Journal of The Gujarat Research Society

ISSN: 0374-8588
Volume 22 Issue 1, January 2020

Ageratum Conyzoides Caused Toxicity in Normal Liver Cell Lines

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ABSTRACT: Intoxicated with Ageratum conyzoides, liver damage is a common clinical development. Since ancient times, medicinal plants have been used in various traditional medicinal systems which makes it very important to evaluate the toxicity of such plants. The hepatotoxic Pyrrolizidine alkaloids (PAs) have been considered as an important therapeutic extract that can be obtained from the medicinal plant Ageratum conyzoides. In this regard butanolic extract of the flowers of this plant were evaluated for its toxicity on a panel of normal mouse & human liver cell lines. Firstly, the flowers were extracted in n-Butanol for 24 h & the extract was treated to the normal mouse liver cells; AML 12 cells & the normal human liver cells THLE-3 cells in an amount dependent manner for 24 h. Both the normal liver cells showed a decrease in cell viability upon increase in the amount of the extract indicating that caution must be exercised while utilizing this plant for medicinal purposes.

KEYWORDS: Ageratum Conyzoides, Butanolic Extract, Hepatoxicity, Normal Liver Cells, Poisonous Plants, Pyrrolizidine Alkaloids.

1. INTRODUCTION

Tibetan traditional system of medicines like other Asian system of medicines is famous globally, which interestingly includes some of the poisonous plants. Some of the medicinal yet poisonous plants who are being used in the Tibetan traditional system of medicines are *Strychnos nux-vomica*, *Aconitum pendulum*, *Anisodus tanguticus* & *Datura stramonium* whose toxic phytochemical composition along with their pharmacological roles have been mentioned in this paper. The very major toxins isolated from these plants are strychnine, aconitine, anisodamine & scopolamine. In Figure 1, some of the toxic alkaloids from commonly used medicinal plants have been illustrated.

