

Growth inhibition of albino rats by *Ageratum conyzoides* Linn. leaves and the possible mechanism involved therein

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Abstract

Ageratum conyzoides Linn. is known as plant growth inhibitor. To know whether the plant can inhibit growth of animal also, male albino rats were divided into two groups. First group of animals took normal diet while the second group took a mixture of normal diet and powdered leaves of *Ageratum conyzoides* Linn. In both the two groups growth of rats was checked up to 40 days. On 20th and 40th days of experiment blood pyruvate level was measured. Intestines of rats were also assayed for pyruvate dehydrogenase and transketolase activity. It was found out that growth of rats which took powdered leaves of *Ageratum conyzoides* Linn. with normal diet was stunted. Rats developed symptom of anorexia and became weak. Blood pyruvate level was elevated and intestinal pyruvate dehydrogenase activity was lowered. Pyruvate thus could not be converted to acetyl coenzyme A needed for generation of energy. Lack of energy caused growth inhibition in rats. Further, intestinal transketolase activity was found decreased. Due to this in pentose phosphate pathway there was insufficient production of NADPH and pentose sugars needed for synthesis of nucleic acid. This also caused growth inhibition in rats.

INTRODUCTION

Ageratum conyzoides Linn. (family, asteraceae) is a plant that grows commonly in the proximity of habitation, thrives in any garden soil and is very common in waste places and on ruined sites^[1]. The plant is distributed throughout India, lower and middle hill in Sikkim and Darjeeling up to 6000 ft. The plant has erect hairy annual 30-90 cm high leaves. Different vernacular names are given to the plant. In Nepali the plant is called as 'Elame'; in Lepcha 'Namyew' and in English the plant is known as 'Goat weed'. Throughout the year the plant gives flower. Purple white flower appears. *Ageratum conyzoides* Linn. is a medicinal plant. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita^[2]. Leaves, root, stem and flower of *Ageratum conyzoides* Linn. are widely utilized in traditional medicine. Leaves are styptic effective in healing of wounds, used in boils and prevent tetanus. Leaf juice is also used as eye lotion. The root juice has antibiotic property. The plant is boiled with oil and applied externally in rheumatism. Phenol, essential oil, friedolin, sitosterol, stigmasterol and unidentified esters are active components of *Ageratum conyzoides* Linn.^[3-8] In spite of these medicinal properties, *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^[9].

With this in view it was thought worthwhile to see whether *Ageratum conyzoides* Linn can inhibit growth of animals also. We started work and found that leaves of *Ageratum conyzoides* Linn. could inhibit growth of albino rats. We then studied the underlying mechanism behind this growth inhibition and in this

communication results of the said experiments are being reported.

MATERIALS AND METHODS

Plant material

Leaves of *Ageratum conyzoides* Linn. were collected from the medicinal plants garden of the University of North Bengal and authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department for future reference. Leaves were sundried and powdered. The powder was used as the test drug.

Animals

Male Wistar 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 experiment was approved by the ethics committee of the Institute. Rats were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and provided water *ad libitum*.

Chemicals

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

Experimental design

Animals 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 test drug in the dose of 1g/kg body weight daily through oral route. Experiment was continued for 40 days. On 20th day 10 rats from each group were sacrificed. Remaining 20 rats of the two groups were sacrificed on 40th day.

Blood samples and intestines were collected. Following parameters were studied.

Growth of rats

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

Blood pyruvate level

Blood pyruvate level on 20th and 40th day of experiment was measured by the method of Neish^[10].

Pyruvate dehydrogenase activity

Intestinal pyruvate dehydrogenase activity of the animals was noted on 20th and 40th day by the method of Bai *et al.*^[11]

Transketolase activity

Intestinal transketolase activity of the rats was recorded on 20th and 40th day as per the method of Bai *et al.*^[11]

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

Table 1 shows effect of powdered leaves of *Ageratum conyzoides* Linn on body weight of rats. It appears from the table that leaves of *Ageratum conyzoides* Linn 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 powdered leaves of *Ageratum conyzoides* Linn in addition with normal diet. The animals also developed anorexia.

Effect of powdered leaves of *Ageratum conyzoides* Linn on blood pyruvate level in rats was given in Table - 2. Blood pyruvate level was elevated in *Ageratum conyzoides* Linn treated rats. Elevation started from 20th day of experiment. Results were statistically significant up to the level of $p < 0.001$.

Table 3 showed the effect of *Ageratum conyzoides* Linn on intestinal pyruvate dehydrogenase activity. Result showed that pyruvate dehydrogenase activity was decreased in rats taking powdered leaves of *Ageratum conyzoides* Linn. daily.

Effect of *Ageratum conyzoides* Linn leaves on transketolase activity in rats was given in Table 4. Intestinal transketolase activity was found significantly decreased ($p < 0.001$) in *Ageratum conyzoides* Linn treated rats when compared to that of normal animals.

