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Induced Toxicity in Normal liver Cell lines by Ageratum conyzoides

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ABSTRACT

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 and human liver cell lines. Firstly, the flowers were extracted in n-Butanol for 24 h and the extract was treated to the normal mouse liver cells; AML 12 cells andthe 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.

KEY WORDS: AGERATUM CONYZOIDES, BUTANOLIC EXTRACT, HEPATOXICITY, NORMAL LIVER CELLS, POISONOUS PLANTS, PYRROLIZIDINE ALKALOIDS.

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* and *Datura stramonium* whose toxic phytochemical composition along with their pharmacological roles have been mentioned in this paper. In Tibet, poisonous plants are mostly found in valleys to mountain peaks and include families, such as Papaveraceae, Thymelaeaceae, Fabaceae, etc. Many such plants have poisonous chemicals having biological roles.The very major toxins isolated from these plants are strychnine, aconitine, anisodamine and scopolamine(L. Ma (2015), Y. Kang (2012), T.Y.K. Chan (2014)). In Figure 1, some of the toxic alkaloids from commonly used medicinal plants have been illustrated.

Species of the genus Aconitum are mostly used for treatment of sepsis, rheumatoid arthritis etc. Moreover, lead agents towards analgesic, anti-arrhythmic diseases. Unfortunately they possess extremely high toxicity due to which processing is needed in Tibetan traditional system of medicines. For example, prior to use the stems of *Aconitum pendulum* are wet in water following which they are boiled for 4–6 h, and then they are sliced off (L. Ma et. al. (2015)).Strychnos L. species are both poisonous and medicinal. The seeds though used in medicine, are quite toxic due to which they should be processed prior use. The seeds are processed by heating in vinegar, sand etc. and are used to treat wounds, paralysis etc. by traditional practioners (L. Ma et. al. (2015)).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.

Literature Review: In the plant *Ageratum conyzoides*, alkaloids like pyrrolizidines were found to be responsible for the induction of hepatotoxicity, lung carcinoma etc. The entire plant which had been extracted in alcohol was reported

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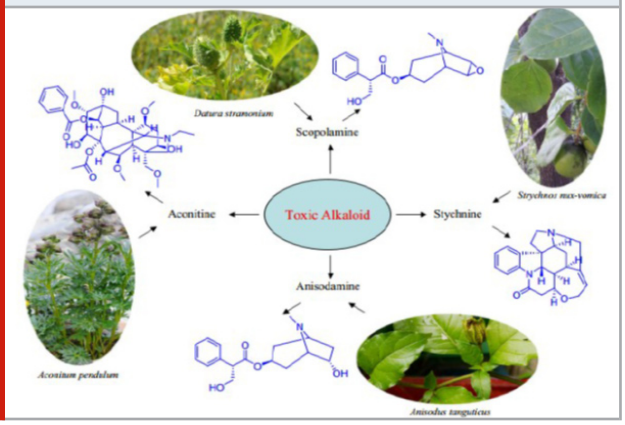
to induce toxicity in mice models of toxicity at amounts of 1500 milligram/kilogram of body weight which can be attributed to the presence of Pyrrolizidine alkaloids (PAs) (N. Yadav (2019), S. E. Atawodi (2017).

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 andDatura stramonium (L. Ma et. al. (2015)



Humans have been exposed to the toxic PAs via ingestion of PA-contaminated botanical medicines or via dietary substituents or by the consumption of PA-laced foodstuffs, like grains, honey etc. The PA-influenced acute liver toxicity shows hepatomegaly, ascites etc. leading to a condition such as hepatic sinusoidal obstruction syndrome (HSOS). Many outbreaks of PA induced poisoning in humans occurs due to PA-influenced HSOS. Moreover certain PAs, like fulvine, monocrotaline (MCT) and seneciphylline, also induces toxicity in non-hepatic organs, where the MCT-influenced lung toxicity has been reported(M. Yang (2016), C.T. Griffin (2012)). Traditional medicinal uses of the plant Ageratum conyzoidesincludes its role of its leaves as an antidote towards snake venom, pneumonia and typhoid fever, its roots are being used against Diarrhoea and for anti-cancer roles whereas its flowers have anti-itch and licicidal roles. In Table 1, the phytochemical profiles of the plant Ageratum conyzoides has been tabulated. In Table 2, the bioactivity of the isolated compounds from Ageratum conyzoides has been tabulated. In this paper, the flowers of Ageratum conyzoides have been evaluated for its hepatotoxicity on a panel of normal mouse and human liver cells. The reason for doing so is as this plant has been used in many medicinal preparations thereby it's important to evaluate its toxicity.

