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**Original Article**

# Seasonal variation in hepatoprotective activity of *Ageratum conyzoides* L. leaves on anti tubercular drugs induced hepatotoxicity in rats

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## ABSTRACT

Seasonal variation in the effect of *Ageratum conyzoides* L. leaves on anti tubercular drugs induced hepatotoxicity in rats was studied. Results showed that *A.conyzoides* L. leaves during the months of July and August had maximum protective effect against anti tubercular drugs induced hepatotoxicity in rats.

***Keywords*:** *Ageratum conyzoides* L., hepatotoxicity, anti tubercular drugs.

## INTRODUCTION

*Ageratum conyzoidesL*. (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. *A. conyzoides* L. 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 *A. conyzoides* L. 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.[3-6] Modern researchers claimed that *A. conyzoides* L. has antibacterial [7] and wound healing effect [8]. It has neurological [9] and gastro protective effect also [10]. The plant acts as analgesic [11] and has effect on circulation [12]. It gives protection against gamma radiation [13].

The plant has anti tumor activity [14] and has allopathic effects[15]. Ita *et al.* [16] demonstrated hepato protective activity of this plant. We also showed hepato protective activity of *A. conyzoides* L. leaves in anti tubercular drugs induced hepatotoxicity in rats (unpublished observation). In present communication seasonal variation in hepato protective activity of *A. conyzoides* L. leaves on anti tubercular drugs induced hepatotoxicity in rats is being reported.

## MATERIALS AND METHODS

**Plant Material:** *A. conyzoides* L. leaves 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 January – February, March – April, May – June, July – August, September - October and November – December in 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 Biochemistry, North Bengal Medical College, Dist. Darjeeling, West Bengal, India for future reference.

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**Reparation of the Test Drug:** Decoction from fresh tender leaves of *A. conyzoides* L. was prepared by the method of Pillai and Santhakumari [17]. The decoction was used as test drug in the dose of 1 ml/kg (1 g leaves per ml of decoction) orally through a feeding tube. Selection of the dose of the test drug was as per the method of Kale *et al.* [18].

**Experimental animals:** Wistar strain albino rats (180 - 200 g) of either sex were used for the study. Rats were housed in colony cages (5 rats / cage) and were kept for at least a week in the experimental wing of the animal house (room temperature 25 – 28 degree centigrade and humidity 60 – 65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. 8 rats were used for each set of experiment. The animal experiment was approved by the ethics committee of the Institute.

**Acute oral toxicity study:** Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh [19]. Test drug developed from the leaves of *A. conyzoides* L. was given orally in doses of 1, 2, 3, 4 and 5 ml/kg to different groups of mice each group containing six animals. 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.

**Chemicals and Drugs:** Isoniazid, rifampicin (R), pyrazinamide (Z)were procured from Plethico Pharmaceuticals Ltd. Indore. All other chemicals were collected from Sigma Chemical Co., USA.

**Experimental design**

Rats were divided into following groups. In each group eight rats were employed.

**Group 1:** Vehicle control – gum acacia was given to rats orally for one month.

**Group 2:** Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension was given to rats orally for one month. Doses are optimum to produce hepatotoxicity in rats [17].

**Group 3:** Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of *A. conyzoides* L. in the month of January – February for one month.

**Group 4:** Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of *A. conyzoides* L. in the month of March – April for one month.

**Group 5:** Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of *A. conyzoides* L. in the month of May – June for one month.

**Group 6:** Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of *A. conyzoides* L. in the month of July – August for one month.

**Group 7:** Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of *A. conyzoides* L. in the month of September – October for one month.

**Group 8:** Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of *A. conyzoides* L. in the month of November – December for one month.

**Assessment of liver damage:** To assess liver damage blood samples were collected from the rats by cardiac puncture under ether anesthesia on the $30^{th}$ day. Serum total protein, serum bilirubin and serum alanine aminotransferase (ALT) were estimated by the methods as followed by Kate *et al*.[18].

**Statistical analysis:** The values were expressed as mean ± SEM and were analyzed using one-way analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) $20^{th}$ versions. 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 test drug (develop from the leaves of *A. conyzoides* L.) did not produce any toxic symptoms when administered orally to mice in doses of 1, 2, 3, 4 and 5 ml/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment. Seasonal variation in hepato protective activity of the leaves of *A. conyzoides* L. is given in table No.1. Results showed that anti tubercular drugs in the given dose could produce hepatotoxicity in rats. Liver markers like serum bilirubin and serum ALT elevated and level of serum total protein decreased after administering the anti tubercular drugs. All

