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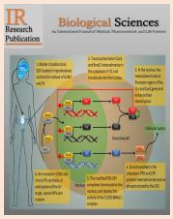

Evaluation of the anti-cancer study of chloroform seed extract of Datura stramonium on EAC cell lines

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ABSTRACT

Including cancer, native medicinal herbs have long been employed for illness prevention and treatment. Herbal supplements are promoted for their wide range of biological benefits, from preventing cancer cell proliferation to boosting the immune system. *Datura stramonium* L., a member of the Solanaceae family, is a common and easily accessible shrub that may grow to a height of 6 feet on fertile soil and a width of 3 feet in poor soil. The purpose of the current study is to determine whether or not a Chloroform seeds extract of *Datura stramonium* may inhibit cancer caused by the EAC cell line in albino mice. The collection of plant material and subsequent chloroform extraction using Soxhlet equipment made this investigation possible. The percentage of cell growth inhibition, tumor weight, packed cell volume, the average increase in body weight, change in food intake, mean survival time (MST), the percentage increase in life span (% ILS), and viable and non-viable tumour cell count were all significantly different between the 100 mg/kg and 200 mg/kg doses of *Datura stramonium* in E.A.C. bearing mice. Here there is therapeutic promise for treating illnesses generated by oxidative stress, and the current investigation confirms that *Datura stramonium* L. is a rich source of considerable natural oxidants with anticancer agents.

Introduction

Plants have historically played an important part in the treatment of human injuries and illnesses all across the world. There has been a rise in the for medicinal plants in both developed and developing nations as people become more aware of the benefits of using natural remedies. Both the ancient and the current medical systems rely heavily on herbal remedies [1].

Datura stramonium L., often known as the "Devils Trumpet," "Thorn," "Apple," "Locoweed," or "Jimson weed," is a wild-growing Solanaceae plant with a huge, coarse shrub that grows to 34 feet tall and 6 feet wide on rich soil [2,3]. The root is big, white, with a taproot system that produces numerous fibres, whereas its stem is either green or purple in colour, without hair cylindrical, upright, leafy, smooth, and forked [3]. At each branch

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junction, leaves, and a solitary, upright bloom emerge [3]. The fruits mature from a green to a brown color, and the seeds may be extracted by cutting them into quarters. In terms of appearance, the seeds are dull, asymmetrical, and black; the outermost layer may be pitted or somewhat reticulated [3]. Ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruising and swellings, fever, asthma and bronchitis, and toothache are only some of the conditions for which this plant is utilized in Eastern medicine, particularly Ayurvedic medicine [3]. When combined with palm oil, the seeds are used to treat severe instances of bug bites and stings in Nigeria [4]. Misuse of this plant for non-therapeutic purposes, despite its long history of usage in traditional medicine, has led to toxic symptoms. The deliriant (anticholinergic) tropane alkaloids found throughout the plants, including the very toxic atropine, hyoscyamine, and scopolamine [2]. Recreational users who consume the plant for its psychotropic properties have a significant risk of deadly overdose, and several hospitalizations have been documented [5]. *Datura stramonium* intoxication is said to cause delirium, high body temperature, rapid heart rate, irrational behavior, severe mydriasis, intense photophobia that might continue for days, and profound amnesia [6]. Poisoning symptoms appear 30 minutes to an hour after smoking the herb and typically last 24 to 48 hours, but can persist for as long as 2 weeks [7]. Neurotoxic symptoms such as disorientation, agitation, mydriasis, and hallucinations were recorded by Adegoke and Alo [8] in the case of two children referred to the Hospital in Ekiti State (Nigeria) after ingesting extract of *Datura stramonium*. When administered intravenously, physostigmine can reverse the effects of anticholinergic poisoning [6]. Caspian Sea areas were likely the original source of *D. stramonium*, from which it moved to Europe in the first century AD. Currently, it may be seen growing in garbage dumps across Europe, Asia, the United States, and South Africa. *D. stramonium* is grown in several locations all over the world [9], including Germany, France, Hungary, South America, and others.

