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A COMPREHENSIVE REVIEW ON CYTOTOXIC POTENTIAL OF ATROPA ACUMINATA

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Abstract
Atropa acuminata it contains various bioactive compounds, like particularly tropane alkaloids, it contributes to its therapeutic properties. Different alkaloids found within this plant include; atropine, hyoscyamine, and scopolamine demonstrating sedative, anti-spasmodic, and antidote properties all of these data are collected through some search-engines like Google Scholar, Science Direct, PubMed, Research Gate, etc. The therapeutic effects of the plant are further enhanced by the other classes of phytochemicals the plant contains, including flavonoids, tannins, saponins, anthraquinones, and phytosterols. Because of the plant's extensive class of phytochemicals and long history of traditional use, scientific research has looked into a variety of impacts of the plant. The phytochemical components of plants have been the subject of extensive scientific research about their anticancer properties. Information for this study was acquired from many credible scholarly sources. All sources of information were employed to identify the technique in which atropine can be produced from dried leaf and root elements of the plant. The study also examined several extraction methods for pharmaceuticals, the effects of induced mutagenesis via sodium azide and ethyl methane sulphonate (EMS) on the yield of alkaloids, and the anticancer properties of plant extract through *in vitro* testing. Atropine was isolated via an acid-base extraction process followed by purification and chemical, chromatographic, and non-aqueous titration methods were used to identify and quantify the isolated compound. Using the MTT test, the cytotoxicity of the ethanol extract was evaluated on HEp-2 human epithelial carcinoma cell lines. The findings confirmed successful isolation and purity of atropine. The extract exhibited strong, dose-dependent cytotoxic action, suggesting intriguing anticancer potential, and mutagenesis showed promise in boosting alkaloid synthesis.

1. Introduction

In the past two hundred years many drugs have come from plants and those, plus thousands of other drug plant extracts, are still used as part of a doctor's main toolkit to combat illness all throughout the world. The researcher must correctly identify, isolate, characterize, and assess the biological activity of indigenous molecularly-based materials in order to seek for novel, safer anticancer medicines. There are many anticancer drugs and drug combinations available to the practicing oncologist today [1] In India, *Atropa acuminata* is a widely used medicinal herb known by its common name, "Indian Belladonna". Indian farmers who have a market for the crop's raw elements can make it. The plant contains several significant alkaloids, including as belladonna, hyoscyamine, and atropine [2]. These are all used to treat convulsive episodes, reverse the toxicity of some toxins, and act as sedatives or antispasmodics. When used in conjunction with a homeopathic treatment, this plant helps patients with breast cancer manage the skin responses brought on by radiation therapy [3,4]. The aerial parts of the plant are used as an antispasmodic and to treat a wide range of conditions, including irregular menstrual

Figure 1: *Atropa acuminata* (Source: e-flora of India)

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cycles, acute infection, and inflammation. Additionally, the use has been historically used for treating issues related to anxiety, arthritis, asthma, bedwetting, bowel disorders, chicken pox, colds, colitis, conjunctivitis, dental problem, diarrhea [3,4]. In addition to being processed to treat diverticulitis, this plant can also be used as a diuretic[5,6,7], earaches, encephalitis, eye conditions (especially pupil dilation), fever, flu, glaucoma, gout, hay fever, hemorrhoids, hyperkinesis, other forms of inflammatory diseases, kidney stones, measles, motion sickness, mumps, muscle/joint pain, muscle spasms, sickness and vomiting associated with pregnancy, organophosphate poisoning, nerve pain, Parkinson's disease, pancreatitis, peritonitis, rashes, scarlet fever, sciatica and many related conditions [8].

Because of the long history of use for many different illnesses and other medical problems [9], this plant has been selected for pharmacological evaluation for many reasons. It is reported to contain several flavonoids including quercetin and kaempferol along with robinin and clitorin. Starch, tannins, resins, and anthocyanins are also contained within this plant material [10, 11]. Through phytochemical analysis, It's been confirmed that this plant contains many types of phytochemicals including saponins, carbohydrates, alkaloids, proteins, anthraquinones, and phytosterols. In conventional medical systems, the uses of this plant range from that of a diuretic, anthelmintic, antidiabetic, antipyretic, and brain tonic [12, 13].

Figure 2: Indian Belladonna (Source: e-flora of India)

The experimental protocol of this study follows procedures for *in vitro* assessing the harmful consequences of an unknown compound by "counting the quantity of viable cells per milliliter" after staining viable cells with a "vital stain" using the MTT assay [14]. The MTT test has been recently developed as a quick and universal biological assay method for assessing bioactivity of natural products. This technique is useful in detecting cytotoxicity of natural goods as well as determining several types of pharmacological activities including anticancer, antiviral, and pesticide [15, 16]. The plant *Atropa acuminata* is considered to have bioactive properties and is applied to diseases such as chronic bronchitis, dropsy, goiter, leprosy, mucous disorders, weak vision, skin diseases, sore throat, and tumors [17].