changes were statistically significant. Leaves of *A. conyzoides* could protect the rats from hepatotoxicity. Maximum activity was noted by the leaves of *A. conyzoides* for the months of July and August. Results were statistically significant up to the level of $p<0.001$. Effect of leaves of *A. conyzoides* L. of different seasons on serum total protein in rats during hepatoxicity as induced by anti tubercular drugs was given in the Table - 1 and fig – 2. Results showed that leaves of *A. conyzoides* of the period July to August could increase level of serum total protein in rats almost to control value during hepato toxicity as induced by anti tubercular drugs. In hepatotoxic rats serum total protein came 5.0 ± 0.20 g/dl while in *A. conyzoides* (July – August) treated rats the value was 6.9 ± 0.41 g/dl (control value, 7.2 ± 0.31 g/dl). Results were statistically significant up to the level of $p<0.001$. Leaves of *A. conyzoides* L.(for the months of May – June and September - October) could also increase serum protein level in rats during hepatotoxicity but the magnitude was less than that of the leaves of *A. conyzoides* for the months of July and August. Leaves of *A.conyzoides* for the months of November – December, January – February and March – April, however, did not show any effect on serum total protein level in hepatotoxic rats. Same results were also found in case of serum bilirubin and serum ALT (Table – 1 and Figs. 3 and 4) when the rats were treated with the leaves of *A. conyzoides* of the period July to August.

Fluck and Pharm [20] 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 [21-26]. In the present work we also noted that leaves of *A. conyzoides* L. of the period July to August had maximum hepato protective effect against anti tubercular drugs induced hepatotoxicity in rats. This is probably due to maximum accumulation of some bioactive compound(s) in the leaves of *A. conyzoides* of that period responsible for hepato protective effect. We are now looking for isolation and characterization of that bioactive compound(s) to see the underlying mechanism of its hepato protective activity. Work in this direction is in progress.

## CONCLUSION

*Ageratum conyzoides* L. leaves of the months of July and August had maximum hepato protective effect on anti tubercular drugs induced hepatotoxicity in rats

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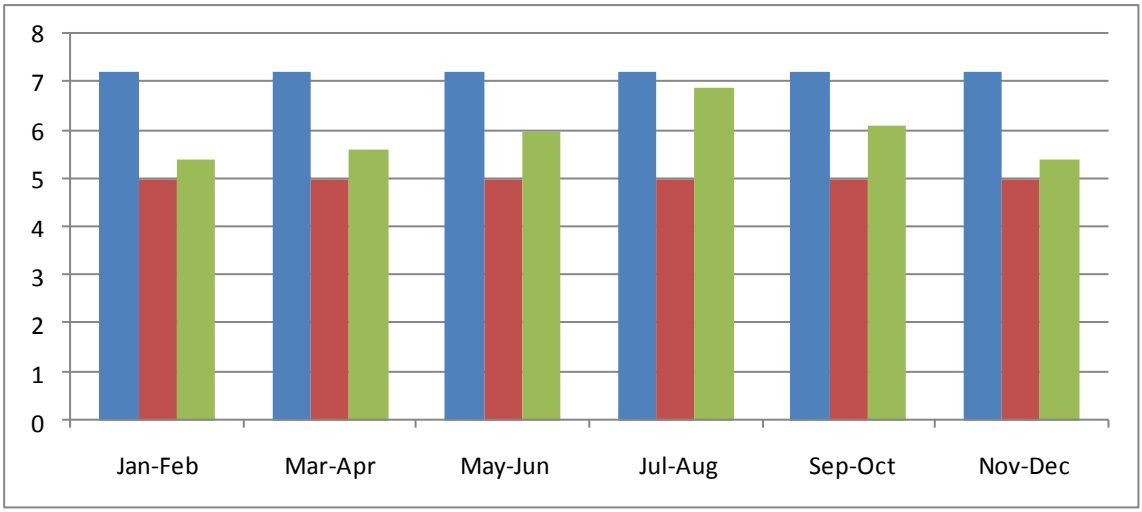
**Table – 1: Showing seasonal variation in hepato protective activity of the leaves of *Ageratum conyzoides* against anti tubercular drugs induced hepatotoxicity in rats.**

| Group | Serum total protein (g/dl) | Serum bilirubin (mg/dl) | Serum ALT (Units/ml) |
|---|---|---|---|
| Control | 7.2 ± 0.31 | 0.94 ± 0.05 | 36.23 ± 3.21 |
| Hepatotoxic rats | 5.0 ± 0.20** | 2.13 ± 0.12** | 172.61 ±5.01** |
| Hepatotoxic rats + AC(Jan-Feb) | 5.4 ± 0.31 | 2.08 ± 0.19 | 168.41 ± 5.01 |
| Hepatotoxic rats + AC (Mar-Apr) | 5.6 ± 0.33 | 1.99 ± 0.20 | 152.12 ± 4.31 |
| Hepatotoxic rats + AC (May-Jun) | 6.0 ± 0.32* | 1.33 ± 0.20* | 148.92 ± 4.31* |
| Hepatotoxic rats + AC (Jul-Aug) | 6.9 ± 0.41** | 1.08 ± 0.11** | 102.38 ± 5.51** |
| Hepatotoxic rats + AC (Sep-Oct) | 6.1 ± 0.30* | 1.31 ± 0.26* | 144.21 ± 4.29* |
| Hepatotoxic rats + AC (Nov-Dec) | 5.4 ± 0.24 | 1.86 ± 0.25 | 160.24 ± 5.09 |

