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Aqueous extract of *Acalypha indica* leaves for the treatment of Psoriasis: *In-vitro* studies

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Abstract: Psoriasis is a chronic inflammatory skin disorder characterized by rapid proliferation of keratinocytes and incomplete keratinization. Discovery of safer and more effective anti-psoriatic drugs remains an area of active research at the present time. A431 and B16-F10 cell lines were used as *in vitro* models. In the present study, we aimed at assessing the Anti-psoriatic activity of aqueous extract of *Acalypha indica*. We analyzed the efficiency of *A. indica* leaf extract in inducing cell death and apoptosis in these cell lines. The cell death (Propidium iodide) and apoptosis (Annexin V) was assessed by fluorescence studies and we observed 80% of cell death and 75% of apoptosis in both cell lines. Therefore, this *in vitro* study suggested that the leaf extract is capable of serving as anti-psoriasis agent or compound.

Key words: Psoriasis; *Acalypha indica*; A431; B16-F10 cell lines; Cell death; Apoptosis.

Introduction

Psoriasis is reckoned as an autoimmune and over proliferative skin disorder with accelerated cell growth, altered keratinocyte differentiation and angiogenesis with marked ectasia of blood vessels impacting 1–3% of the world's population [1, 24]. Psoriasis affects both sexes equally and can occur at any age, although it most commonly appears for the first time between the ages of 15 and 25 years. The word Psoriasis is adapted from Greek, where Psora means itch. Around 3% of people around the world have psoriasis and patients commonly have itchy and painful experience. In the psoriatic patients the skin cells proliferate at a high rate and due to which patches appear on the skin [3]. Normally wound healing is a process where skin cells respond to cellular stimulus and start the repair process, but in case of faulty immune or cellular responses the skin regeneration process goes haywire as a result proliferation of skin cells [2].

Psoriasis is a non-contagious, dry, inflammatory skin disorder, which can involve entire system of person [1]. It is typically inherited and primarily characterized by sharply marginated scaly, erythematous plaques that develop in a relatively symmetrical distribution. The scalp, tips of fingers and toes, palms, soles, umbilicus, gluteus, under the breasts and genitals, elbows, knees, shins and sacrum are the most commonly affected areas on our body [2]. This disease is chronic in nature with a tendency to relapse. Psoriatic patients loose skin as flakes called as psoriatic plaques due to rapid and excessive multiplication of epidermis cells which look like fishy skin and finally peels off as exfoliation [4]. The cause for this phenomenon is not completely understood but it is believed that variations in your genes could be a reason [5]. The prescribed synthetic drugs for the treatment of psoriasis are associated with severe side effects, thus, researchers around the globe are searching for new, effective, and safer drugs from natural resources. Psoriasis can range from being a very mild to a very severe condition [6]. There is no cure for Psoriasis at the moment, but it can be well controlled by using a variety of treatments.

The diagnosis is usually based on the appearance of the skin and there are no specific blood tests for psoriasis. In order to differentiate the psoriasis with fungal infections skin biopsy may be needed to confirm the diagnosis [2]. Another way to diagnosis is look for see pinpoint bleeding from the skin when the plaques are scraped. Diagnosis of psoriasis is made easily by clinical examination. Blood tests in psoriasis patients are usually performed to learn the status of T-cells lymphocytes, ESR etc. [21,24].

Around 60-70% of world population is using the traditional medicines isolated from various medicinal plants as the herbal formulations present lesser side effects when administered to control diseases and their secondary complications and not only that the herbal formulations are economical [7,20]. The search for new and safer plants products has tremendously increased form past decade; according to the literature it is known that the three Thai medicinal herbs, namely *Alpinia galanga*, *Curcuma longa* and *Annona squamosa*, possessed anti-psoriatic activity [8,9].