Cancer cell has uncontrolled growth and suppresses cell death, known as apoptosis. The overall development of multicell organisms and their capacity to maintain homeostasis through cell proliferation is a significant part of how multicell organisms develop [16,17]. There are several chemotherapeutic drugs that is usable to promote apoptosis in cancerous cells, therefore promoting improvement in the way cancer is affected and treated through additional advancements in the fight against cancer [18,19]. Therefore, plant resources from India are an abundant and diverse source of potential anticancer drugs [20].

2. Safety assessment through *in-vitro* and *in-vivo* experiments

The cytotoxicity of the ethanolic leaf extracts of *Atropa acuminata* was evaluated *in vitro* by J. Jayakanthi et al. using the MTT assay against the HEp-2 cell line (2011) [21]. The HEp-2 cell line is a frequently used human laryngeal cell line for carcinoma in anticancer research. The MTT assay, colorimetric analysis for viability decreased the yellow tetrazolium base ion MTT to purple formazan crystals. This is facilitated by the mitochondria's succinate dehydrogenase activity, which provides a small amount of the viability of the cell, and the solubilized formazan which is detectable spectrophotometrically. The results established that the ethanolic extract considerably reduced cell viability in dose dependent manner, indicating its cytotoxicity on proliferating cancer cells. This cytotoxicity was attributed to the inhibiting effect on cellular activity and mitochondrial metabolism, which significantly lowers the proportion of cancer cells that are alive. Cell viability was observed to be reduced to less than 50% when the extract concentration was approximately 0.5 mg/ml, indicating a substantial antiproliferative effect. The plant's crude extracts are physiologically active, as indicated by the $IC\beta_0$ values, which fall between 25 42 to 79 66 µg/ml. These results imply that the ethanolic extract may prevent cancer cells from proliferating and growing by causing damage to their mitochondria [21] .

In another pharmacological study (2015), different solvent extracts of *Atropa acuminata* were screened. Various extracts, including aqueous, methanolic, chloroform, and hexane, were prepared using different parts of the plant (e.g., stems, roots as well as leaves) [22]. In accordance with the plant material and type of extracts used for the

extraction (e.g., methanol, chloroform, hexane), different groups of phytoconstituents (e.g., phenolics, flavonoids, and alkaloids) would be isolated, with polar solvents like methanol providing a superior means for extracting phenolics, flavonoids, and alkaloids. The various extracts were subjected to preliminary cytotoxicity (i.e., cancer cell) screening methods using the brine shrimp lethality bioassay (i.e., a simple, fast, inexpensive way to predict the general cytotoxicity and plant extracts' bioactivity). Enzyme inhibition assays were also performed on these extracts for evaluation of the biological activity at molecular level. The methanolic extract demonstrated the greatest cytotoxic activity among the extracts, which could have been attributed to the presence of more bioactive compounds in that extract. There are also several other studies on Potential anticancer effects of additional medicinal herbs indicating that methanolic extracts will provide lower IC_{50} values and higher anticancer activity compared with other solvent extracts because the active metabolites are better extracted with polar solvents (like methanol). The elevated level of lethality exhibited in the brine shrimp lethality bioassay referred to the active cytotoxic phytoconstituents which must be presented within the samples that could have applications as anticancer, pesticidal, or pharmaceutical products. The data also suggest that solvent polarity has a major influence on extraction efficiency and additionally on phytochemicals' biological activities [23,24].

Phytochemical studies and investigation of *Atropa acuminata* (2020-2021), including tropane alkaloids, flavonoids, tannins, glycosides, and compounds with phenols [25,26]. Phytochemicals, like those present in this plant, perform numerous important roles in delivering pharmacological advantages, such as anticancer, antioxidant, antimicrobial and anti-inflammatory properties. Tropane alkaloids are the primary class of alkaloids present in *Atropa acuminata*, such as scopolamine, hyoscyamine, and atropine, which exert their biological effects by acting on the brain and spinal cord and by modulating cellular signaling pathways. The method by which how these alkaloids exert cytotoxic effects may involve inhibition of DNA replication, disturbance of the potential of the mitochondrial membrane and change in the course of the cell cycle. Additionally, flavonoids and phenolic compounds have antioxidant qualities due to their ability to scavenge reactive oxygen species (ROS). Therefore, they can protect cells against oxidative damage from excess free radical activity [27]. Additionally, studies have demonstrated that phytochemical-rich extracts from *A. acuminata* can modify pro- and anti-apoptotic proteins and activate caspases, among other apoptotic signalling pathways. The pharmacological actions of *Atropa acuminata* as an anticancer agent are influenced by the presence of both alkaloids and antioxidant chemicals in distinctive combinations [28].