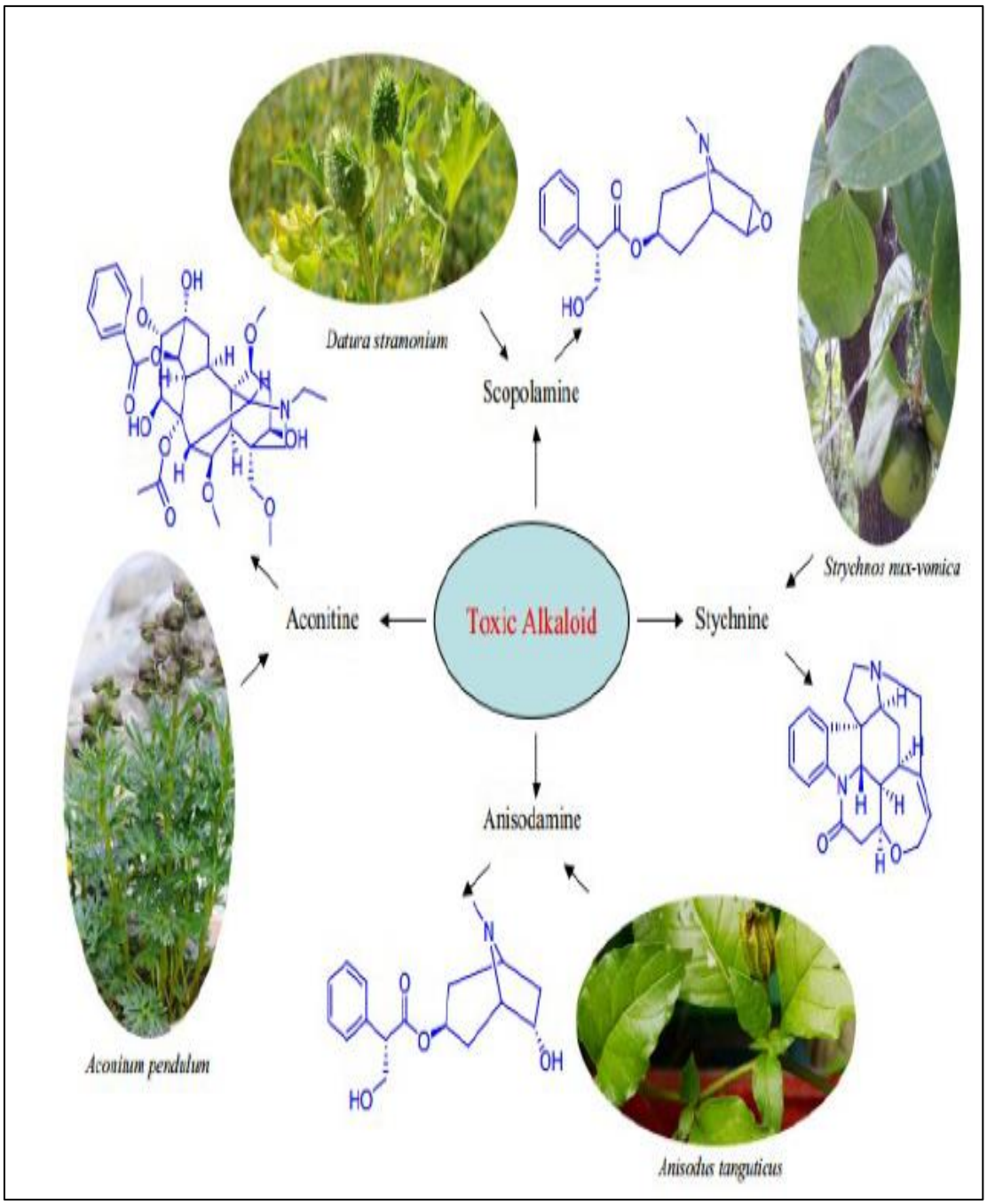
The plant *Ageratum conyzoides* has certain medicinal roles but also reported to have the hepatotoxic Pyrrolizidine alkaloids (PAs) which can cause heath issues in a large number of people. Thereby, in this paper, the hepatotoxic effect of the flowers of *Ageratum conyzoides* has been evaluated [1]. Firstly, the flowers of *Ageratum conyzoides* would be plucked, dried and extracted in butanol. Once the extract is filtered; the extract would be stored in Dimethyl sulfoxide (DMSO) for further use. Varying concentrations of the butanolic fraction of flowers of *Ageratum conyzoides* would be administered on the normal mouse liver cells AML 12 & normal human liver cells THLE-3 for 24 h and the data obtained would be statistically analysed.The flowers were plucked & dried so as to remove moisture following which the flowers were extracted in 60 % n-Butanol for 24 hours after which the extract was filtered using a 0.22 µm filter unit.

Once filtered, the filtrate's butanol content was evaporated and was lyophilized to a powder form, which was then suspended in DMSO for further use. As per available prior publications, normal mouse liver cells AML 12 & normal human liver cells THLE-3 were cultured accordingly. Both the AML 12 cells and THLE-3 cells (5×10^3) were treated with

Journal of The Gujarat Research Society

ISSN: 0374-8588
Volume 22 Issue 1, January 2020

different concentrations of butanolic extract of the flowers of *Ageratum conyzoides* (0, 10, 20, 30, 40 and 50 µg/ml) and cell viability was determined by MTT assay in a procedure as reported earlier. All the experiments were performed three times and $p < 0.05$ value was regarded as statistical significant in a process as reported earlier[2].



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ISSN: 0374-8588
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Figure 1. Some of the major alkaloids which are toxic used in the Tibetan traditional medicine. Some of the plants which have toxic alkaloids are *Strychnos nux-vomica, Aconitum pendulum*, *Anisodus tanguticus* & *Datura stramonium*[3].