Material and methods

Plant material and extraction

Datura stramonium was gathered from Nehru Garden in Jaipur, Rajasthan, for anticancer research against the E.A.C. cell line strain. Botanical Survey of India, Jodhpur, and Rajasthan confirmed *Datura stramonium*. 100 g of air-dried powdered seeds was defatted with petroleum ether under reflux, then witted with 150 ml of NH4OH (25%, m/m) for 4 h, then extracted to exhaustion with CHCl3 using a soxhlet apparatus for 6 h. The organic extract (free alkaloids + lipophilic impurities) was mixed three times with 150 ml 2% aqueous sulphuric acid (m/m). The acid extracts (alkaloids salts) were treated three times with 50 ml NH4OH (25%, m/m) to pH 10 to release the free alkaloids, which were extracted with 150 ml CH2Cl2, dried with Na2SO4, and concentrated under reduced pressure to yield crude alkaloids. This extract yielded 37.5% (w/w).

Experimental animals

Healthy adult Swiss albino mice of both sexes weighing 150-250 g were procured and the experimental protocol was approved by Institutional Animal Ethics Committee CPCSEA No. - 2005/PO/RcBT/S/18/CPCSEA. Animals were housed under standard conditions of temperature (24±2˚C) and relative humidity (30%-70%) with a 12:12 light: dark cycle. The animals were given a standard pellet diet and water ad libitum.

Chemicals

The chloroform extract of Datura stramonium was dissolved in distilled water and administered to the test groups. Bleomycin was purchased from Sigma Chemical Co, USA.

Phytochemical Screening

Preliminary Phytochemical investigation of chloroform extract of Datura stramonium was carried out for the existence or deficiency of phytoconstituents like-Alkaloids, carbohydrates, flavonoids, glycosides, saponins, sterols, Anthocyanins, Terpenes as well as tannins. Phytochemical results have been shown in Table 1.

Acute toxicity studies

The acute toxicity research was conducted using a standardized protocol that calls for a certain number of mice to be used at each stage, as outlined in OECD (Organisation for Economic and Cultural Development) 423 standards from 2006 [10]. Swiss albino male and female young adults (30-35 g) were employed in this research. The next day's dosage experiment was conducted on fasting mice. The dosage was established after measuring the weight of the mice before and after they fasted. A total of 36 mice were randomly assigned to one of six groups (n=6) and given either 250, 500, 750, or 1000 milligrams per kilogram (mg/kg) of plant extract. Six groups of 5 mice each were given intraperitoneal injections of the study extract diluted in distilled water at dosages of 250, 500, 750, 1000, 1500, and 2000 mg/kg p.o. body weight. Mortality rates were recorded after 24 hours to determine LD50. Individual mice were analyzed during the first

24 hours after dosage, beginning 30 minutes after administration.

Evaluation of In-vivo anticancer activity

Six groups of mice (n=6) were weighed and split up into different conditions. Except for the normal saline group, all other groups' mice received an i.p. injection of EAC cells ($1x10^6$cells/animal). This was considered to be Day 1. From Day 1, patients kept receiving bleomycin and a selected plant extract. On day 10 following the final dosage, 24 hours later, four mice from each group were slaughtered, while the others were retained to evaluate the longevity of tumor hosts. Tumor cells were obtained by repeatedly injecting phosphate-buffered saline (PBS) into the abdomens of sacrificed mice [11].

The groups as well as the design of the experiment will be as follows for each plant extract:

Group I: Normal saline (5ml 0.9% w/v)/kg b.wt. i.p.)

Group II: EAC ($2x10^6$ cells/mice i.p.) x Normal saline (5 ml/kg b. wt. i.p.)

Group III: EAC ($2x10^6$ cells/mice i.p.) x Plant extract (50 mg/kg b. wt. p.o.)

Group IV: EAC ($2x10^6$ cells/mice i.p.) x Plant extract (100 mg/kg b. wt. p.o.)

Group V: EAC ($2x10^6$ cells/mice i.p.) x Plant extract (200 mg/kg b. wt. p.o.)

Group VI: EAC ($2x10^6$ cells/mice i.p.) x Bleomycin (0.3mg/kg b. wt. p.o.)

Tumor growth responses

By analyzing parameters such as inhibition of cell growth, the effect of plant extract on both tumor growth and host survival time will be determined. Tumor volume, Increase in average body weight (g), change in dietary consumption, average survival time (MST), and percentage increase in life expectancy (% ILS). Tumor cell count, including viable and nonviable tumor cell counts [12].

Cell growth inhibition

The rodents were sacrificed ten days after the E.A.C. Comparing the viable tumor cells per mouse in the mixed group to those in the control group [10]. The inhibition of cell growth was computed using the following formula:

% cell growth inhibition = (1– Tw/Cw) ×100

Where,

Tw = Mean of number of tumor cells of the mixed group of mice

Cw = Mean of number of tumor cells of the control group of mice.