Research Questions: Ageratum conyzoides being a medicinal plant has been accused of having toxic properties. Thereby the butanolic fraction of its flowers was evaluated for its toxicity towards a panel of normal mouse and human liver cells.

MATERIAL AND METHODS

Experimental design: 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 and normal human liver cells THLE-3 for 24 h and the data obtained would be statistically analysed.

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 andDatura stramonium (L. Ma et. al. (2015).

Number	Class	Name of phytomolecule	Plant source
1	Chromene	Ageratochrome dimer	Oil
2	Sesquiterpene	B-caryphyllene	Oil
3	Sterol	Brassicasterol	Oil
4	Dihydrobrassicasterol	Caffeic acid	Oil
5	Sesquiterpene	Caryophyllene epoxide	Whole plant
6	Sterol	Dihydrobrassica sterol	Oil
7	Alkaloids	Echinatine	Oil
8	Terpenes	Eugenol	Oil
9	Secondary metabolites	Fumaric acid	Oil
10	Flavanoid	Kaempferol-3,7	Oil

Preparation of the butanolic extract fromAgeratum conyzoides: The flowers were plucked and 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.

Cell viability assay: As per available prior publications, normal mouse liver cells AML 12 & normal human liver cells THLE-3 were cultured (S. Mallick et. al. (2013)). Both

the AML 12 cells and THLE-3 cells (5×103) were treated with 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 (S. Mallick et. al. (2013).

Table 2. Bioactivity of isolated compounds from Ageratum conyzoides. Thebioactivities the compounds are due to the presence of many phytomolecules (N. Yadav et. al. (2019).

Number	Class of Phytochemicals	Phytomolecules composition	Bioactivity
1	Alkaloids	5-hydroxy tryptamine	Reproductive
2	Polyphenol	Kaempferol	Anti-cancer
3	-	AC-1	Anti-bacterial
4	Polyphenol	Precocene II	Anti-fungal
5	Polyphenol flavanoids	Chromans & Chromenes	Anti-microbial
6	Sterol	Stigmasterol	Anti-inflammatory
7	Flavonoids	-	Anti-inflammatory
8	Flavonoids	Polymethoxy flavones	Anti-cancer
9	Polyphenol flavanoids	Precocene I & II	Insecticidal

Figure 2: 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. The values were statistically significant with respect to the control or un-treated cells.

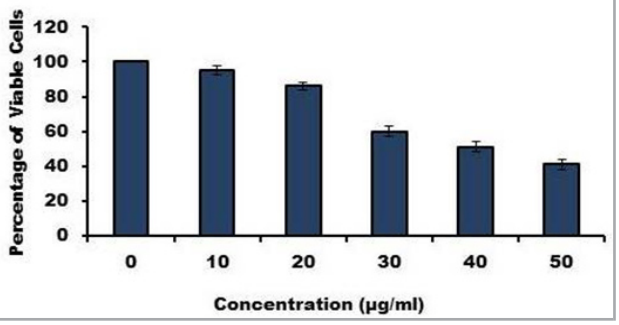
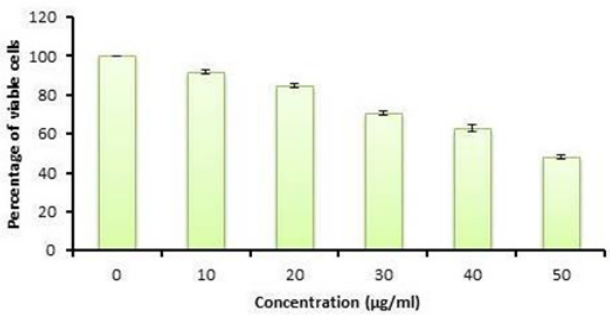