Acalypha indica (kuppaimani) has been part of traditional system of medicine such as Ayurveda and Siddha. *A. indica* belongs to family Euphorbiaceae and it's an annual herb profoundly found all over India and other

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parts near dust bins or road side pavements and hence it is considered as a wild weed plant [6]. The plant parts such of the world [7-9]. The *A. indica* is commonly known as kuppaimani, Indian copperleaf as it's usually found as leaves, roots, seeds are widely been used in treatment of several ailments, most commonly practice in rural regions of India is using the leaf paste for skin diseases [10,11]. The literature review suggests that the *A. indica* has other beneficial effects such as anti-microbial, anti-urolithiatic, wound healing and venom neutralizing activity of organic and aqueous extracts of the leaves and stems[12-23]. Ghani (2003) reported about the chemical composition of leaves which are kaempferol, triacetonamine, acalyphine, acalyphamide, 2-methylanthraquinone, tri-O-methyl ellagic acid, γ-sitosterol, ß-sitosterol, ß-sitosterol glucoside, stigmasterol, n-octacosanol, quinine, tannin, resin and essential oils. The search for the treatment of psoriasis is still on as there is no such convinced treatment available till date. Hence our attempt was to find out if there is any plant product which could help in reducing or curing the skin diseases such as psoriasis and based on the ayurveda reports and literature review, we have chosen *A. indica* leaves for the treatment of psoriasis. In the present study we have taken two dermal cell lines (A431 and B16-F10) as a model for psoriasis and checked the efficiency of *A. indica* in inducing cell death (i.e apoptosis), limiting cell cycle etc.

Materials and Methods

A. indica aqueous extract:

Leaves of *A. indica* are air dried for 4-7 days. Once dried the leaves were grinned into powder and from which 40grams of leaf powder was mixed in 250ml of double distilled water and left for heating at 60°C for 6hr. Once the extract was cooled down, it was filtered using Whatmanns No 1 filter paper. After filtration the extract was lyophilized.

Immortal dermal cell lines:

The A431 and B16-F10 cell lines were procured from NCCS, Pune, India. The cell lines were furthered cultured in our facility using appropriate cell culture media. A431 were cultured in MEM+10FBS media and B16-F10 cell lines.

Dosage and treatment: The dosage concentrations (10-500ug/mL)

Cytotoxicity/Cell death:

Trypan blue dye assay method was performed to evaluate the cytotoxicity potentials of aqueous extracts of *A. indica* in in-vitro conditions. Aqueous extract was dissolved in double distilled water. Different concentrations (100, 300, 500µg/ml) of extract were prepared and exposed to the cell lines for 24 and 48hr and they were incubated at 37°C. Each concentration of the extract was tested in triplicate. All the samples were collected at the end of the experiment. To 10µl sample 10µl of trypan blue dye was added and the number of dead cells was counted in a hemocytometer under a compound microscope. Percentage of cytotoxicity was calculated by the following formula.

% dead cells = Number of dead cells/Sum of dead cells and living cell × 100.

Cell proliferation assay (MTT assay):

MTT assay was used to cytotoxicity efficiency of aqueous extract of *Acalypha indica* leaf powder examined on A431 and B16-F10 cell lines.

Cell viability assay:

MTT assay will help us in determining the viability of the cell lines. 5mg/ml of MTT was dissolved in PBS and vertex it before use. 20 µl of MTT was added to each well of the micro plate and was incubated at 37ºC for 4 hours. After the incubation in order to stop the reaction 100 µl of DMSO was added to each well. The formed formazan was dissolved by shaking the plate and the absorbance was checked at the 570 nm.

Quantification of apoptosis and cell death:

Apoptosis and cell death were determined as described below, using Annexin V Alexa Fluor® 488 conjugate (Apoptosis kit) and Propidium iodide (Cell death assay kit), respectively. The treated cells were cell pelleted by centrifugation and were re-suspended in 200µl of Annexin binding buffer and to approximately 5×10^5 to 5×10^6cells/ml, 10µl of Annexin-V Alexa Fluor® 488, and 5 µl of propidium iodide (PI) was added mixed well and then the mixture was incubated at room temperature in dark for 20 min. Then labeled cell suspension was inserted into the Countess II FL Automated Cell Counter for analysis. The number and percentage of apoptotic and dead cells were assessed by Annexin-V and PI staining, respectively. Unstained cells were used to detect the autofluorescence associated with the cells.