Tumor volume

The peritoneal ascitic fluid was collected after the mice were dissected, and their weight and volume were promptly recorded using a graduated centrifuge tube [13].

Tumor-packed cell volume

The ascitic fluid was extracted from the peritoneal cavity, and the packed cell volume was determined by placing the fluid in a graduated centrifuge tube and centrifuging it at 1000 rpm for 5 minutes [13].

Body weight analysis

Mice from the mixed group and the control group were weighed at the outset of the experiment (day 0) and on every fifth day during the treatment period, and their body weights were calculated on day 15 [14].

Changes in food intake

Feed consumed by 6 mice per cage per week = Total amount of feed offered during the week (gm) - Feed remaining on the final day of the week (gm). Individual Mouse food consumption per week = 6 rodents per cage per weekday. Feed consumed by a single mouse per day = Feed consumed by a single mouse per week [14].

Tumor cell count

A WBC pipette was used to collect the ascetic liquid before it was diluted one hundred times over. Following that, a small drop of the diluted ascitic fluid is deposited on the Neubauer counting chamber, and the total number of tumor cells in the 64 small squares is meticulously enumerated [13].

Viable as well as nonviable tumor cell count

The ascetic tumor cells will then be stained with the dye trypan blue. The cells that did not absorb the dye were viable, while those that did absorb the dye

were nonviable. These viable and nonviable cells were both tallied. The ascetic fluid was drawn into a WBC pipette and diluted numerous times. Then, a drop of the diluted suspension was applied to the Neubauer counting chamber, and the cells were stained with Trypan blue (0.4% in saline) dye. Those cells that did not absorb the dye were viable, while those that did were nonviable. Both viable and non-viable cells were tallied [15].

Cell count = (Number of cells × dilution factor) / (Area × thickness of liquid film)

Percentage increase in life span

We monitored the effect of plant extract on tumor growth by documenting mortality daily for six weeks and calculating the percentage increase in median survival time (%IMST). At least a 25-30% increase in MST was considered indicative of effective anticancer activity.

IMST (%) = [(Median survival time of mixed group / Median survival time of control group)-1] x100

Median Survival Time (MST) = [Day of 1st Death x Day of last Death] /2

Statistical Analysis

The data were represented as a mean ± standard error of the mean (SEM). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests where $P<0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software.

Results and discussions

Antioxidants are substances that scavenge and destroy free radicals, protecting cells from harm. Some antioxidants are produced by the body and are responsible for neutralizing free radicals; these compounds have another name, "free radical scavengers." Dietary antioxidants are a frequent term for exogenous antioxidants. Antioxidants help the body by scavenging harmful free radicals from the circulatory system. Antioxidants may be found in abundance in foods including fruits, vegetables, and grains. Oxidative stress is a fundamental phenomenon in chronic illnesses; it is the result of an imbalance between pro-oxidants and antioxidants in the body. More than a hundred disorders, including Alzheimer's disease (AD) and the aging process in general, are now thought to be linked to oxidative stress. Antioxidants play a crucial role in halting the progression of cancer, aging, and other disorders by protecting cells from harm. The current research looked at *Datura stramonium's* anticancer potential and preliminary phytochemical screening.

Preliminary phytochemical screening

Preliminary phytochemical tests were carried out for the presence or absence of phytoconstituents like- Alkaloids, Carbohydrates, Flavonoids, Glycosides, Saponins, Sterols, Anthocyanins, Terpenes, and Tannins (**Table 1**).

Effect of acute toxicity studies

At doses up to 250 mg/kg of plant extract, the test animals showed no significant changes in behavioral patterns like trembling, diarrhea, salivation, breathing, impairment in food intake, water consumption, postural abnormalities, hair loss, sleep, lethargy, restlessness, or physical appearance like eye color, mucous membrane, salivation, skin/fur effects, body weight, injury, compared to the control after 24 hours of general anesthesia.

Effect of In-vivo anticancer activity

Tumor volume and viable cell count in treated groups were much lower than in E.A.C. control mice, although the number of nonviable cells was significantly higher. **Tables 2** and **3** display the effects of plant extract on the median survival time of E.A.C.-bearing mice, suggesting that plant extract can considerably lengthen the mean survival time of E.A.C.-bearing mice compared to E.A.C. control mice.