In recent years, researchers have investigated application of the extracts from *Atropa acuminata* (commonly named Indian Belladonna), evolving eco-friendly green nanotechnology and for creating functionalized metal nanoparticles (2020) [29]. Applications of the plant extracts to reduce metal ions to nanoparticles (normally) [for example: silver nanoparticles (AgNPs)] may be another alternative to using chemicals. Selection of the characterizing techniques (such as UV-visible spectroscopy, transmission electron microscopy (TEM), and Fourier transform infrared (FTIR) was successful in confirming the size, shape, and functional groups of the biosynthesized nanoparticles, which then subjected to anticancer assays using HeLa cell lines and *in vitro* techniques. Interestingly, the cellular immune defense of the nanoparticles was greater than the crude plant extract because to a rise in surface area, improvement of cellular uptake, and thus, the bioavailability of the active phytochemical compounds. Normal cells show the selection of morphological changes typical of apoptosis, like cell shrinkage, but are less able to divide. These findings suggest the existence of significant potential applicable for nanotechnology to greatly increase the pharmaceutical effects and could be a useful method of treating cancer [30].

Recent studies also demonstrates that extracts of *Atropa acuminata* exert cytotoxic effects through changing cellular metabolism pathways and creating oxidative stress conditions in cells of tumours**.** The plant contains chemicals that are bioactive can elevate Oxygen species that are reactive levels inside cells and cause oxidative damage of important biomolecules, including protein, DNA, and lipids. High ROS disrupts mitochondrial integrity leading to cytochrome-c release and the initiation of interstitial apoptotic processes. This process leads to programmed cell death (apoptosis), which is a target mechanism for anticancer therapy. Furthermore, oxidative stress can stop tumour cells from proliferating by stopping the M phase at particular stages. This mechanism of ROS-evoked cytotoxicity has been described for several other plant derived compounds, demonstrating how oxidative stress is essential for destroying cancer cells**.** Therefore, *Atropa acuminata*'s anticancer activities depend critically on redox balance modification [31].

In fact, chemical studies suggest that *Atropa acuminata* is a particularly rich Tropane alkaloids' source exhibiting substantial cytotoxic and antiproliferative activities alongside other bioactive phytochemicals, such as flavonoids and phenolic compounds. The studies

suggested *in vitro*, using different assays (e.g., MTT, brine shrimp lethality, and enzyme inhibition tests) Its component can cause apoptosis on a number of lines of cancer cells while stopping the proliferation of cancer cells. Most studies are nevertheless limited to preliminary screening and in-vitro models. Further sophisticated research is needed, such as bioassay-guided fractionation to separate certain active chemicals, molecular studies to elucidate pertinent signalling channels, and in-vivo trials to characterize pharmacokinetic behaviour, toxicity profiles, and therapeutic effectiveness. Its potential must pass clinical validation in advance of practicable in mainstream medicine. In this context, thorough research may aid in finding the new phytochemicals with anticancer activity which could produce drug candidates that are more efficient and safer [32,33,34,35].

Table1: Different *in-vitro* and *in-vivo* studies for cytotoxicity assay using cell lines of *Atropa acuminata.*

Sl. No	*In-vitro* and *In-vivo* Model	Cell Line	Outcomes	References
1	Anticancer & Apoptosis Assays [2020-2021]	Rodent tumor cell line	Inhibit cell death regulators and reduce tumor progression	[25,26,27,28]
2	*In-vitro* Cytotoxicity Assays (Green Nanotechnology Study) [2020]	HeLa cell line	Increase anticancer effect and cell death	[29,30]
3	Brine Shrimp Lethality Assay & Enzyme Inhibition Assay [2015]	No human cell line	Safety screen and target protein inhibited	[22,23,24]
4	MTT Assay [2011]	Hep-2 cell line	Cytotoxicity decreased at dose dependent manner	[21]

3. Discussion

Several *in-vitro* studies confirm the high cytotoxic and antiproliferative potential of *Atropa acuminata*. The significant decrease in a cancerous cell viability seen as a dose-related possibility, especially observed in the context of MTT assays, support this as the extract of plant material has been discovered to alter fundamental cellular mechanism such as mitochondrial activity and biochemical processes [21,22]. Then reported IC_{50} for the various extracts indicates moderate to strong cytotoxic efficacy further indicating that it could be a source as cancer preventing substances [21,23]. Interestingly, the increased activity of methanolic extracts highlights that the polarity of solvent directly influences the efficient extraction of biologically active constituents especially Both phenolic and alkaloid chemicals [23,24].