Induced apoptosis and cell death:

For fluorescent imaging of dead and apoptotic cells, $1x10^5$ cells were grown in 12 well plates, serum starved and treated with various extracts for 24- 48 h. At the end of treatment period, cells were washed with DPBS and replaced with 100µl of Annexin-V- binding buffer. The cells were then labeled with 5µl of Annexin-V-Alexa Fluor 488 and 1µl of Propidium iodide and incubated for 20 minutes at 37°C. EVOS Digital inverted fluorescence microscope (Invitrogen) with a 10X LPlanFL PH fluorescence objective was used to image the labelled cells in the plate.

Statistical analysis

Statistical analysis of the data was performed using one way ANOVA (Tukey-Kramer Multiple Comparisons Test) followed by Student T-test. Difference between the values was considered significant, if $*P< 0.05$, $**P<0.01$(n=4).

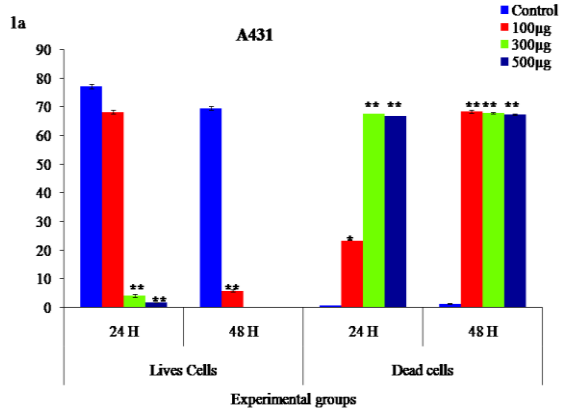
Results and Discussion

Trypan Blue analysis (cell death):

The cell lines were exposed to different concentrations of *A.indica* aqueous extracts for two times (24 and 48h) and then the no of live and dead cells were counted.

A431 cell line

Figure 1a



B16-F10 cell line

Figure 1b

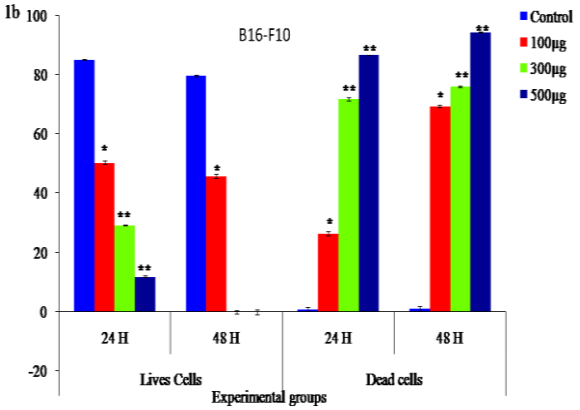


Figure 1a-b: Efficiency of aqueous extract of Acalypha indica in inducing cell death in A431 and B16-F10 cells in different concentrations and time points.

The aqueous extract of *A. indica* treatment on A431 and B16-F10 cells induced in cell death, the no of cells live and dead was observed using trypan blue dye (figure 1a-b). The varied concentrations of extract showed enhanced efficiency in inducing cell death with increased time and dose as are result the number of live cells decreased and whereas the number of dead cells increased.