2. LITERATURE REVIEW

C. T.Y.K in his study discloses about a pharmacophylogenetic analysis of the Ranunculaceae family of medicinal plants, looking into the relationships between their phylogeny, chemical contents, and pharmaceutical qualities. The systematics and molecular phylogeny of the Ranunculaceae were combined with phytochemical, ethnopharmacological, and pharmacological data. Benzylisoquinoline alkaloids, ranunculin, triterpenoid saponin, and diterpene alkaloids were among the chemical components of this family, which contained numerous representative metabolic groups. In some genera, ranunculin and magnoflorine have been shown to coexist. The taxonomy proposed before, in which the family Ranunculaceae was separated into five sub-families: Ranunculoideae, Thalictroideae, Coptidoideae, Hydrastidoideae, and Glaucidioideae, was supported by the pharmacophylogenetic study combined with therapeutic data. It was reasonable to divide the Ranunculoideae subfamily into ten tribes. Each taxonomic group's chemical contents and therapeutic efficacy were examined, demonstrating the underlying linkages between phylogeny, chemical diversity, and clinical application, which should aid in the conservation and long-term use of Ranunculaceae pharmaceutical resources [4].

S. Mallick et al. in their study discusses about the human skin that is permeable to aconitine and mesaconitine, according to an in vitro study utilizing a modified Franz-type diffusion cell. Relevant reports of percutaneous absorption of Aconitum alkaloids and aconite poisoning are examined to characterize the risk of systemic toxicity following topical applications of aconite tincture and raw aconite roots. According to published accounts, aconite tincture and raw aconite roots can be absorbed into the systemic circulation through the skin and produce deadly and non-fatal aconite poisoning. Aconitum alkaloids are found in high concentrations in both aconite tincture and raw aconite roots, allowing penetration of the stratum corneum along the diffusion gradient. If Aconitum alkaloids are kept in occlusive contact with the skin and the epidermis (stratum corneum) is already injured, the danger of systemic poisoning is significantly higher. The public should be warned about the dangers of utilizing topical aconite products and the possibility of systemic poisoning from Aconitum alkaloids absorbed via the skin [5].

Caroline T Griffin in her study focuses on PAs that are found in plants that are used for food, medicine, and as pollutants in agricultural products and food. Although PA contamination in our food chain has posed the greatest health concern, any PA contamination in our food chain should be considered a potential health issue. Retail honeys were evaluated using LC-MS/MS for this purpose. By comparing poisonous retronecine and otonecine-type PAs to reference compounds in a spectrum library, the method allows for specific identification of toxic retronecine and otonecine-type PAs. Within a set of tolerance values, 50 honey samples were matched to the reference spectra. It was possible to perform accurate data analysis and find positive samples quickly. The average PA content in positive samples was 1260 g kg(-1) of honey. Good linear calibrations (R(2)>0.991) were obtained. The LOD and LOQ values varied from 0.0134 to 0.0305 g mL(-1) and 0.0446 to 0.1018 g mL(-1) [6].

3. DISCUSSION

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3.1. Evaluation of the toxicity of butanolic extract of flowers of Ageratum conyzoides towards a panel of normal liver cells:

The butanolic extract of flowers of *Ageratum conyzoides* was administered on the normal mouse liver cells AML 12 cells. After 24 h treatment, it was found that the extract showed an amount dependent toxicity in AML 12 cells, with an IC_{50} value of 36 µg/ml (Figure 5). This implies that the butanolic extract of flowers of *Ageratum conyzoides* has toxic activity on normal mouse liver cells. The results were statistically significant as compared to the control with $P<0.05$. This indicates that this plant maybe toxic in a mouse model of toxicity[7].

Similarly, the butanolic extract of flowers of *Ageratum conyzoides* was administered on the normal human liver cells THLE-3 cells. After 24 h treatment, it was found that the extract showed an amount dependent toxicity in THLE-3 cells, with an IC_{50} value of 47 µg/ml (Figure 6). This implies that the butanolic extract of flowers of *Ageratum conyzoides* has toxic activity on normal human liver cells. The results were statistically significant as compared to the control with $P<0.05$. This indicates that this plant maybe toxic to humans[8].