Conclusion

The current research demonstrates that an extract of a certain herbal plant possesses antiproliferative action against cancer cell lines in vitro, which is very encouraging. Meanwhile, plant extracts can slow the formation of tumors in mice that have had EAC transplanted into them, even in the presence of liver toxicity that can be reversed. According to the findings of this study, the chloroform fruit extract of *Datura stramonium* is a possible source of naturally occurring antioxidants and considerably inhibits free radicals in a dose-dependent manner. This was determined by analyzing the data. The mechanism of action of components in disease prevention will be validated by subsequent research based on clinical trials and animal models of the illness. Plant extracts exhibited selective cytotoxicity against tumor cells but did not affect human primary cell cultures. In an experimental setting using EAC cell

Table 1. Preliminary Phytochemical tests of Chloroform fruits extract of Datura stramonium fruits (CFEDS).

Phytochemical constituents	**Chloroform fruits extract of *Datura stramonium* fruits *(CFEDS)***
Alkaloids	+
Anthocyanins	_
Carbohydrates	+
Flavonoids	+
Glycosides	+
Reducing sugars	+
Saponin	+
Steroids	+
Terpinoids	+
Tannins	+
Proteins	+

+ indicates the presence of compounds; - Indicates the absence of compounds.

Table 2. Effect of Chloroform fruits extract of Datura stramonium (CFEDS) on E.A.C. % cell growth inhibition (in vivo) (n=6) (mean ± SEM).

Treatment groups	**Dose (mg/kg/day, i.p.)**	**No. of E.A.C. cells in mouse on day 6 after tumor**	**% of cell growth inhibition**
Control (E.A.C. cell bearing mice)	-	$(8.99 \pm 0.73)\times10^{6}$	-
Chloroform fruits extract of *Datura stramonium (CFEDS)*	50 mg/kg	$(5.15 \pm 0.35)\times10^{6}$*	42.71
	100 mg/kg	$(3.85 \pm 0.54)\times10^{6}$**	57.17
	200 mg/kg	$(1.92 \pm 0.31)\times10^{7}$***	78.64
Standard Bleomycin	0.3 mg/kg	$(0.91 \pm 0.56)\times10^{6}$***	89.87

*P<0.05, **P<0.01, ***P<0.001, when compared with control.

Table 3. Effect of Chloroform fruits extract of Datura stramonium (CFEDS) on tumor volume, tumor weight, packed cell volume, average increase in body weight, change in food intake, mean survival time (MST), percentage increase life span (% ILS), viable and non-viable tumor cell count in E.A.C. bearing mice.

Parameters	**E.A.C. control**	**E.A.C.+ *CFEDS***	**E.A.C.+ *CFEDS***	**E.A.C.+ *CFEDS***	**Bleomycin**
	(1×10^{6} cell/mouse)	(50 mg/kg)	(100 mg/kg)	(200 mg/kg)	(0.3 mg/kg)
Tumor volume (ml)	5.44±0.31	3.54±0.15*	2.90±0.55*	2.35±0.28*	0.72±0.31*
Tumor Weight (gm)	6.59±0.182	4.39±1.345*	2.85±0.720**	1.56±0.930***	1.56±0.382**
Packed cell volume (ml)	2.59±0.24	1.70±0.37*	1.30±0.16*	0.90±0.18*	0.53±0.12
Average Increase in Body weight (gm)	14.57±0.42	8.3±0.33	7.76±0.98	7.32±0.76	4.80±0.64*
Change in food Intake (gm)	38.88±3.73	44±2.32	42.51±0.68	43.46±0.46**	48.22±1.32***
MST (days)	22.41±0.15	25.71±0.14*	32.08±0.16*	38.61±0.15*	45.70±0.2*
% ILS	-	34.57	53.57	68.41	86.61
Viable cells (x 10^{6} cell/ml)	10.02±0.21	5.02±0.13*	3.02±0.03*	1.04±0.23*	0.51±0.14*
Non-viable cells (x 10^{6} cell/ml)	0.65±0.25	2.43±0.75*	2.23±0.25*	3.19±0.65*	3.33±0.06*
Total cells (x 10^{6} cell/ml)	10.66±0.47	7.44±0.87	5.24±0.27	4.98±0.85	3.83±0.14
Viable %	93.98	67.43	57.56	20.25	13.15
Non-viable %	6.02	32.59	42.46	79.77	86.87

*P<0.05, **P<0.01,***P<0.001, when compared with control.

lines, a plant extract from the *Datura stramonium* plant displayed potent anticancer effects. Based on our findings, whole-plant extracts hold great potential as anticancer medicines.

Contribution of authors

Not Applicable

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Conflict of interest

The authors declare that there are no conflicts of interest.

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