Cell viability using MTT test

In the present study we applied the MTT test to evaluate the potency of selected aqueous extract of *A.indica* various human skin cancer cell lines (A431 and B16-F10) in an *in vitro* cell-based assay. Both A431 and B16-F10 cells were exposed to various concentrations (100, 300 and 500µg/ml) of the aqueous extract for 24 and 48h in order to assess the cytotoxic and anti-proliferative efficiency of the *A.indica.*

Figure2a: A431 cell line

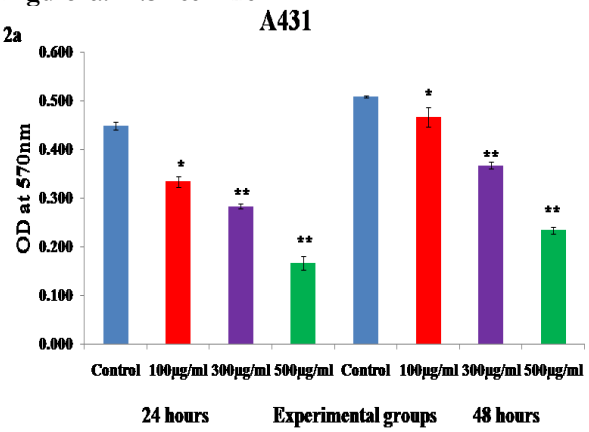


Figure 2b: B16-F10 cell line

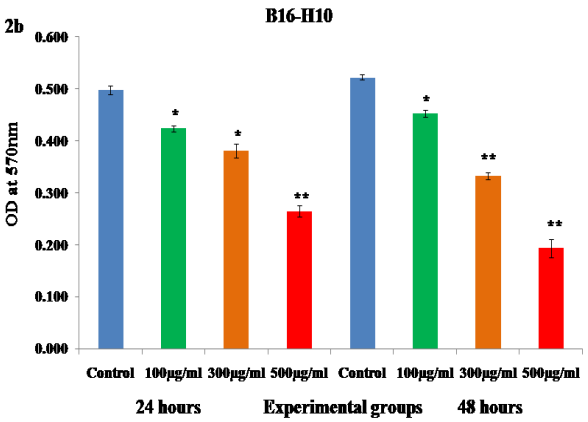


Figure 2a-b: Effect of aqueous extract of *Acalypha indica* on cell viability and cell proliferation in A431 and B16-F10 cells in different concentrations and time points.

The MTT assay measures cell respiration and the number of living cells present in culture can be known by the amount formazan produced, hence based on the live and dead cell number dictates the amount of formazan formation, and indicates the degree of cytotoxicity caused by the drug. All the concentrations showed 50% or greater reduction in the production of formazan product for the both cell lines allowing the determination of an IC50 for each drug with each cell line. The absorbance increased with different concentrations and time points in both the cell lines. However, *A.indica* aqueous extract was inducing less cytotoxicity towards A431 cell line in comparison to B16-F10 cell lines which is represented in through the Figures 2 (a-b). The above results clearly state the anti-proliferative ability of *A.indica* leaf extract against both immortal dermal cell lines, which was increasing dose dependently and also time dependent. The effect of A.indica leaf extract seems less effective with A431 cell line in comparison to the B16-F10 cell line.

Annexin V and Propidium iodide (Apoptosis and Cell death) studies: Cell death by the extracts in dermal cancer cells

Figure 3a

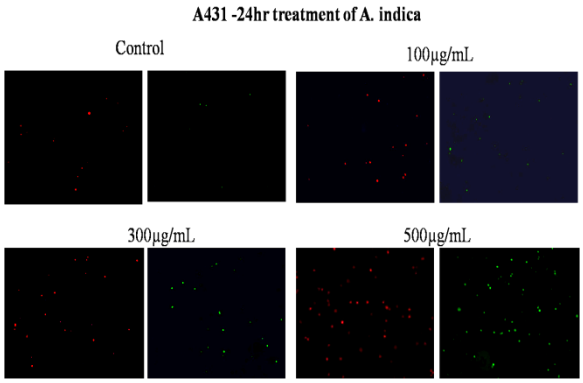
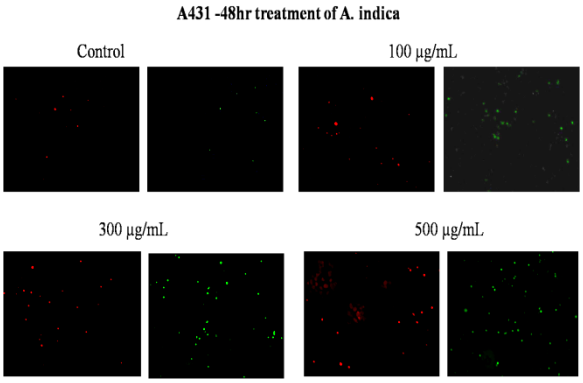