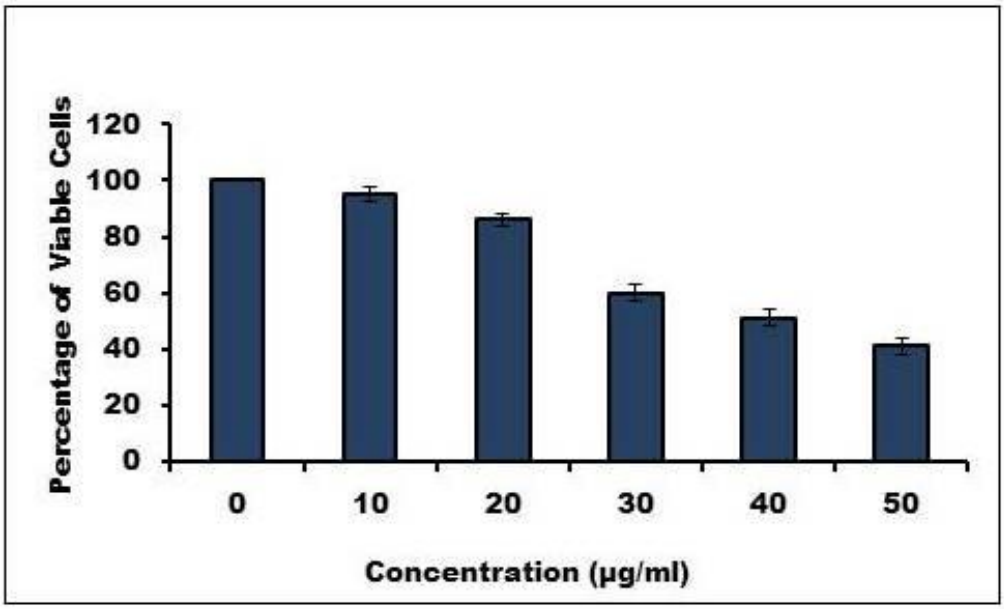

Figure 5: Graph showing toxicity of butanolic extract of the flowers of *Ageratum conyzoides*. The normal mouse liver cells AML 12 showed a concentration dependent decrease cell viability percentage after 24 h[9].

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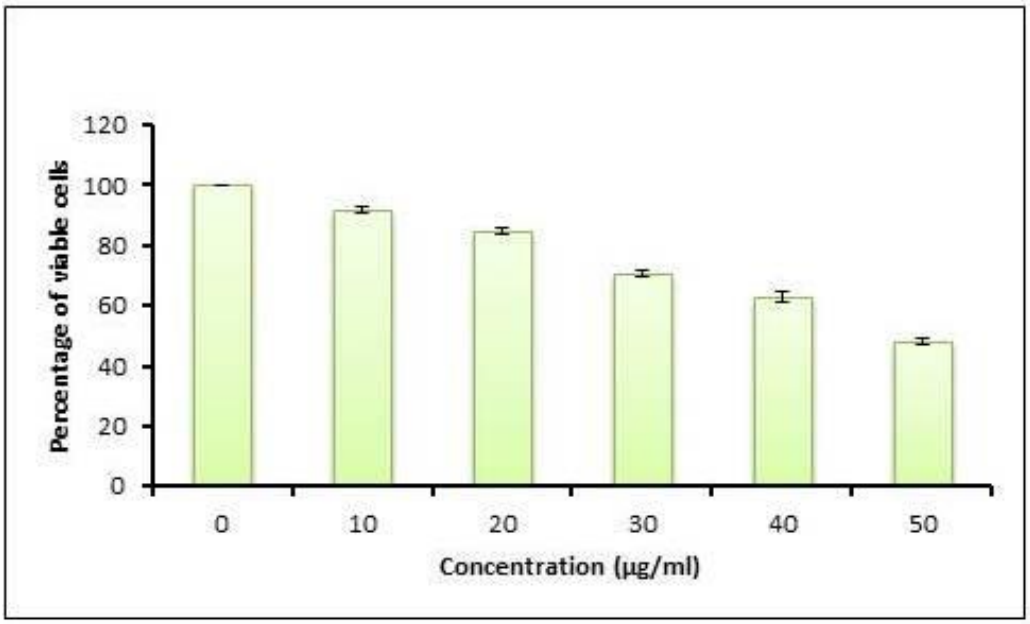

Figure 6: Graph showing growth inhibitory efficacy of butanolic extract of flowers of *Ageratum conyzoides*. The normal human liver cells THLE-3 showed a concentration dependent decrease cell viability percentage after 24 h. The values were statistically significant with respect to the control or un-treated cells with P<0.05[10].