Table 1. Effect of powdered leaves of *Ageratum conyzoides* Linn 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	40.1 \pm 2.0	59.2 \pm 2.4	61.2 \pm 2.5	70.1 \pm 2.4
2	<i>Ageratum conyzoides</i> Linn. leaves	38.0 \pm 0.9	44.1 \pm 1.7*	40.2 \pm 1.1*	40.2 \pm 1.0*

* $p < 0.001$

Table 2. Effect of powdered leaves of *Ageratum conyzoides* Linn on blood pyruvate level (in mg/100 ml blood) in rats

Group	Treatment	20 th day	40 th day
1	Normal	1.21 \pm 0.20	1.19 \pm 0.21
2	<i>Ageratum conyzoides</i> Linn. leaves	4.90 \pm 0.23*	6.92 \pm 0.25*

* $p < 0.001$

Table 3. Effect of powdered leaves of *Ageratum conyzoides* Linn on intestinal pyruvate dehydrogenase activity (in nmole pyruvate/ml/300C) in rats.

Group	Treatment	20 th day	40 th day
1	Normal	39.5 \pm 2.20	36.0 \pm 2.2
2	<i>Ageratum conyzoides</i> Linn. leaves	23.1 \pm 1.10*	15.8 \pm 1.1*

* $p < 0.001$

Table 4. Effect of powdered leaves of *Ageratum conyzoides* Linn on intestinal transketolase activity (in mili Unit / ml homogenate) in rats

Group	Treatment	20 th day	40 th day
1	Normal	15.1 \pm 1.45	14.5 \pm 1.1
2	<i>Ageratum conyzoides</i> Linn. leaves	7.7 \pm 0.90*	3.4 \pm 0.52*

* $p < 0.001$

DISCUSSION

Throughout the world *Ageratum conyzoides* Linn. is widely used in traditional medicine systems. In Asia, South America and Africa, aqueous extract of this plant is used as a bactericide^[12]. In Central Africa the plant is used to treat pneumonia, but the most common use is to cure wounds and burns. *Ageratum conyzoides* is also utilized to treat fever, rheumatism, headache and colic^[13]. Traditional communities in India use this plant as a bactericide, gastro protective, spasmolytic, antidiysenteric, antilithic, anti diabetic etc^[14-17].

Ageratum conyzoides Linn. is also known to inhibit germination of seeds and growth of plants^[9]. We therefore intended to know whether the plant could inhibit growth of animals also. Our idea was that if the plant could inhibit growth of animals then it will be a message for the people who routinely use root, leaves, stem and flower of *Ageratum conyzoides* Linn. to treat different ailments. We thus studied the effect of the leaves of *Ageratum conyzoides* Linn. on the growth of albino rats. It was found out that the plant leaves inhibited significantly ($p < 0.001$) growth of rats. Growth inhibition started from 20th day onwards of the experiment. Rats also developed anorexia. This growth inhibition might be related with energy yielding process. We thus measured blood pyruvate level in rats. It was revealed that leaves of *Ageratum conyzoides* Linn. could elevate significantly ($p < 0.001$) blood pyruvate level in rats. This finding suggests that *Ageratum conyzoides* Linn. might cause a blockade in the conversion of pyruvate to acetyl coenzyme A. Thus blood pyruvate level was increased. At the same time due to not having sufficient amount of acetyl coenzyme A, energy production was poor which might cause growth inhibition in rats.

Elevated blood pyruvate level might be associated with the activity of pyruvate dehydrogenase enzyme^[18,19]. We thus measured intestinal pyruvate dehydrogenase activity in rats and found that leaves of *Ageratum conyzoides* Linn could lower the activity of pyruvate dehydrogenase. Results were statistically significant up to the level of $p < 0.001$. In fact, this was the blockade for which pyruvate could not be converted to acetyl coenzyme A and accumulated in blood. As development of anorexia has relation with the activity of transketolase enzyme^[20], we measured intestinal transketolase activity in rats. Results showed that leaves of *Ageratum conyzoides* Linn could lower transketolase activity significantly ($p < 0.001$) in the animals. Transketolase is the key enzyme in pentose phosphate pathway for production of NADPH and pentose sugar for synthesis of nucleic acids. This might be another reason of growth inhibition in rats. Both pyruvate dehydrogenase and transketolase enzymes depend on TPP (thiamine pyrophosphate) for their activities^[21-22]. TPP is a thiamine dependant coenzyme. Is it possible that leaves of *Ageratum conyzoides* Linn could destroy thiamine *in vivo* thus inactivated both pyruvate dehydrogenase and transketolase enzymes? Work is now in progress in this direction.

CONCLUSION

It may be concluded that leaves of *Ageratum conyzoides* Linn. could inhibit growth of rats. This was due to blockade in energy generation process and interference in pentose phosphate pathway for production of NADPH and pentose sugar for synthesis of nucleic acids.

This study was thus a message to the people who routinely use leaves of Ageratum conyzoides Linn. in different ailments.

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