Numerous phytochemical studies have concluded that *Atropa acuminata* has notable amounts Among the alkaloid's tropane such as scopolamine, hyoscyamine, and atropine, along with flavonoids, tannins and additional phenolic compounds [25,26]. This class of bioactive compounds is shown to have cytotoxic activities through several ways, including the potential of the mitochondrial membrane disturbance, oxidative stress induction and apoptotic pathway activation [27,28]. Producing reactive oxygen species is significant for death induction where in reactive oxygen species causes damage to cellular macromolecules that lead into intrinsic apoptosis [31]. Flavonoids and phenolics also participate in controlling antioxidant defense mechanisms, attenuating signaling pathways connected to apoptosis and cell division [27,28]. The green nanotechnology in recent developments has developed the therapeutic potential of these compounds in *Atropa acuminata*. Green synthesized silver nanoparticles have demonstrated a stronger cytotoxic effect than the crude extracts in lines of cancerous cells [29,30]. This increased efficiency can be associated with phytochemicals enhanced surface area and bioavailability, which is internally conducted through various cells. Apoptosis is an important operating principle confirmed by morphological changes seen in treated cells, including cell shrinkage, membrane blebbing and nuclear condensation [30]. Therefore, these findings imply that nanotechnology-albumin based systems can improve the delivery and efficacy of herbal products [30].

Atropa acuminata has anticancer activity consistent with the wider activity of other medicinal plants. The anticancer activity of natural products has been a source of medicinal agents for an extended period of**.** Vincristine, vinblastine, and paclitaxel, are among the most significant natural product derived anticancer drugs [21,22]. The anticancer activity of plant products involves many different mechanisms, such as suppression of cell division, the apoptotic activation and modifications to the PI3K/Akt, MAPK, and NF-κB signalling pathways [27,28]. The multi-targeted characteristics of these mechanisms can be most beneficial in overcoming drug resistance and improving cancer therapy [32].

Polyphenolic compounds extracted from plants that are herbal, like flavonoids, may protect against some diseases by inhibiting free radicals, decreasing elemental imbalance, controlling angiogenesis and cell cycle events. Quercetin, kaempferol, catechin, and rutin are a few of these substances [25,26,27]. It's been showed that these substances benefit pancreatic, breast, and cell lines for prostate cancer [25,28]. The most important aspect is that cancer activity frequently leads through a combined action of the extract's several components as opposed to one active principle, providing for the concurrent targeting of several carcinogenesis pathways.

The most important challenge is that most of the information on *Atropa acuminata* that was published depends on initial observations. In this regard, no in-vivo investigation has been done on pharmacokinetics, toxicity, or efficacy. Moreover, the issues of rapid metabolism of active compounds, insufficient bioavailability, and variability still remain. Consequently, the adverse effects of

conventional chemotherapy, including myelosuppression, anemia, and related immunosuppression, indicate the critical requirement for better alternatives or additional medications [33].

The abundance of phytochemicals derived from *Atropa acuminata* supports the current scientific inquiry of this plant to complete the necessary bioassay-guided purification of active compounds, as well as the molecular and clinical studies that will ultimately result in the identification of the molecular methods of action and assurance of security of any resulting anticancer treatments [34,35].

4. Conclusion

Because of its high bioactive phytochemical content, particularly flavonoids, Phenolic substances together with tropane alkaloids, *Atropa acuminata* shows considerable cytotoxic and anticancer potential. Strong antiproliferative action is being repeatedly determined using in vitro research using assays including MTT and brine shrimp lethality, and demonstrate a dose-dependent decreases in proliferation of cancerous cells. Several processes, like production the elemental imbalance, Apoptotic pathways are activated and mitochondrial activity is disrupted, are possibly the cause of the observed cytotoxic effects. In addition, the efficiency of different solvent extractions—particularly methanolic extracts—highlights the significance of extraction techniques in isolating potent bioactive substances. The anticancer capacity of *Atropa acuminata* is being further enhanced by major developments in green nanotechnology, especially growth of the plant-mediated silver nanoparticles, that indicates increased bioavailability as well as targeted action of phytochemicals. Despite these promising results, there is a lack of *in-vivo* and clinical data, and present-day studies are generally restricted to experimental in-vitro studies. As a result, it is crucial to implement further investigations on bioassay-guided isolation of active compounds, detailed molecular mechanism studies, and comprehensive toxicity and pharmacokinetic analyses. As a natural source, *Atropa acuminata* has a great deal of room for expansion of the recently created anticancer medications. However, to identify its safety, performance, and therapeutic significance in modern medicine, thorough scientific validation through complex experimental and clinical studies is necessary.

5. Authors' contribution

A.B - Writing original draft conceptualization and reviewing; S.M & R.R - Writing & editing; R.D & J.P.M - Reviewing, conceptualization, editing and validation. All authors reviewed properly and approved the final version of the manuscript.

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