Introduction

Melia azedarach, known by many other names, including Mahanimba, Indian lilac, Chinaberry tree, Pride of India, Kattu vembu, etc., is a species in the Meliaceae family, that is native to Indomalaya and Australasia. It is large evergreen tree found throughout India and very similar to neem. Melia azedarach is traditionally used as anthelmintic, antilithic, diuretic, emmenagogue, astringent and stomachic. Various scientific studies reported the analgesic, anticancer, antiviral, antimarial, antibacterial, anti feedent and antifertility activity of this plant [1-3]. This plant leaves contains Nimbinene, Meliacin, Quercetin, Quercetin-3-O-b-rutinoside, Kaempferol- 3-O-b rutinoside, Rutin and Kaempferol-3-L-rhamno-D-glucoside [4,5] benzyl 3-O-b- D-glucopyranosyl-7-hydroxybenzoate, 1, 7, 8-trihydroxy-2- naphthaldehyde, 2-methoxy-4-(2-propenyl) phenyl b-D-glucoside [6]. The methanolic extract of this plant showed 48 constituents, the major constituents are Phytol (11.04%- diterpene), Quercetin (16.47%- flavonoid), Palmitic acid (15.49%- saturated fatty acid), 9,12,15-Octadecatrienoic acid (3.43%- n-alkanoic acid) serves as an anti-inflammatory, hypocolesterolemic, hepatoprotective, nematicide, insecticide, antithamistic properties [7]. Polycystic ovary syndrome (PCOS) is a disorder of chronically abnormal ovarian function and hyperandrogenism (abnormally elevated androgen levels), women with PCOS can struggle to become pregnant, because the high levels of male hormones prevent the release of an egg ovulation, It affects 5-10% of women of reproductive age. PCOS is one of the most common hormonal disorders among the women. Melia dubia (Malai vembu) leaf extract is used orally to treat polycystic ovary syndrome (PCOS), fertility issues and used as a natural medication to conceive. The species Melia dubia from the Meliaceae family is mainly distributed in India, Srilanka, Malaysia, Australia and Angola [8]. Malabar neem and Malai vembu are other names of Melia dubia. Every part of the plant is being used as traditional herbal medicines, such as anthelmintics, treatment of leprosy, eczema, asthma, malaria, fevers and veneral diseases [9] cholelithiasis, acarisis and pain [10] Piperidinedispay important biological properties like antiviral activity [11] antidepressant effects [12] cytoxic activity [13] antimarial activity [14] and also to encourage conception in women [15]. The phytochemical components of Melia dubia leaf extract, such as Linolenic acid, Palmitic acid, Caryophyllene, Humulene, Aromadendrene, Probucol, Germacrene-D, Phthalic acid 6-ethyl-3-octyl, Butylated hydroxytoluene, Piperidine [16].
Case Report

In this case, a 25 years old village woman gets married and does not conceive as expected by her husband and relatives even after 7 months of her marriage, she has polycystic ovary syndrome along with irregular menstrual cycles. She had been advised to drink Melia dubia leaf extract in empty stomach to recover from the ovary syndrome. One morning, she consumed a leaf extract about 200 mL in her empty stomach, after an hour she was taken to hospital with the symptoms of vomiting, breathing difficulty, fatigue, and unconsciousness. But she was brought dead when examined by the Medical practitioner. During the autopsy, the deceased stomach contains green liquid and multiple petechial hemorrhages were found all over the surface of the lungs. The viscera of deceased was received in Regional Forensic Science Laboratory by the corresponding author to rule out any poisoning substances, with the history of Melia dubia leaf extract consumption.

Forensic Examination

The viscera of the deceased was received here were (i) part of stomach and intestine along with their contents (Figure 1) (ii) part of liver and kidney, Preservative (saturated sodium chloride solution) used. In general, the Forensic toxicologist rules out various poisons like metallic poisons(Arsenic, Antimony, Bismuth, Mercury, etc.), cyanide, yellow phosphorous, Zinc phosphide, Aluminium phosphide, pesticides(organophosphorous, organochlorinated, carbamate and pyrethroid compounds) and plant poisons(Nerium oleander, Cleistanthus collinus , Calotropis gigantea, Strychnos nux-vomica, Abrus precatorius, etc.). In this case all the above said poisons were ruled out, and also conducted chemical analysis for Melia dubia phytochemical constituents using the following methods.

