revealed that percent increase in body weight of control rats from 10th to 20th day was

Table 1: Effect of compound (AP-II) isolated from the leaves of *A. precatorius* L. on body weight of rats (body weight in gram)

Group	Treatment	10 th day	20 th day	30 th day	40 th day
1	Control	40.2 ± 1.1	58.5 ± 1.2	61.6 ± 2.1	75.8 ± 2.0
2	Isolated compound (AP-II) from <i>A. precatorius</i> L. leaves	39.3 ± 0.9	$46.3 \pm 1.1^*$	$43.0 \pm 1.1^*$	$40.2 \pm 1.2^*$

AP-II: 0.1 g/kg, * $p < 0.001$.



■ Control rats ■ AP-II treated rats
Figure 2. Body weight increase (in %) in control and AP-II treated rats.

Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. These plants are from different families but all have medicinal values and are popular for their pharmacological properties like anti diabetic activity, anti cancer potential, anti microbial property, anti oxidant activity, anti ulcer property, liver stimulant, wound healing activity etc.^[25] Medicinal plants, therefore, are widely used in the preparation of traditional medicine. In many developing countries even today a large proportion of population, more than 80% according to World Health Organization (WHO), depends on traditional medicine to meet their primary health care needs (UNESCO,1996)^[26] In China, Sri Lanka, Thailand, Africa, India and Pakistan, about 30 - 40% of the total medicinal consumption is attributed to traditional medicines.^[27]

It has been estimated that more than 3500 million people in the world utilize medicinal plants on a regular basis in the form of traditional medicine.^[28] These people believe that the medicine is cheap, locally available and safe

45.5% but the same for AP-II treated rats was only 17.8%. From 10th day to 30th day as well as from 10th day to 40th day the same values for control animals were 53.2% and 88.5% respectively and for AP-II treated rats the values were 9.41% and 2.29% respectively.

At a glance, percent of the total body weight increase of control rats from 10th to 40th day was 88.5% but the same for AP-II treated rats was only 2.29% (Figure – 2).

because they are 'natural'. However, recent evidence suggests that some of the plants considered to be safe over last many decades have proven to be associated with health hazards.^[29]

A. precatorius L. is one such medicinal plant. It has many medicinal values but at the same time it is toxic. Researchers found that leave and seed of the plant are toxic for animals like dog, chicken, mice, guinea-pigs, cow, rabbit, horse etc. It is also toxic for human.^[30]

Toxicological study of *A. precatorius* L. in rats has been undertaken in detail. In 1971 Genest and co-workers observed^[31] that water extract of seed of *A. precatorius* L. when administered orally to rat produced toxicity (ED₅₀, 2.711 gm/kg). In 2007 Adedapo and co-workers studied toxic effects of aqueous extract of *A. precatorius* L. in rats. Results showed that the extract caused decreased levels of red blood cell count, white blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume and mean corpuscular haemoglobin but increased levels of biochemical parameters like total serum protein, albumin, aspartate amino transferase, alkaline phosphatase and total bilirubin, alanine amino transaminase etc. Histologically, there was testicular degeneration and reduction in sperm cells.^[32] In 2015 Ogbuehi and co-workers^[33] undertook oral acute toxicity (LD₅₀) study of different solvent (aqueous, 70% methanol, acetone, petroleum ether) extracts of *A. precatorius* L. leaves in Wistar rats. Results showed that acetone extract had the lowest LD₅₀ value (187mg/kg) indicating higher toxicity and 70% methanol extract was least toxic (LD₅₀, 3942mg/kg). There were significant changes in body weight and organ weight of the animals took *A. precatorius* L. leave extracts. Histopathological studies were carried out. Results showed possible pathological changes in liver, kidneys and heart at the oral limit dose.

In present study we found that *A. precatorius* L. leaves could cause body weight loss in normal healthy growing albino rats.

CONCLUSION

Due to medicinal values leaves of *A. precatorius* L. are being used for therapeutic purpose through out the world. But studies showed that the plant has toxic effect to animals and humans. Even, the present study has shown that *A. precatorius* L. leaves could cause body weight loss in healthy growing albino rats. Medicinal use of the plant specially leaves of the plant, therefore, should be carefully controlled.

CONFLICT OF INTEREST

None.

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