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EFFECT OF TIME ON SOLVENT EXTRACTION TO OBTAIN ACTIVE MASS FROM *ABRUS PRECATORIUS* LINNAEUS LEAVES RESPONSIBLE FOR BODY WEIGHT REDUCTION IN ALBINO RATS

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ABSTRACT

Effect of time on solvent extraction to obtain active mass from *Abrus precatorius* Linnaeus leaves responsible for body weight reduction in albino rats was studied. Extraction was done with 10 : 1 (v/v) acetone – chloroform mixture. For extraction 10 min, 20 min, 30min, 40min and 50 min time were allowed in different set of experiments. Results showed that 30 min extraction time was sufficient to yield maximum mass responsible for body weight reduction in albino rats.

Key words: *Abrus precatorius* Linnaeus, Effect of time, Extraction process, Body weight reduction activity.

INTRODUCTION

Extraction process is a part of isolation work. In fact, extraction is the first step in isolating active compound(s) from plants. Several general procedures are adopted for extraction (Wall ME *et al*., 1996; Cordell GA, 1981; Hostettmann K *et al*., 1991). Different solvents are used in extraction as they yield different extracts and extract compositions (Zarnowski R and Suzuki Y, 2004). Therefore, a suitable extracting solvent should be selected for extraction of the active compound for its maximum activity (Wang L *et al*., 2006).

Recently, we extracted an active mass from the leaves of *Abrus precatorius* Linnaeus responsible for body weight reduction in albino rats. Extraction was done by 10 : 1 (v/v) acetone – chloroform mixture. As extraction time is important to extract active compound in maximum amount (Cannell RJP, 1998; Huie CW) we studied effect of time on solvent extraction. In this communication experiments and results related with effect of time on solvent extraction to obtain active mass from *A. precatorius* L leaves responsible for body weight reduction in albino rats are being reported.

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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 during the periods of July – August as we have noted that leaves of *A. precatorius L.* had maximum body weight loosing property during this period. 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. Leaves were sundried and powdered. The powder was used as the test drug.

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. The animal Institute. Rats were fed with standard rat pellet diet (Hindustan Lever Ltd.,Mumbai, India) and provided water *ad libitum*.

Acute oral toxicity study

Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh (Ghosh MN, 2005). Powdered 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 also observed for general organ toxicity, morphological behavior and and mortality.

Chemicals

All chemicals used in this study were purchased from Sigma Chemical Company, Mumbai. Chemicals were of analytical grade with high purity.

Experimental design

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. In five sets of experiments different time was allotted for extraction.

a) 10 minutes
b) 20 minutes
c) 30 minutes
d) 40 minutes
e) 50 minutes

Extraction in each case was done on a rotary shaker . It was then centrifuged. Supernatant was collected and evaporated to dryness. Dry brown mass was obtained.

Rats were divided into two groups of 20 each. First group of animals took normal diet while animals of the second group, in addition to normal diet, took isolated dry brown mass obtained after solvent extraction in the dose of 0.5g/kg body weight in watery suspension daily through oral route. Experiment was continued for 40 days. Separate rats were used for different solvent extraction groups.

Growth of rats

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

Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analyses 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 $p < 0.05$. experiment was approved by the ethics committee of the

RESULTS

Acute toxicity studies

Acute toxicity studies revealed that 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 animalwas recorded during seven days of experiment.

Table – 1 shows effect of isolated brown mass, obtained after 10 minutes extraction of *A. precatorius* L. leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture, on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could not decrease body weight of rats even on 40^{th} day of experiment.

Table – 2 shows effect of isolated brown mass, after 20 minutes extraction of *A. precatorius* L. leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture, on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could decrease body weight of rats from 20^{th} day up to 40 days of experiment but the results were not statistically significant when compared with the control group.

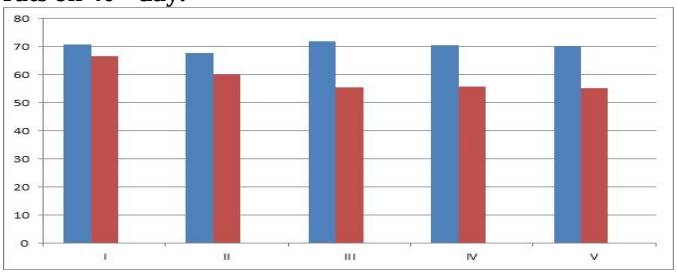
Table – 3 shows effect of isolated brown mass after 30 minutes extraction of *A. precatorius* L. leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could decrease body weight of rats from 20^{th} day up 40 days of experiment and the results were statistically significant up to the level $p< 0.001$ when compared with the control group.

Table – 4 shows effect of isolated brown mass after 40 minutes extraction of *A. precatorius* L. leaves powder(100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could decrease body weight of rats and the decrease was statistically significant from 20 days onwards up to completion of experiment.

Table – 5 shows effect of isolated brown mass after 50 minutes extraction of *Abrus precatorius* L. leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could decrease body weight of rats and the decrease was statistically significant from 20 days onwards up to 40 days (completion of experiment).

Fig 1. *Abrus precatorius* Linnaeus.

Fig 2. Effect of time on extracted mass from leaves of *A. precatorius* L. and its activity on body weight reduction in rats on 40th day.