Thin layer chromatography

The macerated visceral samples about 20g were soaked in 100ml of rectified spirit and 5ml of acetic acid for 12 hrs, the ethanolic extract was filtered and dried, the dried residue was dissolved using hot water and extracted with Chloroform. This chloroform extraction was taken for further chemical analysis. In similar way the Melia dubia leaf extract was taken. Thin-layer chromatography technique (TLC) was used in this case, TLC is a very useful, rapid, and inexpensive chromatographic method. It is especially suitable for screening tests, in which pretreatment of the analytes can be avoided, even with impure samples [17]. There is no specific TLC solvent system (mobile phase) for Melia dubia chemical constituents in literature, but I have used the following two solvent systems based on trial and error. Melia dubia leaf extract and visceral extract were spotted on the TLC plate using micro capillary tube. The plate was developed with the two solvent systems (i) Dichloromethane: Cyclohexane: Methanol [18:1:1] and (ii) Ethyl acetate: Methanol: Water [8:1:1] as mobile phase in a presaturated TLC chamber, then it was removed from chamber and dried at room temperature. The TLC plate was viewed under UV short wave (254 nm) in UV cabinet, but the result was negative for the visceral extract corresponding with the reference sample of Melia dubia extract. So, we extended our analysis for other possible plants from Meliaceae family. We have taken Melia azedarach as one of the reference sample, the above said TLC method was carried out, TLC plate was viewed under UV short wave (254 nm) in UV cabinet (Figure 2a & 2b) The Rf values of Melia azedarach phytochemical constituents’ spots matched with visceral extract chemical constituents. The intensified forensic toxicological examination detected the chemical constituents, which present in the viscera of deceased was Melia azedarach phytochemicals.

Gas chromatography mass spectrum

The visceral extract further examined using Gas chromatography – Mass Spectrum, it shows the major peaks for Quercetin, Palmitic acid, Campesterol, Phytol and many other minor peaks. The reference standard of Melia azedarach extract shows the same peak. Both the methods were revealed that the active constituents of Melia azedarach was present in the visceral samples of the deceased.
Discussion

Melia dubia and Melia azedarach both species are from the same Meliaceae family. The identification of the species are complicated for the common people using the similar type of local names (Tamil language: Malai vembu & Kattu vembu). Major phytochemical constituents are different in both the species. According to this case she unknowingly consumed Melia azedarach leaf extract instead of Melia dubia, though both species have different morphology. Melia dubia leaves are compound with toothed leaflets, greenish-yellow flowers Figure 3 [18]. Melia azedarach leaves are twice-compound with oval to elliptical shaped leaflets, pink to lilac flowers Figure 4 [19]. Melia azedarach is a well-known ethnomedicinal tree used in Ayurveda. Its use in the traditional folk medicine is also well documented [20]. Though, Melia azedarach has many medicinal values, it becomes toxic if it is consumed in excess quantity. Toxicity study on rats and mice using Melia azedarach extract, higher concentration of the extracts depresses markedly the respiratory center by both routes, oral as well as parenteral. This may be due to the direct action on the respiratory centers. In doses where mortality was observed it was noted that death occurs due to the cessation of respiration.

Conclusion

Forensic toxicological examination revealed that the viscera of deceased containing chemical constituents of Melia azedarach, due to the detection, this case turned from Melia dubia poisoning to Melia azedarach fatal poisoning. Because of the toxicology report this case become very rare case of accidental ingestion of Melia azedarach leaf extract. Create awareness among the people about its life-threatening poisonous effects and to prevent accidental ingestion. Forensic toxicologist has major role in establishing the type of poisoning compound from the visceral samples.

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