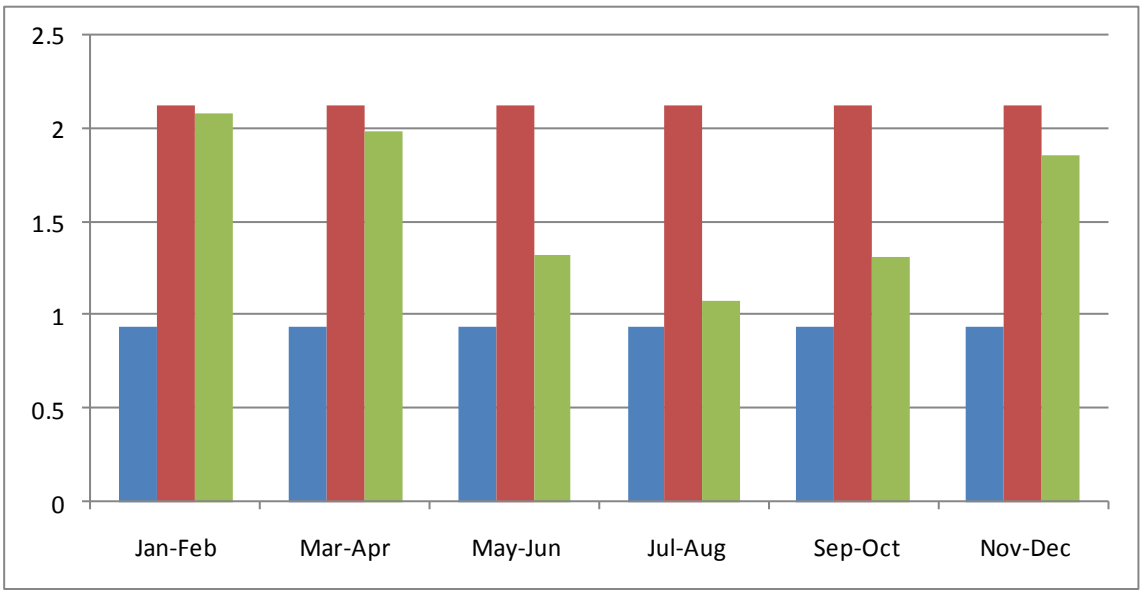
AC: *Ageratum conyzoides*, Values were mean ± SEM of eight animals in each group. * $p< 0.05$,** $p < 0.001$.

**Fig. 1 - *Ageratum conyzoides* L.**



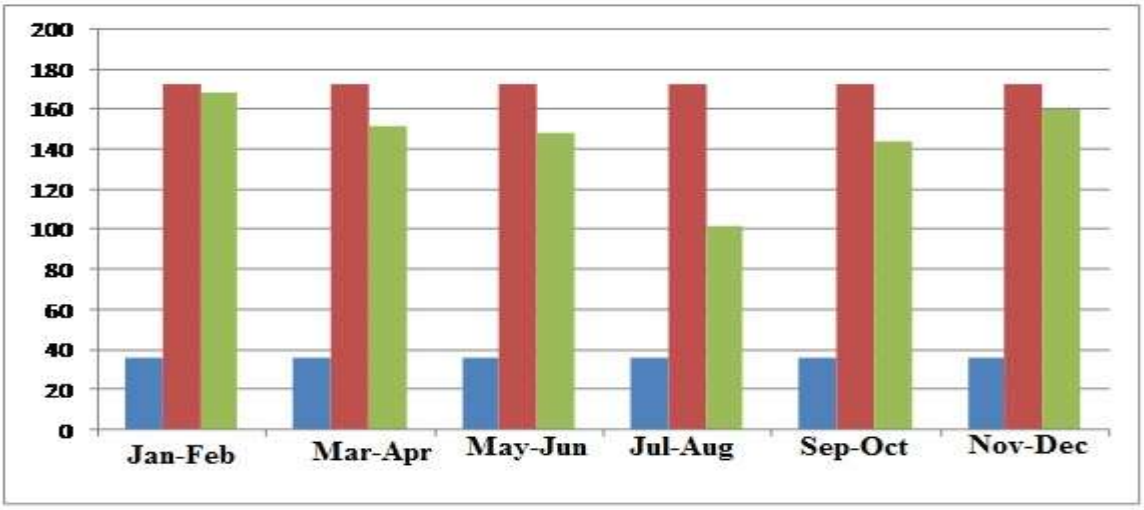

■Control ■Drug treated ■*Ageratum conyzoides* L. (Serum total protein – g/dl)

**Fig 2 :Effect of leaves of *Ageratum conyzoides* of different seasons on serum total protein In rats during hepatotoxicity as induced by anti tubercular drugs**




■Control ■Drug treated ■*Ageratum conyzoides* L. (Serum bilirubin – mg/dl)

**Fig 3 :Effect of leaves of *Ageratum conyzoides* of different seasons on serum bilirubin in rats during hepatotoxicity as induced by anti tubercular drugs**




■Control ■Drug treated ■*Ageratum conyzoides* L. (Serum ALT –Units/ml)

**Fig 4 :Effect of leaves of *Ageratum conyzoides* of different seasons on serum ALT in rats during hepatotoxicity as induced by anti tubercular drugs**

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