PAs (pyrrolizidine alkaloids) are secondary plant compounds that can induce hepatotoxicity in humans and livestock. PAs are found in plants that are used for food, medicine, and as pollutants in agricultural products and food. Although PA contamination in our food chain has posed the greatest health concern, any PA contamination in our food chain should be considered a potential health issue. Retail honeys were evaluated using LC-MS/MS for this purpose. By comparing poisonous retronecine and otonecine-type PAs to reference compounds in a spectrum library, the method allows for specific identification of toxic retronecine and otonecine-type PAs. Within a set of tolerance values, 50 honey samples were matched to the reference spectra. It was possible to perform accurate data analysis and find positive samples quickly. The average PA content in positive samples was 1260 g kg(-1) of honey. Good linear calibrations (R(2)>0.991) were obtained. The LOD and LOQ values varied from 0.0134 to 0.0305 g mL(-1) and 0.0446 to 0.1018 g mL(-1)[11].

The application of QIT-MS in conjunction with a spectral library to allow for fast and accurate PA detection in complex food matrices such as honey is described in this study. A total of 16% of retail honeys were found to be positive, with half of these having a PA content of more than 1000 lg kg1 of honey. The positive samples would surpass all prescribed TDI values using an average intake level of 20 g of honey per serving, as summarized by Edgar. According to the European Food Safety Authority (EFSA), the risk of acute toxicity from PAs in honey is low. The amount of PA in honey is lower than in medicinal and herbal preparations. However, the detected levels of PAs in honey are high enough to pose a risk of chronic toxicity in humans. The method described here has been validated for 11 PAs, and we plan to expand the spectral library to include another 5 PAs and their N-oxides in the future. A large scale retail survey (n > 300) has been initiated as a result of the data obtained from this study in order to assess a comprehensive profile of all retail

ISSN: 0374-8588
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honey in Ireland. EFSA has also called for more research because their findings are based solely on PAs in honey from one EU Member State. The importance of establishing an acceptable level for toxic PAs in food and feed, as well as using validated analytical methods to detect their presence, is becoming more apparent.

Liver injury is a consistent pathological change found intoxicated with Ageratum conyzoides. Adult Wistar rats showed a persistent reduction of body weight gain for 28 days of feeding on 20 percent A. conyzoides diet. Macroscopically liver appeared to have mottling on the capsular surface, and this lesion was the only pathological change found in rats. Megalocytosis and proliferation of bile suct cell were noted in liver microscopically. Similar clinical signs were also seen in rats fed on 10 percent of A. conyzoides diet for six weeks of feeding. Pathological changes did not appear in the liver during the first week of feeding. The pathological changes started to develop in the second week of feeding including anisokaryocytosis, proliferation of bile duct cells and infiltration of mononuclear cells inside and/or outside the veins and arteries of liver in the second week of feeding. Megalocytosis appeared in the third week followed by marked anisokaryocytosis and bile duct cells proliferation. On week-4, focal hepatic necrosis with bile duct cells proliferation were seen in the liver. Bile duct cells proliferation became more extensive in the portal tract where the interstitium of portal tracts were thickened and infiltrated by bile duct cells in the fifth week. The liver lesions were more prominent in sixth week of feeding. Supplementation of diets with normal diet led to liver regeneration in rats.

4. CONCLUSION

Since ancient times, medicinal plants have been used for treating a variety of diseases however, the toxicity of such plants should also be determined. The hepatotoxic Pyrrolizidine alkaloids (PAs) have been reported from the medicinal plant *Ageratum conyzoides*. In this regard the flowers of this plant were evaluated for its toxicity on a panel of normal mouse & human liver cell lines. Firstly, the flowers were plucked, dried & extracted in n-Butanol for 24 h. The extract was then filtered and lyophilized with the resultant powder being stored at room temperature in an airtight container. The normal mouse liver cells AML 12 & the normal human liver cells THLE-3 were cultured on DMEM media. The extract was treated to AML 12 cells & THLE-3 cells in an amount dependent manner for 24 h. Both the normal liver cells showed a decrease in cell viability upon increase in the amount of the extract indicating that caution must be exercised while utilizing this plant for medicinal purposes.

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