■ Control ■ *A. precatorius* L. leaves (extracted mass) treated 1. 10 minutes II. 20 minutes III. 30 minutes IV. 40 minutes V. 50 minutes[Extraction was with 1000ml 10:1 (v/v) acetone – chloroform mixture for 100 gram *A. precatorius* L. leaves powder)

Table 1. Showing effect of isolated brown mass obtained after 10 minutes extraction of *Abrus precatorius* Linnaeus leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. (Changes of body weight - in gram)

Group	Treatment	10th day	20th day	30th day	40th day
1	Normal	40.1 ± 2.2	58.7 ± 1.9	62.7 ± 2.1	70.8 ± 2.5
2	Isolated brown mass from *A. precatorius* L. leaves	38.2 ± 1.5	55.2 ± 1.7	60.5 ± 1.4	66.5 ± 1.5

Table 2. Showing effect of isolated brown mass after 20 minutes of extraction of *Abrus precatorius* Linnaeus leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. (Changes of body weight- in gram)

Group	Treatment	10th day	20th day	30th day	40th day
1	Normal	38.8 ± 1.9	59.9 ± 2.6	61.5 ± 2.6	67.8 ± 2.3
2	Isolated brown mass from *A. precatorius* L. leaves	35.9 ± 1.8	52.8 ± 1.9	55.9 ± 1.8	60.2 ± 2.2

Table 3. Showing effect of isolated brown mass after 30 minutes extraction of *Abrus precatorius* Linnaeus leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. (Changes of body weight - in gram)

Group	Treatment	10th day	20th day	30th day	40th day
1	Normal	41.3 ± 1.7	58.4 ± 1.7	61.9 ± 1.9	71.9 ± 2.1
2	Isolated brown mass from *A. precatorius* L. leaves	39.2 ± 1.6	55.9±1.6*	52.9 ± 1.8*	55.5 ± 1.7**

*p<0.01, ** p< 0.001

Table 4. Showing effect of isolated brown mass after 40 minutes extraction of *Abrus precatorius* Linnaeus leaves powder with 1000 ml of 10 : 1 (v/v) petroleum ether – chloroform mixture on body weight of rats. (Changes of body weight - in gram)

Group	Treatment	10th day	20th day	30th day	40th day
1	Normal	41.9 ± 1.9	58.8 ± 1.5	62.2 ± 1.8	70.5 ± 2.3
2	Isolated brown mass from *A. precatorius* L. leaves	39.8 ± 1.7	50.2± 1.6*	52.7± 1.4*	55.7 ± 1.6**

*p<0.01, ** p< 0.001

Table 5. Showing effect of isolated brown mass after 50 minutes extraction of *Abrus precatorius* Linnaeus leaves powder (100 gram) with 1000 ml of 10 : 1 (v/v) acetone -- chloroform mixture on body weight of rats. (Changes of body weight - in gram)

Group	Treatment	10th day	20th day	30th day	40th day
1	Normal	42.1 ± 1.4	59.5 ± 1.6	62.6 ± 1.6	70.2 ± 2.5
2	Isolated brown mass from *A. precatorius* L. leaves	39.1 ± 1.3	50.8± 1.9*	52.6± 1.5*	55.1 ± 1.4**

*p<0.01, ** p< 0.001

DISCUSSION

Clerodendrum phlomidis L., Blumea mollis (D Don) Merr, Gliricidia sepium, Ocimum canum, Vernonia cinerea (L.), neem, Piper nigrum, Romanomermis yunanensis, Zingiber officinalis Linn, Cinnamonum osmophloem and many other plants are known to act as

insect growth regulators (Park IK *et al*., 2002; Pushpanathan T *et al*., 2002; Cheng SS *et al.*, 2004; Senthilkumar A *et al*., 2009) .

Plant *Ageratum conyzoides* Linn. has growth inhibitory property for seeds and plants. It was found out that the presence of *Ageratum conyzoides* Linn. can be used as seed inhibitor, decreasing development of several herbaceous plants. An aqueous extract of the aerial part or roots of this plant can inhibit germination of wheat and rice seeds (Monago CC, Alumanah EO, 2005; Tailor Chandra Shekhar and Goyal Anj, 2012).

Recently we found that plant *Abrus precatorius* L., a medicinal plant having many pharmacological properties (Noumi Emmanuel and Djeumen Claudette, 2005; Arora Rashmi *et al*., 2011; Saganuwan SA *et al*., 2005; Saganuwan SA *et al*., 2005) could exert body weight loss in albino rats. Solvent extraction in connection to isolation of active compound from the plant was conducted. Results showed that mass obtained after extraction of leaves of A. precatorius L. with 10 : 1 (v/v) acetone – chloroform mixture had maximum body weight reduction activity in albino rats. Results are under communication. As extraction time is important to extract active compound in maximum amount (Cannell RJP, 1998). We studied effect of time on solvent extraction. Results showed that 30 minutes extraction time was sufficient to yield the maximum mass responsible for body weight reduction activity in albino rats.

CONCLUSION

Effect of extraction time on bioactive mass from leaves of *A. precatorius* L. and its activity on body weight reduction in rats were studied. Results showed that mass obtained after 30 minutes extraction with 10 : 1 (v/v) acetone – chloroform mixture could exert maximum body weight loss in rats.

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