Figure 3b



B16-F10 Cell line

Figure 3c

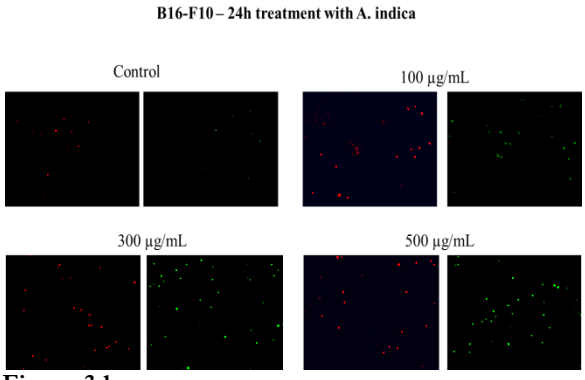


Figure 3d

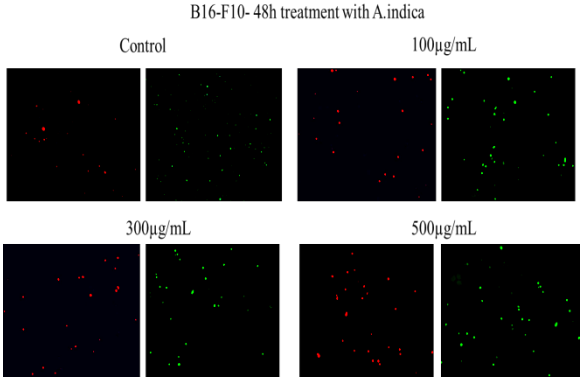


Figure 3a-d: Apoptotic efficiency of aqueous extract of *Acalypha indica* in A431 and B16-F10 cells on exposure with different concentrations and prolonged exposure.

The aqueous extract of *A. indica* induced varied amounts of cell death in the dermal cancer cell lines. Aqueous extract of *A. indica* induced two-fold more apoptosis in A431 (figure 3a) compared to B16-F10 cells (figure 3c) at 24 hrs. A similar increase of 3-fold more apoptotic cells was found in A431 (figure 3b) compared to B16-F10 cells (figure 3d), when they were treated with Curcumin and turmeric extracts. This could be related to the higher sensitivity of the surface receptors on the cells to A431 and B16-F10 or the enhanced activation of pro-apoptotic molecules in both the cell lines. All the different concentration of aqueous extract of *A. indica* induced cell death and increased the number of dead cells after treatment with aqueous extract of *A. indica* in both the cell lines and it increased with concentration and time. The number of dead cells in A431 cells after treatment with *A. indica* extract was similar when compared to B16-F10 cells. The specific proteins which were very sensitive to the treatment with *A. indica* extract in A431 and B16-F10 cells need to be established involved the cell death pathway.

The effect of *A. indica* extract was observed in both cell lines and it is evident that the extract has the ability to induced apoptosis and cell death. The probable mechanism in causing the cell death or apoptosis is by interacting with the cell membrane proteins and making the cell leak its cellular constituents and finally leading death or maybe it is able to interact with the DNA or cell signaling pathways and manipulating the cellular pathways leading or triggering the cell death pathways. The exact mechanism of action has to be studied in details, so that we could understand the exact mechanism of action, as this is could be better source of treatment in treating or controlling the Psoriasis disease or skin related diseases.

Conclusion

This is evident from the results that aqueous extract of *A. indica* could be a probable source in treatment of psoriasis.

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