Figure 3: Graph showing growth inhibitory efficacy of butanolic extract of flowers of Ageratum conyzoides. The normal human liver cells THLE-3 showed a concentration dependent decrease cell viability percentage after 24 h. The values were statistically significant with respect to the control or un-treated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



Statistical analysis: All the experiments were performed three times and p <0.05 value was regarded as statistical significant in a process as reported earlier.

RESULTS AND DISCUSSION

Evaluation of the toxicity of butanolic extract of flowers of Ageratum conyzoides towards a panel of normal liver cells: The butanolic extract offlowers 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 12cells, with an IC50 value of 36 µg/ml (Figure 2). This implies that the butanolic extract offlowers 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.

Similarly, the butanolic extract offlowers 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-3cells, with an IC50 value of 47 µg/ml (Figure 3). This implies that the butanolic extract offlowers 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.

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 and human liver cell lines. Firstly, the flowers were plucked, dried and 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 and the normal human liver cells THLE-3 were cultured on DMEM media. The extract was treated to AML 12 cells andTHLE-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.

REFERENCES

Atawodi, S.E., Adepoju, O.A. and Nzelibe, H.C., 2017. Antihyperglycaemic and hypolipidemic effect of methanol extracts of Ageratum conyzoides L (Asteraceae) in normal and diabetic rats. Tropical Journal of Pharmaceutical Research, 16(5), pp.989-996.

Chan, T.Y., 2014. Aconitum alkaloid poisoning related to the culinary uses of aconite roots. Toxins, 6(9), pp.2605-2611.

Chan, T.Y., 2012. Aconite poisoning following the percutaneous absorption of Aconitum alkaloids. Forensic science international, 223(1-3), pp.25-27.

Griffin, C.T., Danaher, M., Elliott, C.T., Kennedy, D.G. and Furey, A., 2013. Detection of pyrrolizidine alkaloids in commercial honey using liquid chromatography–ion trap mass spectrometry. Food chemistry, 136(3-4), pp.1577-1583.

Kang, Y., Łuczaj, Ł.J. and Ye, S., 2012. The highly toxic Aconitum carmichaelii Debeaux as a root vegetable in the Qinling Mountains (Shaanxi, China). Genetic resources and crop evolution, 59(7), pp.1569-1575.

Ma, L., Gu, R., Tang, L., Chen, Z.E., Di, R. and Long, C., 2015. Important poisonous plants in Tibetan ethnomedicine. Toxins, 7(1), pp.138-155.

Mallick, S., Pal, B.C., Kumar, D., Chatterjee, N., Das, S. and Saha, K.D., 2013. Effect of corchorusin-D, a saikosaponin like compound, on B16F10 melanoma cells (in vitro and in vivo). Journal of Asian natural products research, 15(11), pp.1197-1203.Yadav, N., Ganie, S.A., Singh, B., Chhillar, A.K. and Yadav, S.S., 2019. Phytochemical constituents and ethnopharmacological properties of Ageratum conyzoides L. Phytotherapy Research, 33(9), pp.2163-2178.

Yang, M., Ruan, J., Fu, P.P. and Lin, G., 2016. Cytotoxicity of pyrrolizidine alkaloid in human hepatic parenchymal and sinusoidal endothelial cells: Firm evidence for the reactive metabolites mediated pyrrolizidine alkaloid-induced hepatotoxicity. Chemico-biological interactions, 243, pp.119-126.

Yin, T.P., Cai, L., He, J.M., Dong, J.W., Fang, H.X., Zhou, H. and Ding, Z.T., 2014. Three new diterpenoid alkaloids from the roots of Aconitum duclouxii. Journal of Asian natural products research, 16(4), pp.345-350.